



Multifunctional fluorescent iron quantum clusters for non-invasive radiofrequency ablation of cancer cells

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ABSTRACT

This work reports the potential of iron quantum clusters (FeQCs) as a hyperthermia agent for cancer, by testing its in-vitro response to shortwave (MHz range), radiofrequency (RF) waves non-invasively. Stable, fluorescent FeQCs of size ~ 1 nm prepared by facile aqueous chemistry from endogenous protein haemoglobin were found to give a high thermal response, with a $\Delta T \sim 50^\circ\text{C}$ at concentrations as low as $165 \mu\text{g/mL}$. The as-prepared nanoclusters purified by lyophilization as well as dialysis showed a concentration, power and time-dependent RF response, with the lyophilized FeQCs exhibiting pronounced heating effects. FeQCs were found to be cytocompatible to NIH-3T3 fibroblast and 4T1 cancer cells treated at concentrations up to $1000 \mu\text{g/mL}$ for 24 h. Upon incubation with FeQCs and exposure to RF waves, significant cancer cell death was observed which proves its therapeutic ability. The fluorescent ability of the clusters could additionally be utilized for imaging cancer cells upon excitation at ~ 450 nm. Further, to demonstrate the feasibility of imparting additional functionality such as drug/biomolecule/dye loading to FeQCs, they were self assembled with cationic polymers to form nanoparticles. Self assembly did not alter the RF heating potential of FeQCs and additionally enhanced its fluorescence. The multifunctional fluorescent FeQCs therefore show good promise as a novel therapeutic agent for RF hyperthermia and drug loading.

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1. Introduction

Amongst various therapeutic modalities adopted for cancer, hyperthermia is an ideal treatment option for non-resectable solid tumors [1]. By utilizing hyperthermia, enhanced efficacy of chemotherapy has been reported by overcoming diverse challenges such as poor accumulation of chemotherapeutics in tumor, high intratumoral pressure, cell heterogeneity and altered tumor vasculature [2,3]. Invasive hyperthermia has been performed using various sources including microwave, radiofrequency as well as near infrared probes [4]. Clinical reports have demonstrated a modest increase in the median survival of liver cancer patients subjected to invasive localized therapies using microwave or radiofrequency needles, as compared to surgical resection and liver transplantation alone [5]. Among the various hyperthermic treatment options, radiofrequency ablation (RFA) therapy is a clinically practiced minimally invasive technique used for the hyperthermic treatment of localized tumors of breast [6] and liver [7,8] and for the treatments

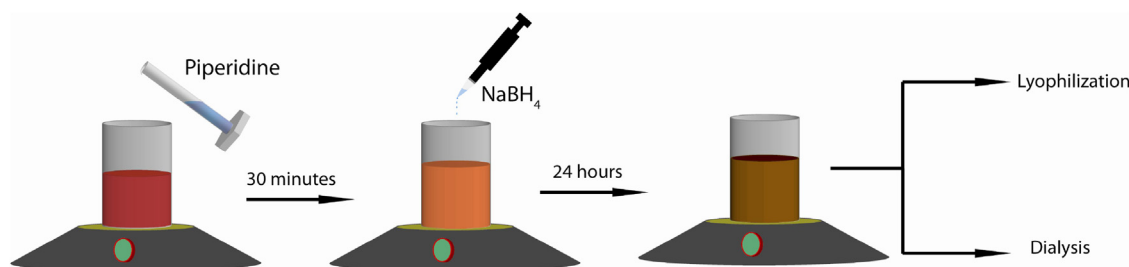
of cardiac arrhythmias [9,10], varicose veins [11], etc. In clinics, RFA is employed using needle probes that are inserted into the diseased site so as to supply high frequency focused RF wave exposure to enable heat generation and ablation [12]. However, the high resistance to heat conduction by body tissues, and the inconvenience of probe insertion, limits the therapeutic potential of RFA therapy [13]. To counter this, whole body, non-contact, non-invasive RFA therapy is presently under investigation to enhance the efficiency of heat generation and delivery [14].

Non-invasive RFA therapy relies on the fact that different tissues in the body show different properties of complex permittivity in the same RF range and therefore show different RF heating behaviour. However, to enable specific and quick temperature elevation within the diseased site, it is imperative to enhance the tissue conductivity at the treatment site to prevent undesirable heating effects in other parts of the body [15]. Recently, a number of literature reports try to elucidate the use of nanoparticles to achieve this aim [16–19]. A wide variety of nanoparticles including, gold [14,20–22], quantum dots [23], semiconductor nanoparticles [17], single walled carbon nanotubes [24,25], fullerenes [23], graphene [26,27], etc, have been shown to exhibit high RF heating and applicability for tumor ablation. The use of water structuring molecules (kosmotropes), to alter

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Scheme 1. Synthesis of FeQCs from haemoglobin. The synthesized FeQCs are purified either through lyophilization or dialysis.

local tissue conductivity has also been investigated and its conjugation with a nanomaterial for delivery has been reported [28].

Even though the exact mechanism of interaction between nanomaterials and RF waves leading to heat generation is not entirely understood, a few theories have been proposed [29–31]. Theoretical reports suggest that under high field strength and high nanoparticle concentrations, conductivity of the host medium gets altered due to the ionic stabilizing agents present on the nanoparticles, which impart increased RF heating [30]. Another report suggests that RF heating was observed only for small sized (<10 nm) nanoparticles, that may undergo electrophoretic movement under an alternating electromagnetic field, leading to the generation of frictional heat [32]. Based on the above two theories, we hypothesize that extremely small sized, well stabilized metal clusters may show good RF heating capability due to its high surface charge that may impart electrophoretic mobility. Further, such metallic clusters also show the ability to exhibit fluorescence emissions in the visible, [33–41] thus making them imageable. Thus, we investigate the theranostic capability of small sized metallic clusters for non-invasive RF heating and fluorescence imaging application against cancer.

To demonstrate this, water soluble iron quantum clusters (FeQCs) of ~1 nm were synthesized using an endogenous protein and evaluated for its potential for RF hyperthermia at different particle concentrations, RF power and exposure period. In-vitro cytotoxicity, anti-cancer activity on RF exposure and cancer cell imaging properties of the synthesized quantum clusters were also studied. Spectrofluorimetry revealed the fluorescence characteristics of the clusters independently as well as when self assembled with cationic polymers. Such a self assembly would permit concurrent loading of multiple agents such as drugs, biomolecules, dyes, etc in the matrix [42], without compromising the inherent capabilities of the iron clusters.

2. Results and discussion

2.1. Synthesis and characterization of FeQCs

The protocol for the synthesis of FeQCs was directly adopted from literature wherein haemoglobin was used as the source of Fe [43]. Scheme 1 shows the method of preparation of FeQCs. Briefly, piperidine was used to extract Fe ions from haemoglobin followed by its reduction using NaBH_4 . Since RF heating is highly dependent on the presence of free ions in the medium, it was required to remove the contaminants in the as-synthesized FeQC suspension. For this, Fe QCs were subjected to either lyophilization or dialysis to remove free piperidine and other un-reacted components.

TEM analysis (Fig. 1A) of as-synthesized FeQCs showed that the clusters have a size of ~1–2 nm and EDS confirmed the presence of Fe (Fig. S1). Spectrofluorimetry studies were used to determine their characteristic excitation and emission wavelengths (Fig. 1B). It was observed that the FeQCs showed an emission peak maximum at 575 nm, with a characteristic bright yellow fluorescence, for excita-

tion at 451 nm. The absorbance spectrum of FeQCs (Fig. 1C) was also recorded to determine the presence of molecular band-like absorption spectra which are characteristic of metallic clusters. It can be seen that the sample showed shoulder-like absorbance bands centered at 330, 423, 578 and 640 nm, similar to that reported earlier [43]. The surface charge of FeQCs was measured in PBS, cell culture medium and MilliQ water (Fig. 1D) by zeta potential analysis. It was found that the zeta potential values of FeQCs did not change significantly in PBS and cell culture medium (DMEM) as compared to MilliQ water, suggesting that the particles remained stable even in a highly ionic medium.

To further confirm the formation of FeQCs, MALDI-MS was done, with haemoglobin as the control (Fig. 2A). The mass spectrum of Hb had peaks at m/z values of 15638 Da and 16399 Da due to the α and β -chains of haemoglobin respectively. The shift in m/z of FeQC sample to 15924 and 16719 Da respectively corresponds to the formation of FeQCs in the haemoglobin chains. The number of iron atoms calculated from the shift in m/z value accounts to 5–6 atoms per FeQCs. Schematic representation of FeQCs containing Fe atoms within haemoglobin is depicted in Fig. 2B.

2.2. RF response of FeQCs

Owing to their small size, we hypothesize that FeQCs could generate heat upon exposure to RF waves. To test this, the RF heating potential of FeQCs was investigated in an RF heating equipment operated at 13.56 MHz (Fig. S2) at different RF power, time and material concentrations. Fig. 3A shows the results of RF exposure of lyophilized FeQCs (LS FeQCs) against different controls including NaBH_4 , haemoglobin, and 0.9% Saline, at an RF power of 100W for 1 min exposure. To eliminate the effects of contaminant heating [32], FeQCs were also subjected to rigorous dialysis to form dialyzed FeQCs (DS FeQCs) which were then exposed to RF waves. From Fig. 3A, it can be seen that both LS FeQCs ($\Delta T \sim 50^\circ\text{C}$) and DS FeQCs ($\Delta T \sim 18^\circ\text{C}$) showed a significant increase in temperature upon RF exposure as compared to the above controls which showed a thermal response of only $\Delta T \sim 8^\circ\text{C}$. It is important to note that neither haemoglobin nor the reducing agent NaBH_4 showed any significant RF heating response. This implies that the reduction of haemoglobin by NaBH_4 lead to the formation of RF responsive, small sized FeQCs.

On comparing the RF heating of LS and DS FeQCs, both materials showed a concentration dependent RF heating response (Fig. 3B). It can be seen that LS FeQCs showed a much higher thermal response as compared to DS FeQCs at 165 $\mu\text{g}/\text{mL}$ ($\Delta T \sim 50^\circ\text{C}$). This is possibly due to the presence of ions associated with the clusters which could contribute towards the RF heating of LS FeQCs. However, upon removal of these free ions, DS FeQCs showed considerable RF heating only at high concentrations of 500 $\mu\text{g}/\text{mL}$ ($\Delta T = 18^\circ\text{C}$). Thus both LS and DS FeQCs showed significant heating activity when exposed to RF waves, although at different concentrations. This data is amply supported by the thermal images of LS and DS FeQCs at identical concentrations upon RF exposure (Fig. 3C), revealing the high RF heating displayed by LS over DS FeQCs.

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