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Evaluation of ferromagnetic fluids and suspensions for the site-specific radiofrequency-induced hyperthermia of MX11 sarcoma cells in vitro

Nikolai A. Brusentsov[a], V.V. Gogosov[b], T.N. Brusentsova[b], A.V. Sergeev[a], N.Y. Jurchenko[a], Anatoly A. Kuznetsov[c], Oleg A. Kuznetsov[c,e,*], L.I. Shumakov[d]

[a]*Blokhin Oncological Research Center RAMS, 115478, Moscow, Russia*
[b]*Institute of Mechanics, Moscow State University, 117192, Moscow, Russia*
[c]*Institute of Biochemical Physics, Russian Academy of Sciences, Kosygin St.4, Moscow 117977, Russia*
[d]*Russian Radiotechnical Research Institute, Moscow, Russia*
[e]*University of Louisiana at Lafayette, P.O. Box 42451, Lafayette, LA 70504-2451, USA*

Abstract

Seventeen different ferromagnetic fluids and suspensions were prepared and evaluated for application in radiofrequency-induced hyperthermia. Specific power absorption rates were measured at 0.88 MHz to range from 0 to 240 W per gram of iron for different preparations. Survival of MX11 cells mixed with ferrofluids and subjected to radiofrequency was much lower than with RF without ferrofluid or ferrofluid alone.

Keywords: Ferrofluids; Specific power absorption rate; SAR; Hyperthermia; Sarcoma; Dextran-ferrite; Carboxymethyldextran-ferrite; Ferro-carbon; MX-11 sarcoma; Radiofrequency ablation

1. Introduction

Hyperthermia is an established technique in experimental and clinical oncology, which takes advantage of higher sensitivity of tumor tissues to heat [1–6]. Therapeutic effects can be further increased by combining heating with chemotherapy and radiation, since malignant cells are more sensitive to these treatments at higher temperatures [2]. Several techniques are used to increase the temperature in the tumor to 42–46°C, with magnetic fluid hyperthermia attracting increasing attention. This method involves the introduction of ferromagnetic or superparamagnetic particles into the tissue and their irradiation with an alternating electromagnetic field at radiofrequencies (RF) of 10^4 to 10^7 Hz, typically about 1 MHz [2–6]. The particles dissipate the energy of the radiowaves into heat by several physical mechanisms [4]. Thus, combined with a targeted delivery of the particles into the desired zone, this method allows to generate heat locally even in tumors, located deep inside the patient's body, while minimizing heating of the rest of the organism. Effectiveness of transformation of the RF field energy into heat strongly

* Correspondence address: University of Louisiana at Lafayette, P.O. Box 42451, Lafayette, LA 70504-2451, USA. Tel.: 1-337-482-5230; fax: 1-337-482-5834.
E-mail address: oleg@louisiana.edu (O.A. Kuznetsov).

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depends on the frequency and on the nature of the particles [3–6], and is typically characterized by specific power adsorption rate (SAR, measured in W/g of Fe) of the particles as a function of the frequency. Therefore, proper selection of the particles and the field parameters is very important for effective treatment.

The purpose of this work was to evaluate a variety of ferromagnetic fluids and suspensions produced by our group for application in the site-specific radiofrequency-induced hyperthermia.

2. Materials and methods

We tested four water-based dextran-ferrite (DF) and four carboxymethyldextran-ferrite (CMDF) ferrimagnetic fluids (FF); several ferri- and ferromagnetic suspensions (FS) of uncoated Fe_3O_4, -Fe_2O_3, -Fe_2O_3, $Fe(OH)_3$, $Fe(OH)_2$ and reduced Fe; and four water-based ferro-carbon suspensions. DF and CMDF ferrofluids and ferrosuspensions were prepared by a procedure modified from [7–11]. Ferro-carbon particles and their suspensions were prepared according to the procedures described in [11–14], electron micrographs and physical characterizations of these particles are presented in [13]. Dextran-coated colloidal magnetic iron oxides (DCIO) were prepared as in [5,15]. A half of each ferrosuspension was treated with 150–200 W ultrasound for 30 min [3–5,10,15]. Both sonicated (UFS) and non-sonicated ferrosuspensions were tested.

Transmission electron microscopy (TEM) of ferrofluids and ferrosuspensions was performed using a Phillips EM-400 microscope (300,000× magnification, 0.4 nm resolution) and the images were analyzed using a control SEM IPS image analyzer.

Saturation magnetization (M_s) and initial magnetic susceptibility (χ_{init}, susceptibility at zero field) of dry particles, ferrofluids and ferrosuspensions were determined using a Faraday balance magnetometer (Bruker).

Specific power absorption rates (SAR) of ferrofluids and ferrosuspensions were measured by a technique modified from [5]. 3 ml samples of ferrofluids or ferrosuspensions were placed inside a 60 × 200 mm air-cooled inductor with a matching high-Q-resonator fed with RF power. The device was developed in the Russian Radiotechnical Research Institute and operated at $f = 0.88$ MHz with peak magnetic field intensity $H_p = 90$ Oe. The sample temperature was monitored using an organic liquid thermometer and the SAR of the sample was calculated from the time–temperature dependencies as the amount of power converted into heat per gram of Fe.

Cytotoxicity of DF in combination with RF was studied on peritoneal ascitic sarcoma MX11 cells in vitro. 2.4 ml of MX11 cells suspension (3×10^6 cells/ml) was mixed with 0.6 ml of sterilized ferrofluid and exposed to RF as described above for up to 6 h. The temperature was maintained in the range of 43–45°C. Mixture of 2.4 ml of the same cell suspension and 0.6 ml of physiological solution (0.9% NaCl (w/v)) served as a control. Alternatively, the same mixtures were placed into a water bath at 44°C or at 37°C. After 20 min, 40 min, 1 h, 3 h or 6 h of one of the above treatments survival of MX11 was analyzed by a hemacytometer and by an intraperitoneal injection of 1 ml of the MX11 suspensions into C57Bl/6j mice, according to procedures in [16]. Statistical analysis was done as described in [16].

3. Results and discussion

There was no detectable heating of a physiological solution (0.9% NaCl) by the 0.88 MHz RF. Therefore, the observed heating of ferrofluids and ferrosuspensions was due to an interaction of RF with ferromagnetic particles in them. Data on particle size (measured by TEM), initial magnetic susceptibility (χ_{init}) and SARs of the ferrofluids and ferrosuspensions are summarized in the Table 1. SARs of DF ferrofluids are up to 210 W/[g of Fe]; of DF ferrosuspensions — up to 180 W/[g of Fe]; of DF UFSs — up to 240 W/[g of Fe]. SARs of ferrosuspensions of uncoated $Fe(OH)_3$, $Fe(OH)_2$, -Fe_2O_3, reduced Fe, -Fe_2O_3, Fe_3O_4 ranged from 0 to 45 W/[g of Fe]. SAR of ferrofluids and ferrosuspensions strongly depends on the nature, structure and composition of the ferromagnetic core and the stabilizing coating. Treatment with ultrasound causes not only dispersion of particle

Table 1
Physical properties of various ferromagnetic fluids (FF) and ferromagnetic suspensions (FS): particle diameter, initial magnetic susceptibility (χ_{init}) of dry particles and specific power absorption rates (SAR) at 0.88 MHz and 90 Oe peak magnetic field. ($\pm$ the accuracy of the measurements)

Preparations	Type	Particle diameter (nm)	χ_{init} (cm^3/[g of Fe])	SAR (W/[g of Fe])
Dextran–ferrite 363	FF	10–12	0.58 ± 0.02	210 ± 8
Dextran–ferrite 540	FS	10–90	0.53 ± 0.02	180 ± 7
Carboxymethyldextran–ferrite 543	FF	6–12	0.22 ± 0.02	90 ± 4
Carboxymethyldextran–ferrite 544	FS	6–120	0.24 ± 0.02	93 ± 4
Dextran–stabilized magnetic colloidal iron oxide	FS	3–60	0.17 ± 0.02	42 ± 3
Sonicated dextran–stabilized magnetic colloidal iron oxide	FF	3–9	0.18 ± 0.02	60 ± 3
Sonicated dextran–ferrite ferromagnetic suspensions	FSs	6–12	$0.05 - 0.65$	$12 - 240$
Ferro-carbon 3013	FS	60–1200	0.045 ± 0.005	9.3 ± 1
Sonicated ferro-carbon 3013	FS	10–150	0.045 ± 0.005	15 ± 2
Ferro-carbon 3014	FS	60–1200	0.025 ± 0.005	1.5 ± 0.2
Ferro-carbon 3015	FS	60–1200	0.020 ± 0.005	1.2 ± 0.2
Fe_3O_4	FS	100–150	–	45 ± 3
α–Fe_2O_3	FS	100–150	–	0 ± 0.1
γ–Fe_2O_3	FS	100–150	–	42 ± 3
$Fe(OH)_3$	FS	10–150	–	0 ± 0.1
$Fe(OH)_2$	FS	10–150	–	0 ± 0.1
Reduced Fe	FS	1000–2500	–	21 ± 2

agglomerates but also at least partial destruction of the coating, which leads to higher SARs (Table 1). However, such loss of stabilizing coating also leads to aggregation instability of the ferrofluids and ferrosuspensions, making them unsuitable for medical applications.

Dextran-ferrite and carboxymethyldextran-ferrite ferrofluids were determined to be the most promising type of the particles for magnetic fluid hyperthermia and were selected for further studies. DF contained 10–12 nm diameter -Fe_2O_3 cores (Fig. 1), surrounded by ca. 120 nm thick hydrated dextran coating. Dried DF contained 36% of -Fe_2O_3 and 64% of dextran; M_s of dried DF was 22 emu/g. M_s of ferrofluids was proportional to the concentration of the DF particles. In earlier studies [11] we have determined that DF has low toxicity and is well tolerated by mice: LD_{50} was 5 g/kg. CMDF contained 6–12 nm diameter -Fe_2O_3 crystals surrounded by a 130 nm layer of hydrated carboxymethyldextran. DF and CMDF ferrofluids were resistant to gravitational forces, magnetic fields and can be re-suspended after lyophilizing.

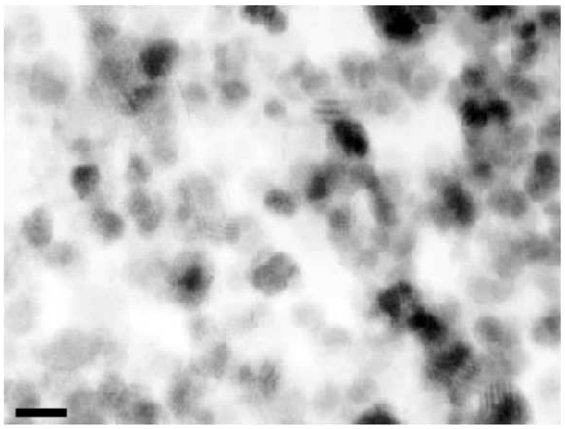

Fig. 1. Transition electron micrograph (TEM) of dextran–ferrite 363. The bar is 10 nm.

Treatment with ultrasound led to a 10–30% increase in SAR of DF and CMDF ferrofluids, but reduced their aggregation stability, presumably due to destruction of the dextran coating. These data support the hypothetical structure of DF and

CMDF particles as a subdomain iron oxide core surrounded by a hydrated layer of dextran molecules, and suggest, that the main heating mechanism is the magnetization relaxation loss process ("Néel mechanism" [4]). Even without ultrasonic treatment DF and CMDF ferrofluids have sufficiently high SARs and produce a satisfactory heating in the tested RF field.

No long-term toxicity or acute death of ascitic sarcoma MX11 cells was detected when the cells were exposed to DF ferrofluids (up to 80 mg of DF/ml) alone at 37°C, or to the RF field without a ferrofluid. After 6 h the survival rate (SD) was 9.76% and 9.47%, respectively. However, when MX11 cells were exposed to 43–45°C temperatures either by RF-induced ferrimagnetic hyperthermia in the presence of 40–80 mg/ml of sterilized DF (10–20 mg of Fe per ml) or by conventional water bath heating at 44°C, survival of MX11 cells was low. The survival rate (SD) after a 40 min treatment was 1.48% for DF + RF and 1.89% for the water bath, and only traces in both cases after 6 h of treatment. Typical exposure-dependent cytotoxicities were observed for both treatments with no significant differences ($P < 0.05$) between cytotoxic effects of the two heating methods. Hemacytometer data were confirmed by intraperitoneal injections of 1 ml of the treated MX11 suspensions into C57Bl/6j mice. Therefore, the cytotoxicity of ferrofluids with RF-heating should be attributed mostly to the effects of heat itself.

Several authors [4–6] studied similar dextran-ferrite magnetic fluids for magnetic fluid hyperthermia and measured their SARs at different frequencies and peak magnetic field intensities. Since SAR is proportional to $\boldsymbol{f}$ and $\boldsymbol{H}^2$ [4,5], to compare SAR values of ferrofluids produced by our group with the literature data, the SAR values need to be "normalized" to 1 MHz and 100 Oe field, as was done in [5]. Therefore, SAR values of our ferrofluids need to be multiplied by the factor of 1.4, and $\mathrm{SAR}_{1\ \mathrm{MHz},100\ \mathrm{Oe}}$ of DF will be 250–330 W/g of Fe, that of CMDF will be 130 W/g of Fe. Chan et al. [5] reported $\mathrm{SAR}_{1\ \mathrm{MHz},100\ \mathrm{Oe}}$ of 400–500 W/g of Fe for "optimally" and 100–300 for "sub-optimally" synthesized dextran-coated ferrocolloids. Jordan et al. [4,6] synthesized dextran magnetite with $\mathrm{SAR}_{1\ \mathrm{MHz},100\ \mathrm{Oe}}$ 80–200 W/g of Fe. Thus, DF ferrofluids produced by our group have sufficiently high SAR values, comparable with the literature data. This allows to estimate [4], that only about 2 mg of Fe in the DF is needed per gram of body tissue for heating it 5°C/min. Further experimental studies are needed to confirm these estimates, and methods of targeted delivery of the DF particles into the desired zone of an organism need to be developed. The targeted delivery technique might include antibody-based recognition, magnetic concentration, injection through a catheter into a local artery combined with magnetic particle retention [12], or a combination of the above.

Considering their low toxicity, high SAR and convenience of use, dextran-ferrite and carboxymethyldextran-ferrite ferrofluids appear to be promising materials for application in magnetically controlled RF-induced ferrimagnetic hyperthermia.

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