FERROFLUID FOR RADIOFREQUENCY CAPACITIVE HYPERTHERMIA: *IN-VITRO* STUDY

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Abstract

The current work deals with preparation and characterization of electrically lossy ferrofluid which can be used as a mediator for radio frequency (RF) - capacitive hyperthermia method. To this end, ferrofluid that can absorb the energy of alternating electrical field at the frequencies commonly employed in RF-capacitive hyperthermia (13.56 and 27.12 MHz) has been prepared by co-precipitation method. This ferrofluid comprises of electrically conductive component with core-shell structure, i.e. magnetite nanoparticles (NPs) coated by dextran, organized in chain-like structure. The effect of RF - capacitive hyperthermia in the presence of mediator was studied on the series of tests performed on HaCaT and HepG2 cell lines using MMT test. The RF-electrical field (13.56 MHz) with controllable power output was applied using the EHY-110 SA (Oncotherm group) to increase the temperature of samples from 37 °C up to target temperature of 44 °C. The results of *in-vitro* test clearly indicate that the usage of capacitive heating of obtained ferrofluid substantially contribute to cytotoxic effect of hyperthermia treatment.

Keywords: Iron oxide nanoparticles, Dextran, Core - shell nanoparticles, Hyperthermia

1. INTRODUCTION

Presently, the rationale for using hyperthermia, as a medical heat treatment of tumors in temperature range 42 °C – 45 °C, is well established [1]. The molecular mechanisms for cell death by hyperthermia are understood now, with protein denaturation being the main mechanism among these. Simultaneously, the structural and enzymatic changes of proteins in temperature window between 42 °C and 45 °C cause an increase in sensitivity of tumor cells to radiation and chemotherapy drugs. It is considered now that the main intention of hyperthermia is to improve the results of the conventional treatment strategies within a framework of multimodal treatment: chemotherapy and radiotherapy followed by hyperthermia [2-5]. The existing hyperthermia systems (applicators) are based on the following methods: (i) impedance, inducting and capacitive heating in kHz and MHz regions; (ii) antenna (phase) array in 100 MHz region; (iii) microwave radiation over 200 MHz [6]. In hyperthermia, the final temperature of tumour is mainly dependent on the energy deposition and its localization that can be improved by embedding specific material into the tumor, so called mediator, which absorbs and subsequently dissipates energy of electromagnetic radiation into heat resulting in selective heat treatment of tumour on the microscopic level. Depending on the mediator type, different hyperthermia methods can be used: inducting heating by using of magnetic materials (Magnetic Hyperthermia, MH) [7] and capacitive heating by using electrically lossy materials [8, 9]. The lack of mass production of the alternating magnetic field applicator systems is one of the reasons that hinders the realization of the MH in clinical practice. However, capacitive heating of tumors using a radio-frequency (RF) electrical field has been adopted in practice worldwide. Examples of commonly employed RF-capacitive heating applicator systems are Thermotron RF-IV (Yokogawa ElectronicsCo., Tokyo, Japan) or Thermotron RF-8 (Yamamoto Vinyter Co., Osaka, Japan), and SYNCHROTHERM RF (Due.R Srl. Co, Italy) and Yacht-5 (Istok, Fryazino, Russia). Current work deals with synthesis and *in-vitro* study of ferrofluid (FFL) with high dielectric losses at basic frequency(ies) of actual RF–capacitive hyperthermia device with a view to enhance heating efficiency of treatment.

2. EXPERIMENTAL PART

2.1. Synthesis of FFL

FFLs were prepared by coprecipitation method in alkaline media starting from a mixture of $FeCl_3 \times 6H_2O$ and $FeCl_2 \times 4H_2O$ in twice distilled water; thereafter, an equal volume of stabilizer solution (dextran; 40 kDa; 15 wt. %) in distilled water was added to the iron salts solution. Aqueous ammonia was used as a precipitating agent. An approximately equal volume of NH_4OH (1 M) was added dropwise (1 drop/min) to an iron salts-polymer mixture under permanent stirring up to pH = 10. After that, the solution was boiled for 15 minute at 60 °C to form an almost black precipitate, which was dialyzed against unreacted iron salts during 48 hours with fourfold changing of water to maintain pH ≈ 6.5. Finally, the subsided iron oxide nanoparticles (NPs) were separated by ultracentrifugation and re-dispersed in distilled water by sonication. Substantially, seven types of FFLs (N1-N7) with different structures, dielectric and magnetic properties were prepared under the different Fe(III)/Fe(II) molar ratios (**Table 1**).

Table 1 Characterisation of FFL samples

Sample	Fe (III) / Fe (II)	a_0, (Å)	d_{TEM}, (nm)	d_{DLS}, (nm)	c_{Fe}, (g l^{-1})	M_S, (emu g^{-1})	ε'', [13.56 MHz]	ε'', [27.12 MHz]
N1	1.7 : 1	8.325	8-15	60	5.44	0.39	20	9.8
N2	1.4 : 1	8.315	8-15	60	5.70	0.39	10	5.1
N3	1.25 : 1	8.333	8-15	70	5.60	0.39	7	3.2
N4	1 : 1	8.320	8-10	80	6.00	0.39	140	73.8
N5	1 : 2.9	8.350	8-15	70	4.26	0.39	10	4.2
N6	2 : 1	8.319	8-15	70	4.60	0.39	30	14.7
N7	2.5 : 1	8.355	8-15	100	5.81	0.39	45	21.5

2.2. Characterization

The iron concentration in magnetic liquids were determined by Energy Dispersive X-ray Fluorescence spectroscopy by ARL Quant'X EDXRF Analyzer, Thermo Scientific. The morpho-structural properties of FFLs and average NPs size (d_{TEM}) were examined by Transmission Electron Microscopy (JEOL JEM - 2100F). Hydrodynamic size (d_{DLS}) of NPs based aggregates were measured by Dynamic Light Scatering (DLS) and Laser Doppler Velocimetry by Zetasizer Nano ZS, Malvern Instruments. X-ray diffraction patterns of samples were obtained by using an X'Pert PRO X-ray diffraction meter with Co Kα radiation at λ = 0.179026 nm. Lattice parameter (a_0) was refined with software package (HighScore Plus, PANanalytical, Netherlands). Magnetization curves and magnetization saturation (M_S) were obtained using a Vibrating Sample Magnetometer (Lake Shore 7404) in the field up to 10 kOe. Dielectric properties of FFLs were investigated by impedance method in frequency range from 10 Hz to 100 kHz using LCR Hioki 3522, whereas in RF-range (from 10 MHz - 20 GHz), the open reflection method was performed by Agilent 2-Port PNA-L Microwave Analyzer N5230A. In last method, the samples were measured by immersing the open-ended coaxial probe into a FFL. *In-vitro* test of the effect of electrically lossy type FFL on the heating of malignant cells was performed in Biological Safety Cabinet: HERAsafe KSP (Thermo Electron LED Gmbh, Germany) by using a lab type RF-applicator, EHY 110 Oncontherm, operating on a fixed frequency of 13.56 MHz. Two different cell lines were used: human immortalized non-tumorogenic keratinocyte cell line (HaCaT) (Catalog No. 300493, CLS Germany), and human hepatocellular carcinoma cell line (HepG2) from ATCC (HB-8065). Cell viability was measured using the MTT assay (Invitrogen Corporation, USA). The absorbance of MMT assay was measured at 570 nm by Sunrise microplate absorbance reader (Tecan, Switzerlald). Hyperthermia treatment was designed in a following manner. FFL was mixed with cells suspension with the iron concentration of 3.5

g/l and 4.67 g/l in the tested volume. All samples were preheated to 37 °C. Further heating was realised via capacitive applicator; temperature was measured using a pair of thermocouples. Specific power employed during ramping was about 2.5 W/ml and 1.5 W/ml for steady state. Samples were heated from 37 °C up to a target temperature of 44 °C which was maintained for 30 minutes making sure it was not overshot. Microscopic observation of cell culture before and after treatment was carried out by Inverted phase contrast microscope Olympus CKX 41(Olympus, Japan), and Inverted fluorescent microscope Olympus IX51 (Olympus, Japan).

3 RESULTS AND DISCUSSION

FFLs have been developed that represent a stable colloidal dispersion of magnetic iron oxide NPs stabilized by a thin layer of biodegradable and biocompatible polymer, dextran. Among FFLs obtained, only one is characterized by high dielectric losses (**Figure 1**) resulting from its specific morphology, where NPs are organised into chain-like structures, which are interconnected to form an infinite cluster (**Figure 2a**). The microstructure of nanoparticles assembly of FFL is given by synthesis condition, namely polymer-stabiliser molecular weight (Mw) and its concentration, as well as corresponding molar ratio of ferrous chloride and ferric chloride.

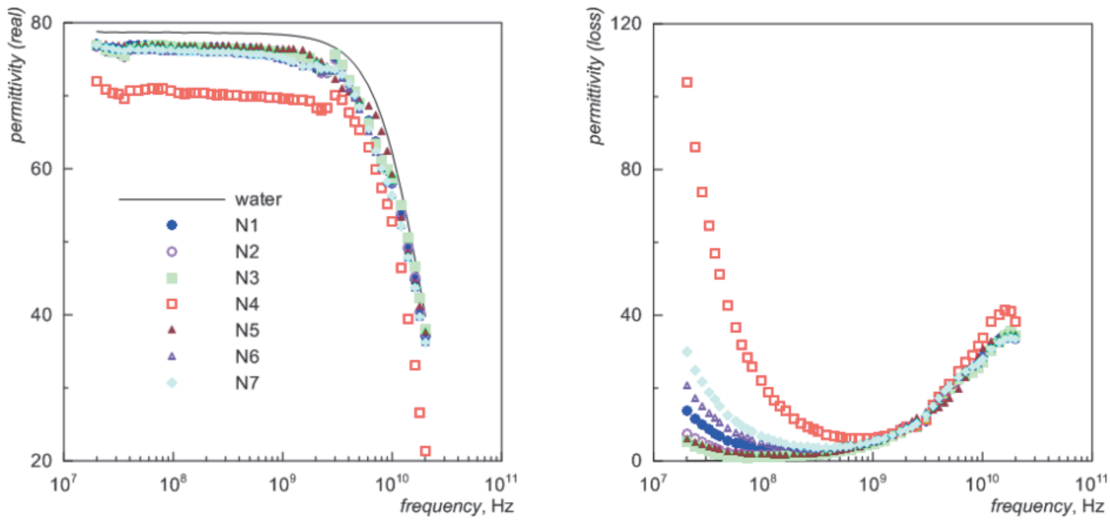
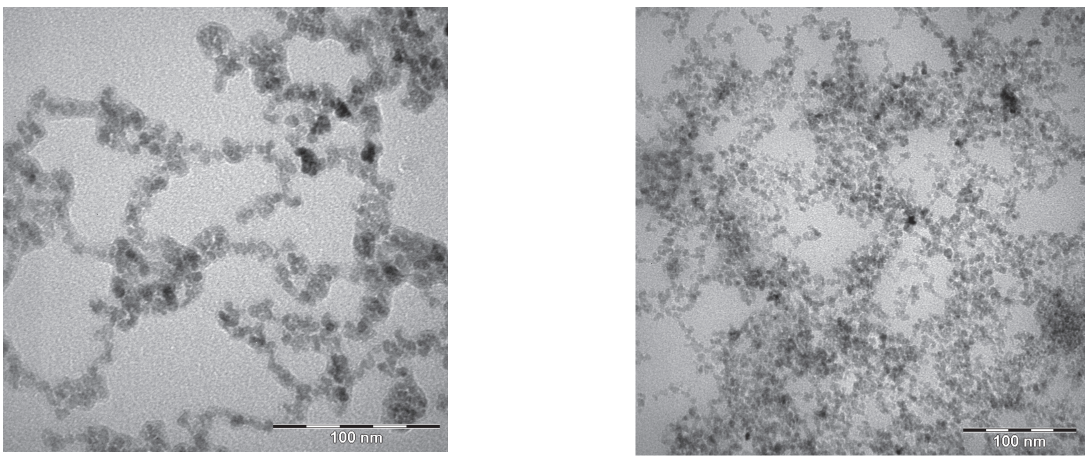


Figure 1 Dielectric spectra of FFLs with different microstructure (samples N1-N7) compared with the spectrum of distilled water



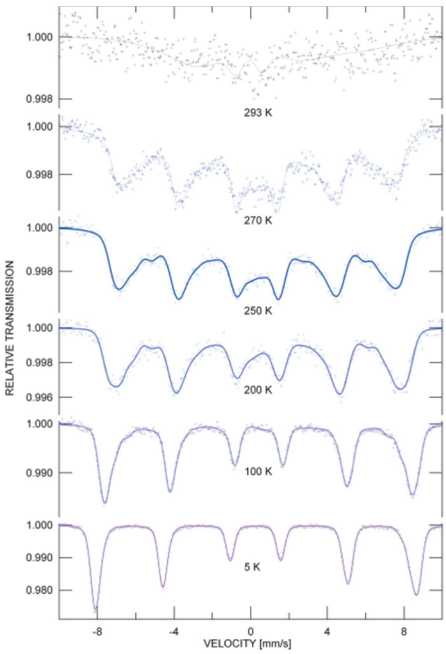
(a) (b)

Figure 2 TEM images of samples N4 (a) and N1 (b)

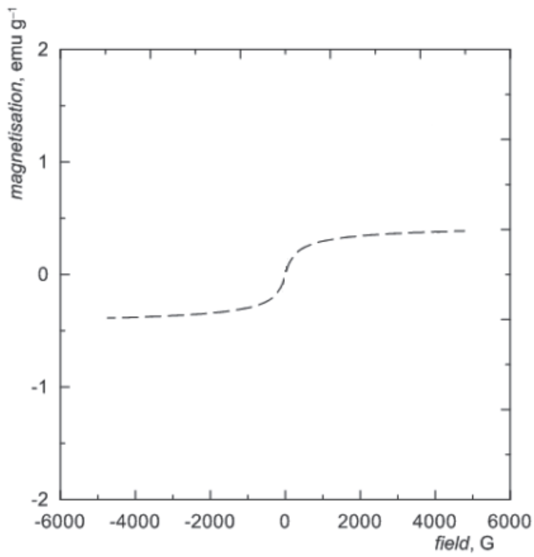
In accordance with TEM images, NPs size is ranging from 8 to 15 nm (**Figure 2**). According to X-Ray diffraction analysis, NPs are more probably nonstoichiometric magnetite as long as the value of lattice constant a_0 is lower than for magnetite (a_0 = 8.396 Å) (**Table 1**). NPs are superparamagnetic as it was determined by Mossbauer spectroscopy and magnetostatic measurements. Thus, Mossbauer spectra demonstrated that the superparamagnetic relaxation occurs at temperatures above 270 K according to the depression of the magnetically split signal (**Figure 3a**), and magnetization curve shows anhysteretic behavior (**Figure 3b**).

Consequently, NPs have core-shell structure, where core consists of nonstoichiometric magnetite and shell comprises dextran. The choice of a dextran-type polymer and its concentration is a crucial item since it affects the thickness of the dextran overlayer (shell) which in turn determines nanoparticle size and interparticles interaction. It is known, that the increase of dextran molecular weight leads to increase of the dextran-shell thickness [10], and the increase of dextran concentration leads to the smaller particle size and formation of longer chain-aggregates [11]. Dextran with molecular weight of about 40 KDa promotes precipitation within a polymer random coil structure. The well folded polymer creates confined space within which iron oxide crystal growth is limited and thus resulting in roughly spherical particles with a size of about 10 nm were the polymer overlayer is about several nm. Each particle having magnetic moment is involved into interparticle interaction and consequently in creation of different types of aggregates (chain-like, globular, ring-like, etc.). According to DLS analysis, the mean size of aggregates varies from 60 to 100 nm (**Table 1**). It is established that, for 15 wt. % of dextran with M_w = 40 kDa only molar ratio of Fe^{III}/Fe^{II} = 1:1 yields particles organised into chain-like structures forming an infinite cluster, contrariwise, other molar ratio of Fe^{III}/Fe^{II} with the same concentration of dextran does not lead to said self-organized structures (**Figure 2**).

Topological differences in FFLs microstructure in their turn influence the dielectric properties. When the particles are arranged into chains forming conducting network structure (sample N4), the dielectric losses are significantly higher than values recorded for other morphologies (**Table 1**). The enhancement of imaginary part of complex permittivity was observed in frequency range from 1 MHz up to 30 MHz (**Figure 1**), the frequencies adopted in RF– capacitive hyperthermia. The average length of chain-like aggregates is around 100 nm and thus, it is possible to consider these as nanowires with high aspect ratio. It is well known that the dielectric losses for such type of structure are significantly higher than for individual particles [12].



(a)



(b)

Figure 3 Mossbauer spectroscopy and magnetization curves of FFL (sample N4)

Electrically lossy FFl (sample N4) has been chosen for *in-vitro* study as a mediator for RF-capacitive hypethermia, since it can generate sufficient heat through dielectric losses by the application of RF field at the frequencies of 13.56 and 27.12 MHz. Obtained results confirmed that use of electrically lossy FFL has significant impact on cell proliferation, i.e. usage of capacitive heating in the presence of mediator substantially contribute to anti-proliferative effect of hyperthermia treatment. Thus, MMT test showed the significant regress of number of malignant cells during short period after treatment (**Table 2**). It should be noted also that effect increases with concentration of mediator: unlike 3.5 g/l concentration of mediator, the 4.67 g/l concentration leads to noticeable decrease in cells survival rate by 13 - 40 % depending on used cell line. Although the observed effect on cell viability can be mainly put down to the exposure of cells to enhanced temperature, we cannot exclude the influence of the factors such as interconnections between cells and mediator.

Table 2 Results of *in-vitro* tests for two cell lines determined using MTT. The ratio of surviving cells.

Cell line	No. days after treatment	Control	Water bath	Mediator 3.5 g / l	Mediator 4.67 g / l
HepG2	2	1.00	0.53	0.47	0.34
	8	1.00	0.86	0.66	0.32
HaCaT	2	1.00	0.36	0.59	0.28
	8	1.00	0.53	0.75	0.34

4 CONCLUSION

FFL based on dextran-coated magnetite NPs with different dielectric properties were obtained by coprecipitation. A significant difference in dielectric properties of FFLs is attributed to their microstructure, namely, whether or not NPs form conductive network structure. Cluster formation is determined by magnetic dipolar interaction between NPs and thus can be controlled by changing of the ratio between average particle diameter and the thickness of dextran coating through synthesis conditions. This structured FFL exhibits high value of dielectric losses in frequency range from 10 to 30 MHz which makes it a promising heat-generating medium for RF-capacitive hyperthermia. *In-vitro* study of electrically lossy FFL on HaCaT and HepG2 cell lines under exposure to RF field at 13.56 at lower power output shows quicker attainment of the target temperature (44 °C) and significant regress of number of malignant cells during short period after treatment.

ACKNOWLEDGEMENTS

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REFERENCES

[1] VAN DER ZEE, J. Heating the patient: a promising approach? *Annals of Oncology*, 2002, vol. 13, pp. 1173-1184.

[2] FOTOPOULOU, C., CHO, C. H., KRAETSCHELL, R., GELLERMANN, J., WUST, P., LICHTENEGGER, W., SEHOULI, J. Regional abdominal hyperthermia combined with systemic chemotherapy for the treatment of patients with ovarian cancer relapse: Results of a pilot study. *International Journal of Hyperthermia*, 2010, vol. 26, pp. 118-126.

[3] WUST, P., HILDERBRANDT, B., SREENIVASA, G., RAU, B., GELLERMANN, J., RIESS, H., FELIX, R., SCHLAG, P. M. Hyperthermia in combined treatment of cancer. *Lancet Oncology*, 2002, vol. 3, pp. 487-497.

[4] VAN DER HEIJDEN, A. G., JANSEN, C. F. J., VERHAEGH, G, O'DONNELL, M. A., SCHALKEN, J. A., WITWJES, J. A. The effect of hyperthermia on mitomycin-C induced cytotoxicity in four human bladder cancer cell lines. *European Urology*, 2004, vol. 46, pp. 670-674.

[5] FRANCKENA, M., STALPERS, L. J. A., KOPER, P. C. M., WIGGENRAAD, R. G. J., HOOGENRAAD, W. J., VAN DIJK, J. D. P., WARLAM-RODENHUIS, C. C., JOBSEN, J. J., VAN RHOON, G. C., VAN DER ZEE, J. Long-term improvement in treatment outcome after radiotherapy and hyperthermia in locoregionally advanced cervix cancer: An update of the Dutch Deep Hyperthermia Trial. *International Journal of Radiation Oncology Biology Physics*, 2008, vol. 70, pp. 1176-1182.

[6] VORST, A. V., ROSEN, A., KOTSUKA, Y. *RF/microwave interaction with biological tissues*. Hoboken: Wiley, 2006.

[7] JORDAN, A., WUST P., FAHLING, H., JOHN, W., HINZ, A., FELIX, R. Inductive heating of ferrimagnetic particles and magnetic fluids: Physical evaluation of their potential for hyperthermia. *International Journal of Hyperthermia*, 2009, vol. 25, pp. 499-511.

[8] KANZIUS, J. *Enhanced systems and methods for RF-induced hyperthermia*. US Patent, 2009.

[9] SHINKAI, M., UEDA, K., OHTSU, S., HONDA, H., KOHRI, K., KOBAYASHI, T. Effect of functional magnetic particles on radiofrequency capacitive heating: An in vivo study. *Japanese Journal of Cancer Research*, 2002, vol. 93, pp. 103-108.

[10] KAWAGUCHI, T., HANAICHI, T., HASEGAWA, M., MARUNO, S. Dextran-magnetite complex: conformation of dextran chains and stability of solution. *Journal of Materials Science-Materials in Medicine*, 2001, vol. 12, pp. 121-127.

[11] LEE, K. M., KIM, S. G., KIM, W. S., KIM, S. S. Properties of iron oxide particles prepared in the presence of dextran. *Korean Journal of Chemical Engineering*, 2002, vol. 19, pp. 480-485.

[12] LANDAU, L. D., LIFSHITZ, E. M. *Electrodynamics of continuous media*. Pergamon Press, 1984.