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Local radiofrequency-induced hyperthermia using CuNi nanoparticles with therapeutically suitable Curie temperature

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Abstract

Copper–nickel (CuNi) alloy nanoparticles with Curie temperatures (T_c) from 40 to 60 °C were synthesized by several techniques. Varying the synthesis parameters and post-treatment, as well as separations by size and T_c, allow producing mediator nanoparticles for magnetic fluid hyperthermia with parametric feedback temperature control with desired parameters. In vitro and in vivo animal experiments have demonstrated the feasibility of the temperature-controlled heating of the tissue, laden with the particles, by an external alternating magnetic field.

Keywords: Magnetic fluid hyperthermia; Radiofrequency heating; Curie temperature; Temperature control by parametric feedback; Nanoparticle; Copper–nickel alloy; Cancer treatment

1. Introduction

Hyperthermia, a rapidly developing method of cancer therapy, exploits greater sensitivity of tumor cells to heating at 42–46 °C [1–7]. Magnetic fluid hyperthermia, which involves introduction of ferromagnetic nanoparticles (mediators) into the tumor tissue and heating them with alternating magnetic field (AMF), allows localized heating of tumors located deep inside the patient [1–7]. For safe and effective hyperthermia therapy of cancer, it is very important to maintain the temperature in the entire tumor within ±0.5 °C from the optimal value during the entire hour-long procedure. Lower temperature reduces effectiveness of the treatment, while higher temperature damages healthy tissue. For most particles suggested so far for this application uniform and controlled heating is very challenging, since it is very difficult to achieve uniform distribution of the particles throughout the tissue. Nonuniform distribution of the particles creates zones with different levels of AMF power absorption, and thus, different heating rates. Heat losses due to thermal conductivity and due to cooling by the blood flow are also difficult to account for, compounding challenges for achieving uniform and controllable heating of the tissue. This requires introduction of heat probes into the tissue to control the temperature at different locations of the tumor, significantly increasing the complexity of the medical procedure [7]. However, the temperature control can be achieved by application of mediators with intense AMF absorption at normal body temperature and Curie temperature (T_c) ranging from 42 to 60 °C. Such particles can be effectively heated by AMF at low temperatures, but they stop absorbing AMF energy as their temperature approaches T_c and the heating stops. As soon as the particles cool below T_c, magnetic ordering re-establishes and the heating resumes. Sufficient power of the AMF

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generator guarantees constant temperature within the tissue laden with the particles even with nonuniform distribution of the particles throughout the tissue and uneven dissipation of the evolving heat [1]. Ferromagnetic materials with T_c in this range include alloys, amorphous structures, ferrites, manganites, and other multi-component systems consisting of metals and metalloids [1]. Magnetic particles for hyperthermia should be suitable for injection into a human organism, be ultradisperse (10–1000 nm), should effectively absorb AMF, and have a steep magnetization vs. temperature curve. Because individual particles tend to vary in their chemical composition and structural organization, creating particles meeting these requirements is a challenging scientific and technological task: different magnetic materials have different rates of thermal demagnetization (determined by the processes of magnetic domain formation); therefore, the particles vary in their magnetization and T_c.

The thermal demagnetization rate of the mediator particles near T_c is another important parameter for their application in hyperthermia. The higher the value of this parameter is, the faster the target temperature can be established. We studied AMF absorption rates and thermal demagnetization rates of nanoparticles made from various ferromagnetic materials (alloys and complex oxides) with reasonable T_c values [1]. Copper–nickel (CuNi) alloys have emerged as a promising group of such materials. This well-studied alloy is chemically stable and is used to make surgical instruments because of its good biocompatibility; it also has good magnetic properties. Therefore, the CuNi nanoparticles should satisfy most of the above requirements.

2. Materials and methods

2.1. Synthesis and characterization of CuNi alloy nanoparticles

We prepared CuNi alloy (25–30 at% of Cu) particles by several methods: mechanical grinding of alloy pieces, plasmachemical re-condensation [9], co-precipitation of salts from solution followed by reduction in hydrogen and thermal treatment (sol–gel method), VHF treatment of solutions of the salts [9], and by the cryochemical technique [1].

The method of salts co-precipitation followed by reduction produced the best results. Solutions with proper ratios of Cu and Ni salts were co-precipitated by Na_2CO_3 at room temperature under sonication and the precipitate was dried at 80–90 °C. In order to avoid particle agglomeration during thermal processing CuNi oxide particles were insulated in a NaCl matrix. Then the particles were reduced by hydrogen at 300–1000 °C. An additional thermal treatment in argon was performed at 600–1000 °C. The temperatures were varied to determine the best conditions for the particle synthesis. The size of CuNi alloy particles produced by this method can range from 5 to 1000 nm, depending on reduction and annealing conditions, according to TEM measurements; however, individual samples reduced and annealed at particulate temperatures demonstrate more uniform size distributions (Fig. 1).

Magnetic measurements of dry powders were performed using a Faraday balance (Bruker) with a custom temperature control unit. Our measurements have shown that Curie temperature of CuNi alloy particles, as well as their thermal demagnetization rate, can be adjusted both by varying the Cu/Ni ratio and by the proper selection of hydrogen reduction temperature and additional thermal treatment (Figs. 2–4). The reduction and annealing conditions appear to influence uniformity of phase composition and the domain structure of the particles, as well as their size. Particles with 28 at% of Cu, reduced in hydrogen at 700 °C, annealed at 450 °C for 23 h and at 650 °C for 2.5 h were selected for animal experiments.

2.2. Particle separation by size

Particles produced by this technique are polydisperse in size and vary in their chemical and phase composition. To produce particles with more uniform in T_c and thermal demagnetization rate we employed several grinding and separation techniques. Both T_c and thermal demagnetization rate were found to correlate well with the particle size and the history of mechanical treatment of the sample (Figs. 5 and 6). This allows modification of the particle properties by mechanical grinding and separation by size.

2.3. Magnetic separation of the particles by T_c

To further improve uniformity of T_c and demagnetization rate of the particles we employed magnetic separation. Suspension of the particles was sonicated for 30 min (with cavitation), and magnetic separation at different temperatures of the suspension was used to obtain fractions of the particles with more uniform T_c (Fig. 7). Heating of a 10 mg/ml suspension of the particles with AMF (410 kHz), resulted in heating to 43 °C, at which point the heating

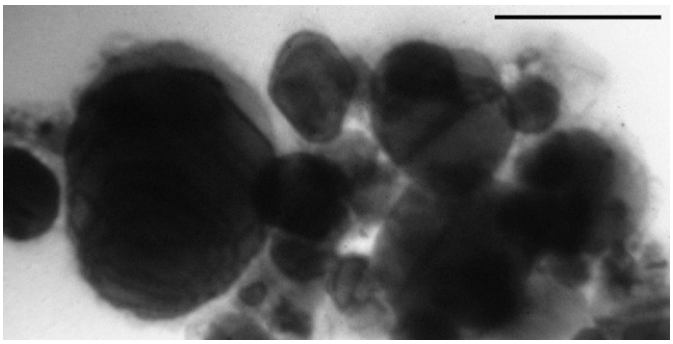

Fig. 1. CuNi particles (reduction at 300 °C for 1 h, annealing at 700 °C, surface area 23 m^2/g, TEM, bar = 30 nm).

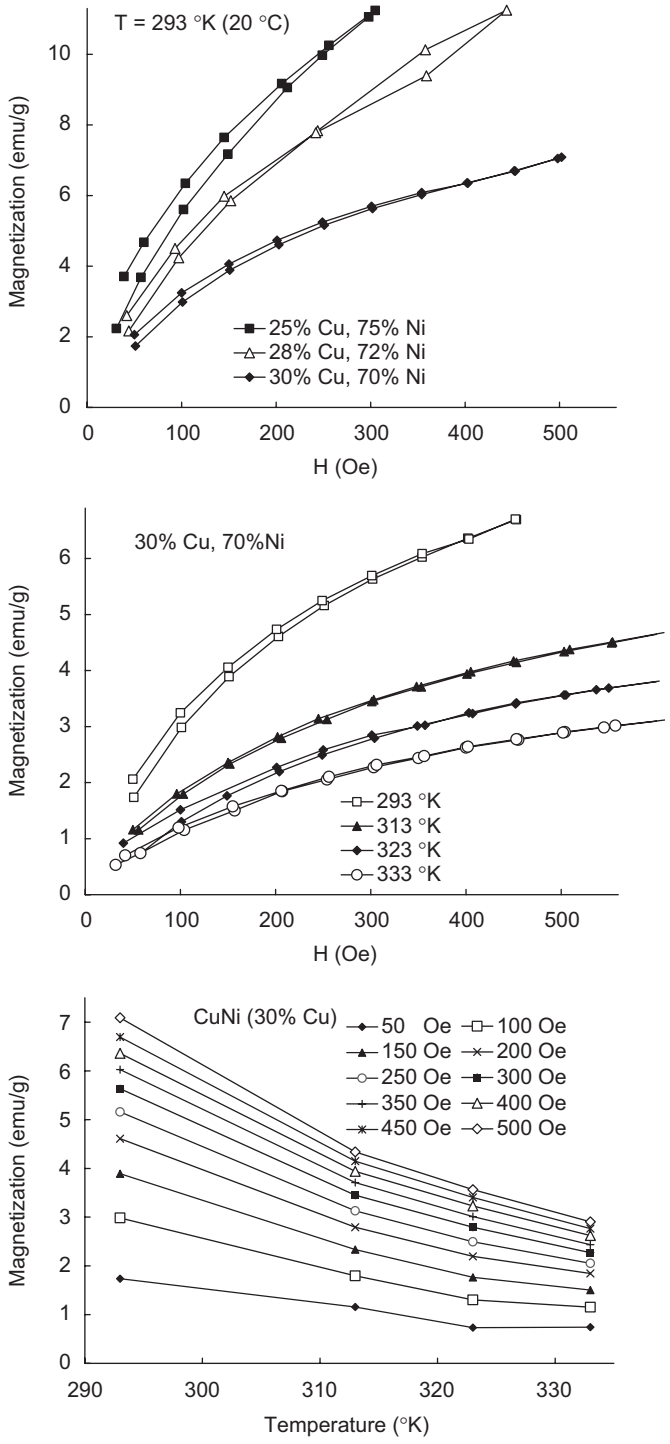

Fig. 2. Top: Magnetization of copper–nickel alloys with different Cu content at 20 °C. Middle: Magnetization of 30% Cu, 70% Ni alloy at different temperatures. Bottom: Thermal demagnetization of 30% Cu, 70% Ni alloy particles in different magnetic fields.

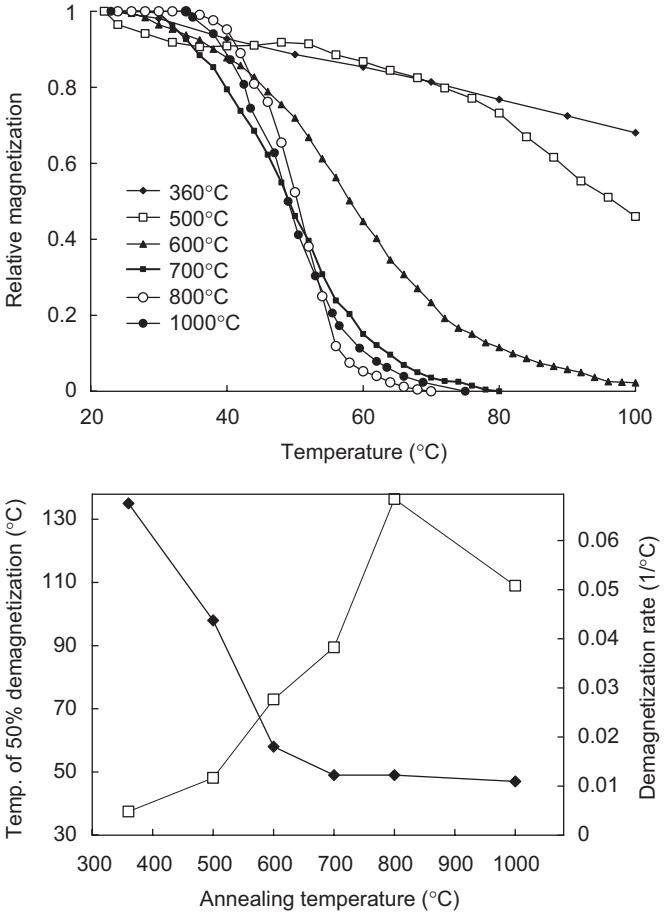

Fig. 3. Top: Magnetic transition in CuNi particles produced by reduction in hydrogen at different temperatures. Bottom: Temperature of 50% demagnetization (diamond) and demagnetization rate (open square) as a function of the annealing temperature.

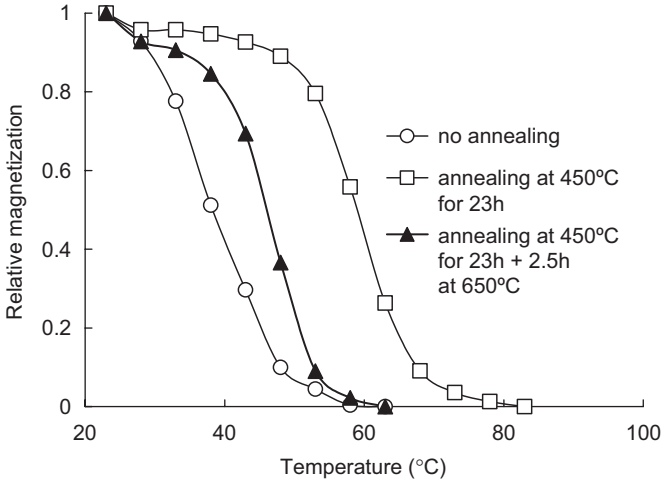

Fig. 4. Influence of additional annealing on magnetic transition of CuNi alloys.

stopped (Fig. 7), while initial (unseparated) mixture of the particles continued heating well above this temperature. This demonstrates the potential of magnetic separation for obtaining particles with more uniform thermal demagnetization characteristics.

2.4. AMF power absorption by particles

We considered physical mechanisms of energy transfer from AMF to the tissue laden with particles, and have

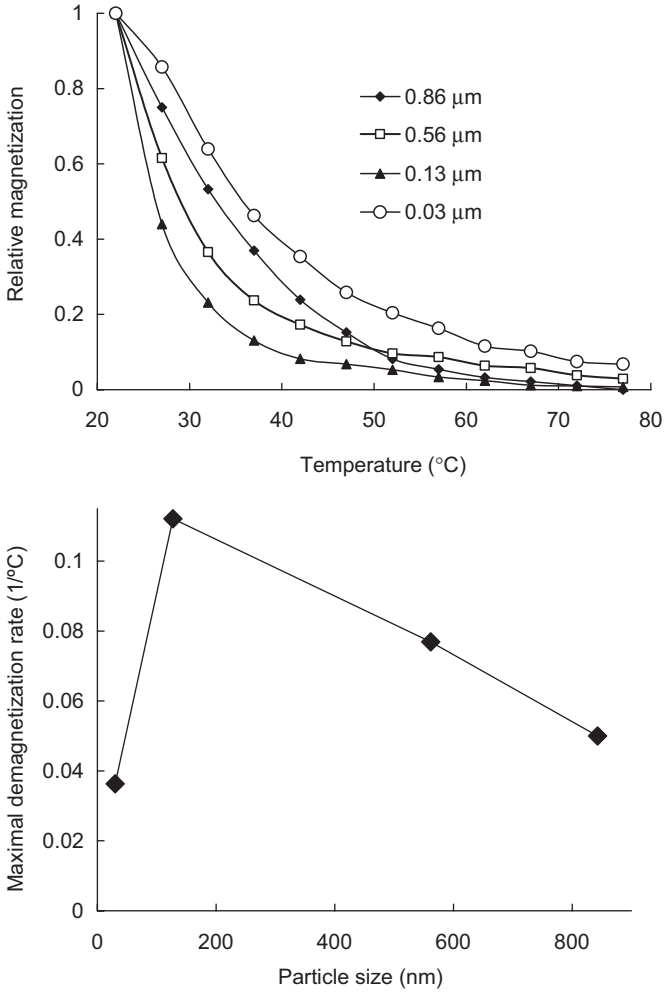

Fig. 5. Top: Magnetic transition as a function of the particle size. Bottom: Maximal thermal demagnetization of CuNi particles as a function of their size.

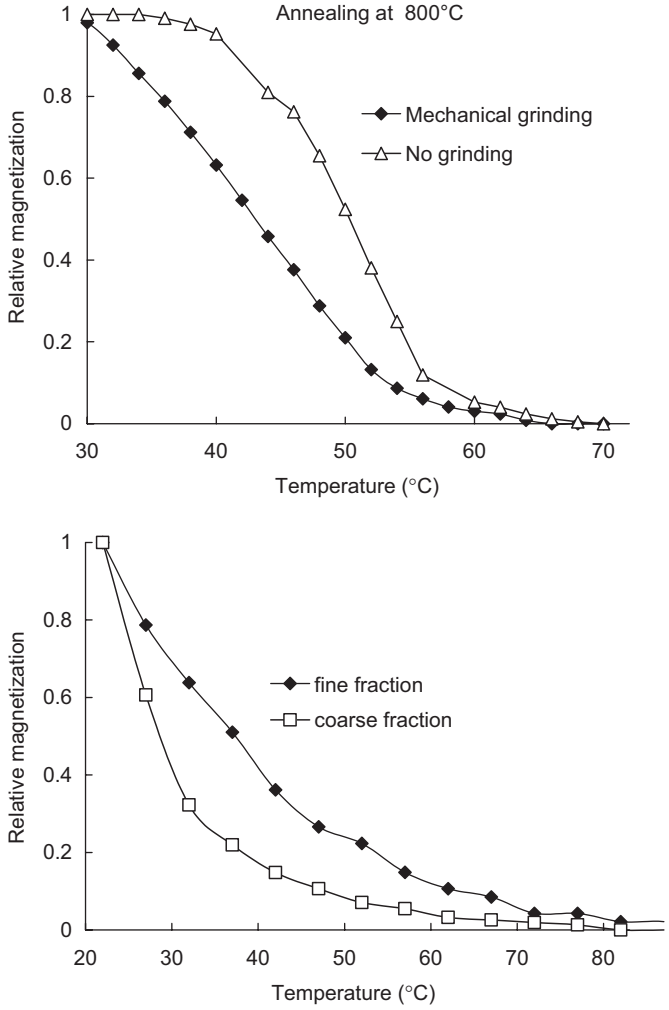

Fig. 6. Top: Influence of mechanical grinding on magnetic transition. Bottom: Separation by particle size.

calculated, that after reaching thermal equilibrium near T_c, any further heating occurs only in a relatively thin layer at the surface of the area loaded with the particles, compensating for heat dissipation. For a spherical area with the radius R, uniformly filled with particles, the temperature distribution is described by the following equations:

$$T(r) = \begin{cases} T_c & \text{when } 0 \leqslant r \leqslant R_1, \\ T_0 + q/k(R^2/2 - r^2/6 - R_1^3/3r) & \text{when } R_1 \leqslant r \leqslant R, \\ T_0 + q(R^3 - R_1^3)/3rk & \text{when } R \leqslant r \leqslant \infty, \end{cases} \quad (1)$$

where $R_1^2 = R^2 - 2k(T_c - T_0)/q$, T_0 is the temperature of the medium, T_c the Curie temperature, q the heat production per unit volume, k the heat conductivity of the medium. The temperature varies only in the spherical layer with the thickness of $(R - R_1)$. This distribution is shown in Fig. 8. Therefore, even with nonuniform distribution of the particles throughout the tissue, after reaching thermal equilibrium near T_c, any heating occurs only in a relatively thin surface layer of the particle-loaded region of the tissue. When the temperature in any other part of the tissue falls below T_c, it is again heated by the AMF to T_c. This assures constant temperature throughout the tissue, thus avoiding many problems typical for magnetic fluid hyperthermia with magnetite-based mediator particles [7].

Specific power absorption rate (SAR) of water suspensions of CuNi nanoparticles was measured by heating 1 ml of 10 mg/ml suspension in a thermally isolated cell in 410 kHz AMF (300 Oe peak). The cell was calibrated by heating the same amount of water by a coil with a known electric current and measuring the temperature increase. Comparison of the temperature increase produced by the AMF heating of the suspension and Joule's heat produced by the current allowed estimating the SAR of the CuNi nanoparticles to be 12 W/g of nickel.

2.5. Model experiments on rats and proposed method of clinical application

For clinical application, magnetic particles will be injected into a local artery feeding the tumor through a

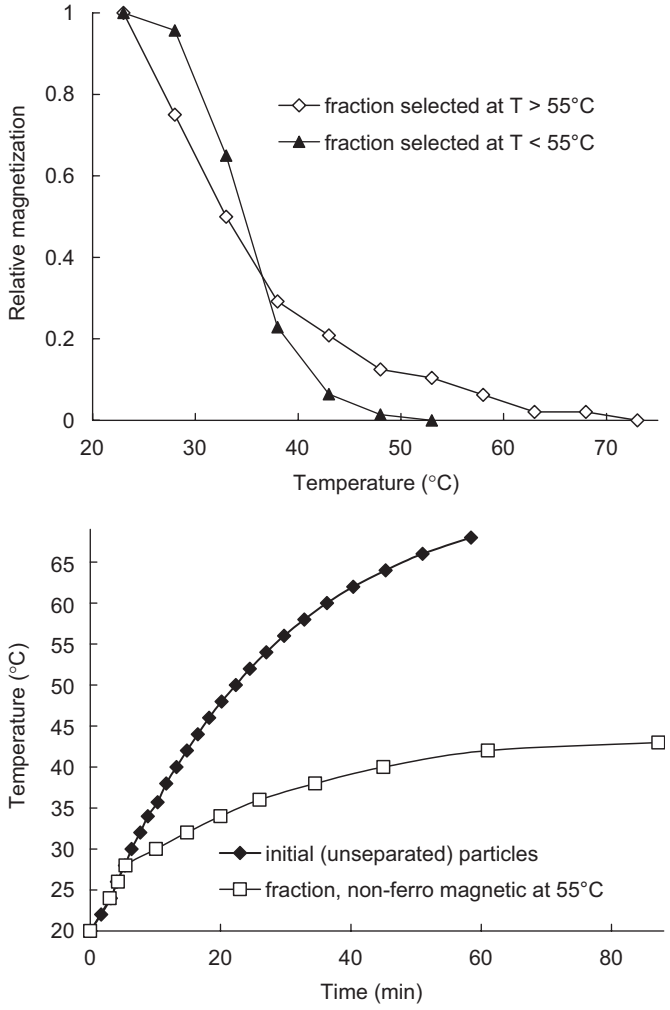

Fig. 7. Top: Magnetic separation by the Curie temperature. Bottom: Heating by alternating magnetic field. AMF: 410 kHz, 10 kW, 300 Oe peak, 10 mg of particles per 1 ml of water.

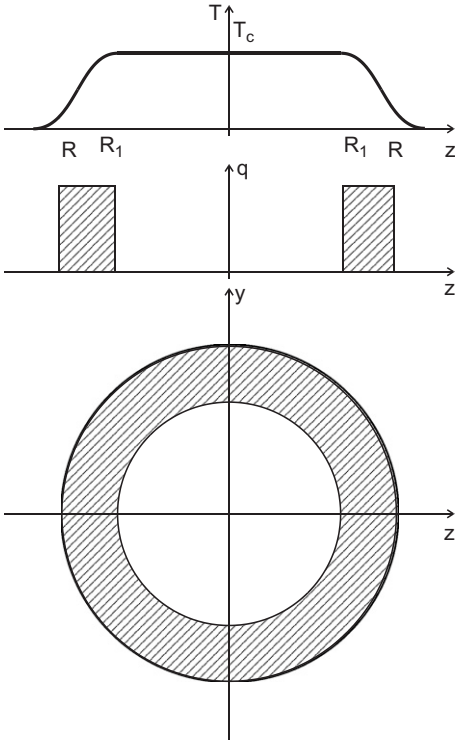

Fig. 8. Mathematical model of the equilibrium distribution of temperature (T) in a spherical zone with the radius R, which is filled with particles with the Curie temperature T_c, heated by an AMF. Only particles located in the spherical layer ($R_1 > r > R$) have temperatures below T_c and continue to absorb AMF energy ($q > 0$, hatched areas).

catheter, and a permanent magnetic field will be superimposed onto the tumor area, forcing magnetic particles to agglomerate and to become trapped in the tumor's capillaries. This clinically tested method of microparticles introduction into the tumor [8,9] allows localizing the thermal treatment in the tumor zone.

Acute toxicity of CuNi nanoparticles was tested on male BALB/c mice, weighting 18–20 g. Injections were made into the tail vein. The maximal tolerable dose for intravenous injection was estimated to be 150 mg of the particles per kg of body weight.

For AMF hyperthermia experiments on animals, we produced a custom setup with a large water-cooled Helmholtz-rings type inductor: 180 mm diameter, 110 mm gap. The generator outputs up to 10 kW of 440 kHz AMF with the 375 Oe amplitude of the field in the center of the gap of the inductor. This large volume of the AMF field allows experiments not only on small animals (mice, rats), but also on medium-sized animals (rabbits, even dogs).

We used rat liver as a model of a solid tumor. It weighs about 25 g, comparable to several types of tumors, and has a vast vascular network, also similar to many tumors. Despite obvious limitations (healthy liver tissue is different from cancer tissue), this model allows to test the concept of particles application described above: introduction of the particles locally in the bloodstream, magnetically controlled embolization of the capillaries and, thus, immobilization of the particles. The distribution of the particles in the tissue can be expected to be similar to that of the particles introduced the same way into a real tumor. Thermal characteristics (thermal conductivity, heat capacity and cooling by the remaining blood flow) of the liver is also comparable to that of a real tumor. Thus, this is a rather realistic model for testing the effectiveness of AMF heating with parametric feedback. Our group at this time has limited capabilities to conduct animal experiments; therefore this model was used for initial tests.

Rats were anesthetized with chloroform and novocain, and their abdominal cavities were open. Care was taken to preserve intact diaphragm, and the heart was working during the entire procedure, ensuring working bloodstream. One to four ml of 3% suspension of the particles (magnetite or CuNi alloy (28 at% of Cu)) was injected through a catheter into the liver vein. Magnetic field (3 kOe) was superimposed on the liver during the injection.

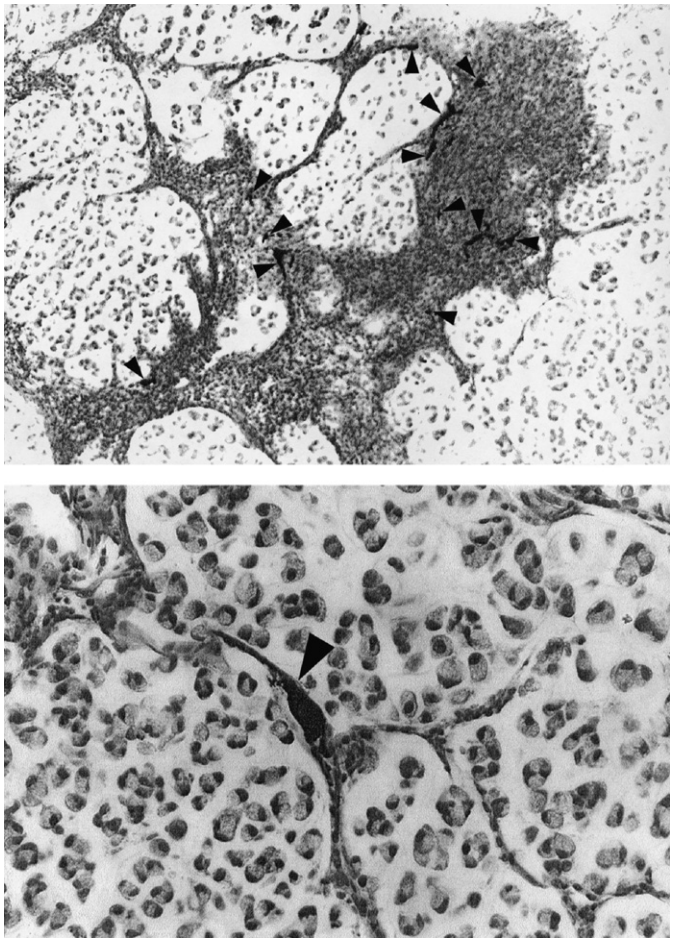

Fig. 9. CuNi particles localization in rat liver. Arrowheads indicate locations of the particle aggregates in capillaries.

After the procedure, the liver appeared darkened. Microscopy has shown that most of the particles were located in blood vessels of the liver (Fig. 9). During the AMF exposure the temperature was measured with an alcohol-based thermometer to prevent heating by AMF. In the 440 kHz AMF magnetite nanoparticles laden tissue was heated fast without any signs of temperature stabilization, while the temperature of CuNi alloy nanoparticles loaded liver stabilized at 42 °C (Fig. 10).

3. Conclusions

By varying composition of CuNi particles and parameters of their synthesis and post-treatment it is possible to manufacture nanoparticles with high RF power adsorption rates and T_c in the range suitable for local hyperthermia with the parametric feedback. We optimized parameters of CuNi nanoparticles synthesis for these purposes. The particles have reasonably low acute toxicity.

These particles were tested on an animal model of a tumor (rat liver) with working bloodstream. Most of the particles were captured in capillaries in the liver. The liver was heated by 440 kHz field, and the temperature stabilized

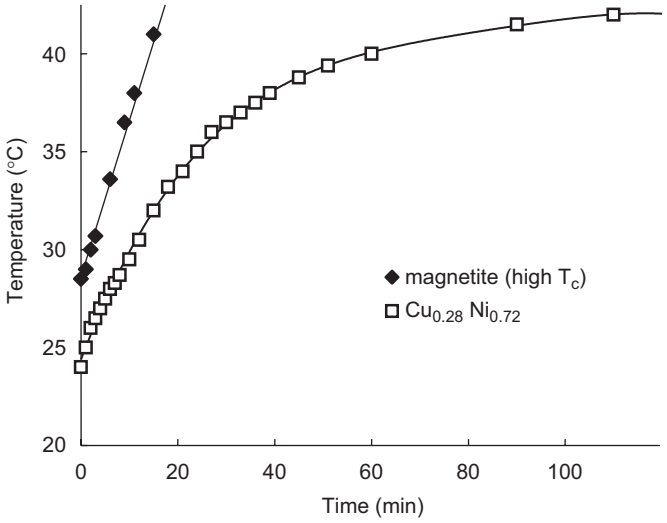

Fig. 10. AMF heating of the rat liver using magnetite and CuNi particles (28% Cu). AMF: 440 kHz, 10 kW, 375 Oe peak.

at 42 °C. These preliminary animal tests demonstrate the viability of the concept of application of CuNi alloy nanoparticles as mediators for magnetic fluid hyperthermia with parametric feedback temperature control. Further animal studies are required for testing on real tumors, for studies of the uniformity of the heating, long-term toxicity of the particles and possible side effects.

Application of CuNi alloy nanoparticles for magnetic fluid hyperthermia treatment of cancer can be combined with localized chemotherapy, radiotherapy, phototherapy, etc. [1,8–11], when these particles are functionalized and used as magnetically guided drug/radiation/photoagent carriers. Such combined treatment can further improve effectiveness of the medical procedure.

Acknowledgment

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