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Human and Animal Physiology

Development and Testing of Nanoparticles for Treatment of Cancer Cells by Curie Temperature Controlled Magnetic Hyperthermia

Archil Chirakadze[*], Nodar Mitagvaria[**], David Jishiashvili[*], Marina Devdariani[§], Gia Petriashvili[#], Lena Davlianidze[§], Nugzar Dvali[θ], Ketevan Chubinidze[#], Alexander Jishiashvili[#], Zakaria Buachidze[θ], Irina Khomeriki[θ]

[*] *Department of Engineering Physics; Vladimer Chavchanidze Institute of Cybernetics, Georgian Technical University, Tbilisi, Georgia*
[**] *Academy Member, Department of Cerebral Circulation and Metabolism, Ivane Beritashvili Center for Experimental Biomedicine, Tbilisi, Georgia*
[§] *Department of Cerebral Circulation and Metabolism, Ivane Beritashvili Center for Experimental Biomedicine, Tbilisi, Georgia*
[#] *Vladimer Chavchanidze Institute of Cybernetics, Georgian Technical University, Tbilisi, Georgia*
[θ] *Georgian Technical University, Tbilisi, Georgia*

A vast amount of nanoparticles has been developed and proposed for the local hyperthermia of cancer during the last decades, but only a few of them correspond to the mandatory requirements of having therapeutic range Curie temperature (TC=41-45^{0}C), high-rate crystallinity and "strong" magnetic properties, strictly controlled homogeneity and dispersion of the nanoparticles, good biocompatibility and harmless decomposition products. Among them are the nickel-copper (Ni-Cu) and silver doped lanthanum manganite ($Ag_xLa_{1-x}MnO_3$) nanoparticles. The developed research showed that the materials obtained at lower than usual temperatures using microwave enhanced synthesizes and annealing can be successfully used for local hyperthermia revealing high magnetic properties. Behavioral toxicity testing of the developed nanoparticles was enhanced by blood oxygen saturation measurements using noninvasive oximetry in white rats. Both of the developed nanomaterials revealed a lower toxicity level than the commercially available Fe_2O_3 nanoparticles.

Cancer, magnetic hyperthermia, behavioral tests, toxicity, nanoparticles, synthesis, magnetic properties, microwave, blood oxygen saturation

Reviewing of more than 500 scientific publications on cancer statistics, cancer incidence and mortality projections, the effectiveness of cancer therapy, financial performance of leading pharmaceutical companies and medical institutions, analysis of the market research reports, etc., explicitly indicates the continuous increase in demand for the more effective and convenient, less invasive and less expensive combined modalities for cancer treatment, at least up to 2035, not only for adults but also for children and adolescents [1, 2]. A vast amount of nanoparticles has been developed and proposed for the local hyperthermia of cancer during the last decades using the mechanical treatment, micro-emulsion technique, the sol-gel process, polyol reduction method, electrochemical deposition, hydrothermal reduction method, combustion method, explosion method, etc., but only a few of them correspond to the mandatory requirements of having therapeutic range Curie temperature (T_C=41-45^{0}C), high-rate crystallinity and “strong” magnetic properties, strictly controlled homogeneity and dispersion of the nanoparticles, good biocompatibility and harmless decomposition products, which is required to provide the designed properties. Among them are the nickel-copper (Ni-Cu) and silver doped lanthanum manganite ($Ag_xLa_{1-x}MnO3$) [3-9] which are highly prospective materials for the Curie temperature limited magnetic hyperthermia and, therefore, the issues related to volume production and practical use of these materials (especially for the local combined therapy) are quite acute. Recently we reported several advantages of the microwave enhanced synthesis of uncoated and zinc sulfate/boron nitride coated Ni-Cu and $Ag_xLa_{1-x}MnO_3$ nanoparticles yielding the improved crystallinity, higher capacity and better effectiveness of silver doping. In the frame of the reported research, the dependence of magnetic properties (Curie temperature, saturation magnetization, remnant magnetization and coercivity) on the nickel content was studied at eight various Ni to Cu atm% ratios in eighty Ni-Cu nanoparticles synthesized and annealed at 7000-1200 microwave power range.

Methodical Approach

Microwave enhanced synthesis and characterization of nanoparticles for the Curie temperature controlled magnetic hyperthermia. Eighty samples of Cu-Ni nanoparticles (10±0.05 grams each) with Ni to Cu atm% ratios R=1.9 to 3.0±0.05, have been synthesized using a 1500 W microwave reactor OLT-CR-50. Special attention was paid to the content range of R=2.3–2.6, because it was expected to correspond to the pharmaceutical Curie temperature range. The microwave enhanced synthesis was conducted according to [4, 5] at two microwave power levels (700 and 1000 W) to investigate the possible dependence of the capacity of synthesis and properties of the yielded nanoparticles on the applied microwave power. Another important task was to investigate the magnetic properties of nanoparticles (coercivity H_c, remnant magnetization M_r and saturation magnetization M_s which can be strongly dependent on particle size, uniformity and morphology. Scanning Electron Microscopy (SEM-VEGA 3, LMU) and Energy Dispersive Spectroscopy (EDS) were used to analyze the size (d_c), morphology and composition of nanomaterials. The structure of synthesized materials was studied by X-ray diffraction (XRD) method using a Shimadzu XRD-6000 diffractometer. Magnetic characterization of obtained materials was executed using a vibration scanning magnetometer (VSM EZ9). Curie temperature was measured using a Thermo Gravimetry/Differential Thermal Analyzer TG/TDA 7000 and MX BAOHENG WBF Y201 based microwave reactor with an additive magnetron was used for microwave heating. The temperature was controlled to reach 650^{0}C and then was kept with accuracy ±5^{0}C, and the processing time was determined as the time when the temperature was constant. Eighty samples of silver doped lanthanum manganite nanoparticles were prepared with Argentum content x=0.15 using the comparatively low-temperature combustion synthesis method described in [6-9] and annealing at microwave power levels 700 or 1000 W.

We used the stoichiometric compositions with a slightly (for 5-10%) increased amount of glycine as fuel. The prepared solution was heated under continuous mixing to 65°C. The obtained gel was put through a water-pipe previously heated to 300-320°C. After boiling, the process products were annealed (calcinated) in an air atmosphere at 700 or 1000 W power and controlled annealing temperature of 650°C for 240 minutes. All obtained samples were compared to a sample calcinated (annealed) in a conventional oven at 800°C for 5 hours (Fig. 1) to estimate their crystallinity rate. Temperature again was controlled to reach 650^0C and then was kept constant: the calcination time was determined as the time when calcination temperature was constant. Samples with x=0.15±0.05 were selected.

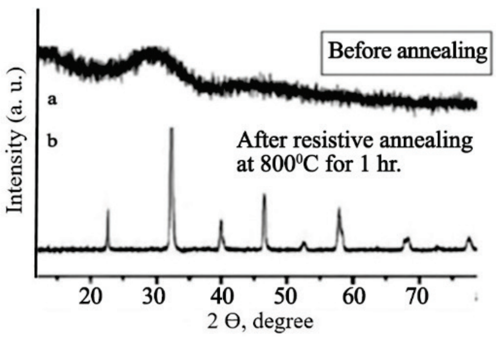

Fig. 1. XRD patterns of $La_{0.85}Ag_{0.15}MnO_3$ samples: a) pretreated, ignited and b) annealed using conventional heating (annealing at 800^0C for 5 hours).

Toxicity testing of the developed nanoparticles. Taking into account biomedical applications of the developed nanoparticles, assessment of their acute general toxicity is one of the most important parts of the research. In addition to the behavioral testing method [7-10] we used the noninvasive oximetry [11] (pulse oximeter Nellcor N-100) with a disposable sensor placed proximally on the tail) in rats, which in the oxygen saturation range of 0.75–0.95 has an acceptable accuracy and good agreement with arterial blood sampling data. Behavioral effects and oxygen saturation as toxicity indicators were studied. Various maze techniques for studies of behavioral responses related to learning and memory processes in animals using a variety of maze constructions have been applied in experiments on rats. An accurate preliminary testing of toxicity of the developed pilot materials was carried out by a modified methodic using the Complex Toxicity Index (CTI) based on the normalized data on passing time, number of mistakes and time spent in the "closed" and "open" parts of the route:

$$CTI = [(N_l / N) + (T_t / T_d)] / S^2, \quad (1)$$

where T_d is the total time spent in dark parts of the route, T_t is the total time needed for passing the route, N_l is the total number of mistakes, N is the total number of decisions made by the test animal during passing the route, and S is the average saturation rate (usually, 0.85–0.98) during passing the route.

Results

Microwave enhanced synthesis and characterization of Ni-Cu nanoparticles. The main results of the reported research are the data on the synthesis parameters and properties of Ni-Cu nanoparticles given in Fig. 2. The above data clearly show that the developed materials with nickel content (71.25-71.75)±0.25atm% can be successfully used for the Curie temperature controlled hyperthermia in the therapeutic range 40-45^{0}C, and the deviation in Ni content should not be more than 0.25atm%. Materials with (71.75-73.5atm%) could be if needed, used for thermal ablation. The magnetic properties in these temperature ranges are already high, although much "weaker" than in samples with a higher content of nickel. We have not found any reliable dependence of the magnetic properties on the utilized microwave power level, but a 2–fold decrease in the required processing time (until the solution reached the spontaneous combustion point) was fixed, which can be important in case of volume production.

Microwave enhanced synthesis and characterization of $Ag_xLa_{1-x}MnO_3$ nanoparticles. One of the main aims of the reported research was to investigate the possible direct impact of the annealing power on the magnetic properties of the synthesized and annealed samples, as well as the capacity of the synthesis process. Magnetic moment of samples with x=0.15, annealed for 38-40 minutes at 650^0C using two different microwave power levels (1000 W and 700 W) was determined using VSM room temperature measurements. The main results of the study are shown in Fig. 3, which contains two curves: curve 1 (blue) corresponds to annealing (calcination) at 1000 W microwave power, and curve 2 (red) corresponds to annealing at 700 W microwave power. Under the conditions of our experiment, there should not be a noticeable difference between the two measured curves if the magnetic moment depends only on the annealing temperature and duration, and does not depend on the applied magnetic field.

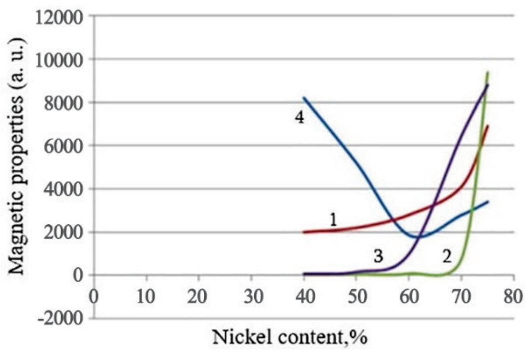

Fig. 2. Dependence of magnetic properties of Ni-Cu nanoparticles (developed at 1000 W power level) on nickel content (atm%): 1. C_T (^OC)$\cdot 10^2$ (red curve), 2. M_r ($A\cdot m^2\cdot kg^{-1}$)$\cdot 10^3$ (green curve); 3. M_s ($A\cdot m^2\cdot kg^{-1}$)$\cdot 10^4$ (violet curve); 4. H_c ($A\cdot m^2\cdot kg^{-1}$) (blue curve); averaged crystallites' size (27-32)±3 nm.

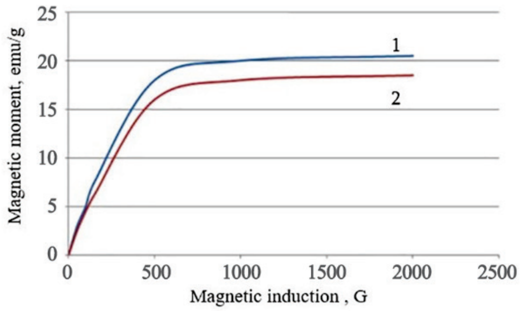

Fig. 3. The typical dependence of the magnetic moment (emu/g) of the $Ag_{0.15}La_{0.85}MnO_3$ samples on the magnetic field (G): 1. heating power 1000 W (blue curve); 2. heating power 700 W (red curve); annealing for 240 minutes.

Experiments carried out clearly showed, that the measured values of the magnetic moment in all tested samples annealed at 700 W were significantly (up to 12%) lower than in samples annealed at 1000 W. In our opinion, this means that the appearance and growth of the magnetic moment are determined not only by thermal effects but, maybe, also by the electromagnetic wave affecting the condensed matter. However, more experimental statistics and theoretical considerations are needed to approve and explain the fact, or to refute it.

Toxicity testing. The dynamics of averaged CTI characterized toxicity of Fe_2O_3, $Ni_{0.715}$-$Cu_{0.285}$ and $La_{0.85}Ag_{0.15}MnO_3$ nanoparticles are given in Fig. 4. The initial (first day) value of CTI for Fe_2O_3 nanoparticles is taken as 100. We can see that the lowest CTI characterized toxicity was revealed by $Ni_{0.715}$-$Cu_{0.285}$ despite the high content of nickel and the fairly common opinion that nickel-containing compounds have significant toxicity, which is not supported by behavioral tests and, inparticular, measurements of oxygen saturation in the blood.

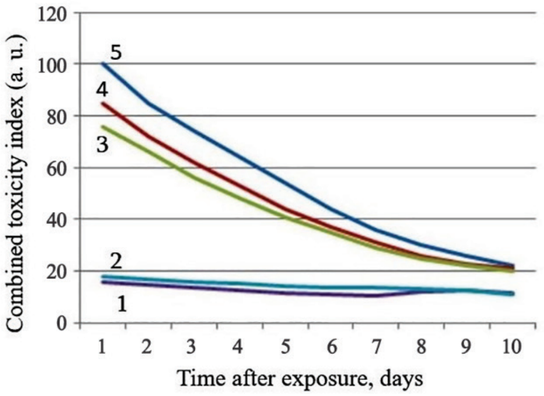

Fig. 4. Dependence of CTI characterized toxicity on the days of the testing experiment: 1. saline solution injections; 2. intact; 3. CuO; 4. $La_{0.85}Ag_{0.15}MnO_3$; 5. Fe_2O_3 nanoparticles.

The $La_{0.85}Ag_{0.15}MnO_3$ nanoparticles (probably, due to the moderately toxic lanthanum) revealed a higher CTI characterized toxicity than the $Ni_{0.715}$-$Cu_{0.285}$ nanoparticles. Both of them revealed a lower toxicity level than the commercially available Fe_2O_3 nanoparticles.

Discussion

The experiments showed that Ni-Cu nanoparticles with nickel content (71.25-71.75 atm%) are most suitable for the therapeutic range local magnetic hyperthermia (41-45^{0}C). Materials with (71.75-73.5) atm% of nickel could be, if needed, used for thermal ablation of cancer cells. We have not found any reliable direct dependence of the magnetic properties on the utilized microwave power level, but we fixed a significant decrease in the required processing time (until the solution reached the spontaneous combustion point) using 1000 W microwave power compared to 700 W power. So, the high-power microwave enhanced synthesis can increase the capacity in case of volume production. Study of magnetic properties of the $Ag_{0.15}La_{0.85}MnO_3$ annealed at 1000 W and 700 W microwave power level showed that the measured values of the magnetic moment in the samples annealed at 700 W level in all tested samples were significantly lower than of annealed at 1000 W level, which can be caused by some uninvestigated "direct" influence of the microwave electromagnetic field on the crystalline structure or the defects annealing in the samples. A modified methodology of toxicity testing including the measurement of oxygen saturation of the blood of the white rats was developed and a modified Combined Toxicity Index (CTI) was used to characterize the acute general toxicity of the developed nanoparticles. The measured toxicity was lower than that of the commercially available Fe_2O_3 nanoparticles. The use of the modified methodology should enhance the accuracy and reliability of toxicity testing. If the correlation of these data with the data of toxicity testing based on lethal doses and concentration is sufficiently high, the CTI method can find a wide application due to its high tolerance to animals' welfare. Taken together, the results obtained show that due to their high heating efficiency, low invasiveness, Curie point controlled temperature and low acute toxicity the developed and tested materials should be regarded as highly promising modalities for the combined treatment of cancer [12-14].

Conclusions

Statistical analysis of available literary data explicitly indicates the continuous increase in demand for the more effective and convenient, less invasive and less expensive combined modalities for cancer treatment, at least up to 2035.

The developed research showed that the nickel-copper (Ni-Cu) and silver doped lanthanum manganite ($Ag_xLa_{1-x}MnO_3$) nanoparticles synthesized applying microwave heating and annealing at lower than usually utilized temperatures can serve as highly efficient and low-toxic agents for the Curie temperature controlled magnetic hyperthermia in the therapeutic range 41-45^{0}C.

Magnetic moment measured in $Ag_xLa_{1-x}MnO_3$ samples annealed at different microwave power levels were significantly different which can be caused by some uninvestigated "direct" influence of the microwave electromagnetic field on the crystalline structure or the defects annealing in the samples.

Behavioral toxicity testing of the developed nanoparticles was enhanced by blood oxygen saturation measurements using noninvasive oximetry in white rats. Both of the developed nanomaterials revealed a lower toxicity level than the commercially available Fe_2O_3 nanoparticles.

Results of noninvasive oximetry were in good correlation with data of the behavioral testing. CTI values determined for the commercially available Fe_2O_3 nanoparticles were used as a control providing the comparative measurement of toxicity and enhancing the accuracy and reliability of testing.

The use of an enhanced methodology including 10 days data both on behavioral and blood oxygen saturation showed more acceptable toxicity of the developed materials in comparison to the commercially available Fe_2O_3 nanoparticles. CTI method may find a wider application due to its high tolerance to test animals' welfare.

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ადამიანისა და ცხოველთა ფიზიოლოგია

ნანონაწილაკების მიღება და ტესტირება კიბოს უჯრედების სამკურნალოდ კიურის ტემპერატურით კონტროლირებადი მაგნიტური ჰიპერთერმიის გამოყენებით

ა. ჭირაქაძე*, ნ. მითაგვარია, დ. ჯიშიაშვილი*, მ. დევდარიანი§, გ. პეტრიაშვილი#, ლ. დავლიანიძე§, ნ. დვალიθ, ქ. ჩუბინიძე#, ა. ჯიშიაშვილი#, ზ. ბუაჩიძეθ, ი. ხომერიკიθ**

**საქართველოს ტექნიკური უნივერსიტეტი, საინჟინრო ფიზიკის დეპარტამენტი; ვ. ჭავჭანიძის კიბერნეტიკის ინსტიტუტი, თბილისი, საქართველო*

***აკადემიის წევრი, ივანე ბერიტაშვილის ექსპერიმენტული ბიომედიცინის ცენტრი, თავის ტვინის სისხლის მიმოქცევის და მეტაბოლიზმის განყოფილება, თბილისი, საქართველო*

§ივანე ბერიტაშვილის ექსპერიმენტული ბიომედიცინის ცენტრი, თავის ტვინის სისხლის მიმოქცევის და მეტაბოლიზმის განყოფილება, თბილისი, საქართველო

#საქართველოს ტექნიკური უნივერსიტეტი, ვ. ჭავჭანიძის კიბერნეტიკის ინსტიტუტი, თბილისი, საქართველო

θსაქართველოს ტექნიკური უნივერსიტეტი, თბილისი, საქართველო

ბოლო ათწლეულების განმავლობაში შემუშავდა და გამოიცადა მრავალი სახეობის ნანონაწილაკი კიბოს ლოკალური ჰიპერთერმიისთვის, მაგრამ მხოლოდ რამდენიმე მათგანი აკმაყოფილებს კიურის ტემპერატურის თერაპიულ დიაპაზონში (T_C = 41-45^0 C) შენარჩუნების, კრისტალურობის მაღალი ხარისხის, „ძლიერი“ მაგნიტური თვისებების, შემადგენლობის მკაცრად კონტროლირებადი ერთგვაროვნების, მონოდისპერსიულობის, კარგი ბიოთავსებადობისა და დაშლის პროდუქტების უვნებლობის მკაცრ მოთხოვნებს. მათ შორისაა ნიკელ-სპილენძის (Ni-Cu) და $Ag_xLa_{1-x}MnO_3$. კვლევებმა აჩვენა, რომ ჩვეულებრივზე უფრო დაბალ ტემპერატურებზე მასალები, მიღებული მიკროტალღოვანი სინთეზის და კალცინაციის გამოყენებით, ამჟღავნებს მაღალ მაგნიტურ თვისებებს და წარმატებით შეიძლება იქნეს გამოყენებული ლოკალური ჰიპერთერმიისთვის. მიღებული ნანონაწილაკების ტესტირება მოხდა როგორც ექსპოზირებული ცხოველების (თეთრი ვირთაგვები) ქცევით მაჩვენებლებზე დაკვირვებით, ასევე ტესტირების პროცესში სისხლის ჟანგბადით გაჯერების გაზომვით არაინვაზიური ოქსიმეტრიის გამოყენებით. ორივე ტესტირებული ნანომასალა ხასითდებოდა ტოქსიკურობის უფრო დაბალი დონით, ვიდრე კომერციულად ხელმისაწვდომი Fe_2O_3 ნანონაწილაკები.

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