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Human and Animal Physiology

Microwave Synthesis, Characterization and Testing of Acute Toxicity of Boron Nitride Nanoparticles by Monitoring of Behavioral and Physiological Parameters

Archil Chirakadze[*], Nodar Mitagvaria[**], David Jishiashvili[*], Gia Petriashvili[§], Nugzar Dvali[#], Zeinab Shiolashvili[§], Ketevan Chubinidze[§], Nino Makhatadze[§], Alexander Jishiashvili[§], Zakaria Buachidze[#], Irina Khomeriki[#]

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

Hexagonal boron nitride nanoparticles, nanosheets and nanotubes (BNNPs) are even more promising materials for biomedical application than carbon nanotubes (CNTs) and nanoparticles (CNPs) due to their negligible cytotoxicity. The reported research yielded in development and testing of two distinctive microwaves enhanced comparatively low-temperature methods of synthesis of the hexagonal boron nitride nanoparticles and nanosheets with reduced distortion of the crystal lattice, and an improved method of general toxicity testing of the developed nanomaterials utilizing continuous observation of behavioral effects in white rats in combination with blood oxygen saturation, systolic blood pressure and body temperature measurements in full agreement with the 4R principles of animal welfare in scientific research. The obtained results allow us to expect that the developed materials can be a good basis for developing highly effective modalities for anticancer (in combination with chemotherapy, hyperthermia and radiotherapy) and antiviral (in combination with chemotherapy and hyperthermia) treatment. *© 2021 Bull. Georg. Natl. Acad. Sci.*

Cancer, low-temperature synthesis, microwave radiation, boron nitride, nanoparticles, turbostratic effect, behavioral testing

The research needed in the field of cancer therapy involves the development and testing of high-efficient and less toxic nano-liquid based therapeutic materials and carriers, including chemical and herbal therapy agents, materials for the Curie-temperature (TC) limited magnetic hyperthermia, nanoparticles and nano-sheets for radiotherapy and photodynamic treatment, and assessment of the efficiency of the synergy of the developed modalities [1], utilizing the joint

therapeutic effect of drugs and enhancement of the immune system [2]. The general toxicity of developed nanomaterials should be low or very low, while the testing methods should be highly reliable and fully comply with the 4R principles of humane treatment of experimental animals [3].

The numerical data on the incidence and mortality from cancer in the USA, Germany and globally are alarming [4-7]. The obvious stabilization of a number of deaths against a quasi-linearly increasing incidence looks encouraging, but taking into account various sources of uncertainty, a noticeable increase in mortality may be expected in the next few years.

Nanostructured hexagonal boron nitride (h-BN) is a highly prospective material for the tumor localized boron capture therapy characterized by high biocompatibility, and significant efforts have been made to reduce the conventional synthesis and annealing temperature, improve the crystalline structure, as well as for more detailed study of the microwave enhanced synthesis and microwave properties of the hexagonal boron nitride and its compositions, encapsulating capacity and luminescence properties [8-14] of BN nanoparticles synthesized using various methods. One of the main disordering factors hindering synthesis of h-BN with improved crystalline structure is the so-called turbostratic effect [13]. Based on the gained experience, new routes and improved methodic for the development of BN nanoparticles with higher crystallinity utilizing the ordering impact of microwave synthesis and additional annealing on the disordered (including turbostratic) BN samples was investigated and tested in the frame of the reported research. According to the literary data, hexagonal boron nitride nanoparticles and nanotubes (BNNCs) are even more promising materials for biomedical application than carbon nanotubes (CNTs) and nanoparticles due to their negligible cytotoxicity [14]. Testing of the general toxicity of developed samples was carried out using the behavioral testing method [3] and the so-called "Complex Toxicity Index" (CTI).

The energy effective low-cost and high capacity production of high-performance and low-toxic magnetic nanoparticles can form a good basis for the future active research focused on the combined use of coated and uncoated magnetic nanoparticles and their dispersions in water, saline, acetic acid, sodium stearate, hydroxychloroquine, chloroquine solutions for the efficient anti-cancer (in combination with radiotherapy) and antiviral (including new coronavirus) treatment. In both cases, a not antagonistic local magnetic hyperthermia could be an effective adjuvant treatment modality.

Methodical Approaches

Ammonium tetrafluoroborate reacts with potassium chloride yielding in ammonium chloride and potassium tetrafluoroborate: $3NH_4 \cdot BF_4 + 3KCl = = 3K \cdot BF_4 + 3NH_4 \cdot Cl$. Gaseous ammonia and boron trifluoride were simultaneously blown in equal volumes into a water-cooled 21 volume reactor with a velocity of 0.71 per minute. After 180 minutes 648.5 g of the reaction product in form of white powder was accumulated in the reactor. The XRD analysis showed that it was a solid complex compound trifluoroammineboron NH_3BF_3. The obtained powder was treated in a 1500 W microwave reactor OLT-CR-50 at 250^0C for 2.5 hours in a porcelain crucible placed in another porcelain crucible filled with cuprous oxide powder as a microwave-induced heating agent, and after cooled to the room temperature. After XRD analysis the yielded product was identified as a mixture of boron nitride BN and ammonium tetrafluoroborate NH_4BF_4, a well water-soluble salt. The analyzed powder was poured into a borosilicate glass vessel with deionized water and thoroughly stirred. Suspended BN particles were precipitated by centrifugation and dried. The weight of the dried boron nitride (BN) powder was 46.1 g, which equals to about 91% of the theoretically possible limit.

The study of cytotoxicity of h-BN nanomaterial is of high concern for developing and implementing new simple, rapid, highly effective and safe modalities for cancer therapy and many other medical applications, probably including prevention and antiviral modalities for treatment of COVID-19. At the same time, the general toxicity of the developed modalities is no less important, especially for critical and serious patients. The recently reported behavioral method is based on monitoring of passing of various kinds of mazes by white rats unexposed and exposed to the whole-body hyperthermia. In the current research the number of measured characteristics of the tested animals was increased from one (total passing time) to eight parameters: T_t – total passing time; T_d – total time spent in the "closed" (darkened) parts of the route; N_1 – correct decisions during passing the route; N_2 – mistakes during passing the route; ΔABP – relative change of systolic blood pressure during passing the route, indicated by a noninvasive blood pressure controller NIBP SYSTEM IN135/R; ΔT_b – relative change of body temperature during passing the route, indicated by an infrared thermometer BIO-IRB153; S – the averaged oxygen saturation rate in blood, indicated before and after passing the route by a non-invasive pulsoxymeter BET P-55 VET. The relative changes were calculated by dividing the difference between the measured values immediately before and after passing the maze by the value measured before passing the route. A modified CTI (complex toxicity index) was used to provide the sufficiently accurate and reliable assessment of the general toxicity of the developed and the materials

$$CTI=[(N_2/N_1)+(T_t/T_d)]\cdot[(\Delta ABP+\Delta T_b)/S^2] \quad (1)$$

All data obtained were compared to the toxicity of commercially available carbon nanotubes C-E-015-NT, hexagonal boron nitride nanotubes BO-N-0_2-NT (supplied by American Elements) and commercially available iron oxide nanotubes measured utilizing the method described above. CTI of carbon nanotubes measured on the first day of testing was taken as 100 arbitrary units.

About two weeks later the tested animals underwent hyperthermia treatment followed by the testing procedure to assess the expected combined acute toxicity of the therapeutic range hyperthermia and the tested nanomaterials.

Results

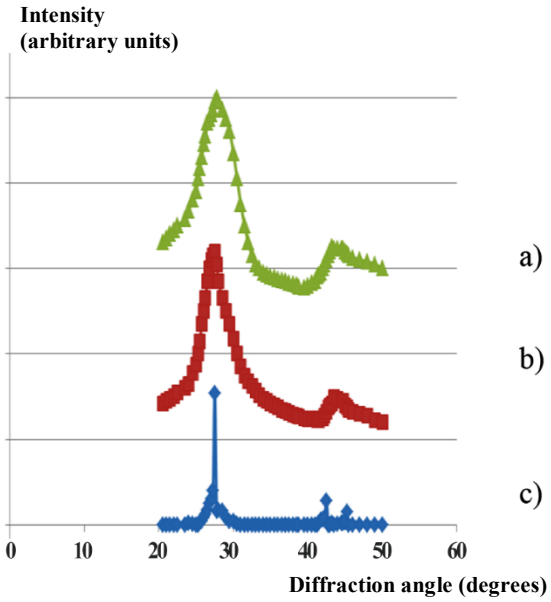

Fig. 1. Representative XRD peaks of the h-BN nano-particles with different turbostratic disorder synthesized using various methods and treated at maximum temperature: a) 900^{O}C [9], b) 450^{O}C [12] and c) 250^{O}C (the given research).

XRD patterns of the obtained boron nitride powders in comparison with the data of papers [9] and [12] are given in Fig. 1. Despite the rather low temperature of crystallization and absence of high-temperature annealing, the developed samples (Fig. 1, c) have significantly less disordered (turbostatic) crystal lattice than the materials treated at maximum temperatures of 450^{0}C (Fig. 1, b) [12] and ≈ 900^{0}C (Fig. 1, a) [19]. The gaseous state of the reacting components makes it possible to regulate synthesis reaction rate according to the supply of the components; reaction product $NHBF_3$ is thermally unstable and

decomposes above 125^{0}C giving two components: BN and NH_4BF_4 (ammonium tetrafluoroborate). The last one is a highly soluble product, which facilitates purification of the target product. During the thermal decomposition of the synthesized complex – ammonia trifluoride+boron ($NH_3 \cdot BF_3$) – a nanopowder of boron nitride (BN) is obtained with the average nanosheet thickness size which is significantly less than 5 nm, as evidenced by both x-ray phase analysis and SEM studies. The half-width of the maximum peak of sample c) is about 5-times smaller than of the corresponding peak of sample b) and about 10-times smaller than of the corresponding peak of sample a).

Five groups of ten white rats each were exposed during 3 days to equal number of intramuscular injections of equal dispersions of developed samples (1 – h-BN synthesized using boron trifluoride and ammonia precursors, 2 – h-BN synthesized using boric acid, 3 – BO-N-0_2-NT boron nitride nanotubes, 4 – boron nitride encapsulated copper nanoparticles; and 5 – C-E-015-NT carbon nanotubes). The sixth and seventh groups of ten white rats each were exposed to injections of saline solution with commercially available iron oxide nanoparticles and of pure saline solutions to be used as control. The experimental data of the average normalized magnitude of CTI characterized general toxicity of developed samples during 15 days after cessation of treatment clearly showed that carbon nanotubes revealed the highest toxicity which (on the 4th day of trial) was for about (8±1)% higher than of iron oxide nanotubes and for about (30±2)% higher than of all h-BN samples.

10 days after all groups of tested animals well divided to sub-groups to underwent the five-minute hyperthermia treatment at 40 and 43.5^{0}C (see Fig. 2), followed by the full testing procedure given above. The experimental data clearly showed that the hyperthermia pretreatment at 40^{0}C reduced the CTI characterized toxicity for about 15-20%, while the hyperthermia pretreatment at 43.5^{0}C increased it for about 25-30%.

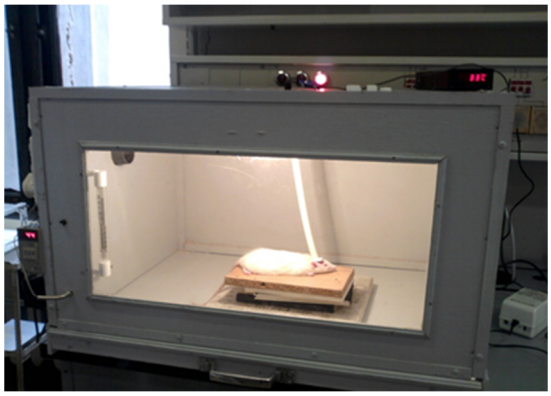

Fig. 2. The tested rat in a hyperthermia chamber (HC): the temperature in HC is automatically maintained to the needed value in the range of 39–45^{O}C.

Discussion

The majority of all most common cancers in Germany and a considerable part of most common cancers in the US shows the tendency of increase of incidence and deaths numbers, and no one of them could be characterized as being eliminated (despite of stabilization or even a sharp dropdown of the number of deaths).

Experiments showed that the first of the above described newly developed routes of nanoparticle synthesis provides a sufficiently rapid and capacitive synthesis of hexagonal nanoparticles and nano-sheets with nanosheet thickness about 3-5 nm at a maximum processing temperature of 250^{0}C, significantly lower (as far as we know) than it has been reported in scientific publications until now [12]. Comparing the samples synthesized using different methods and temperature and reported research we can conclude, that in case the highest disorder is observed in the samples treated at the highest maximum temperature, while the lowest disorder is observed in the samples treated at the lowest maximum temperature.

So, in our opinion, the synthesis temperature is not always the main factor which determines the turbostratic disorder, but just one of numerous physical and chemical factors differing for various methods. Toxicity of the developed uncoated nanoparticles was significantly higher than that of

the coated nanoparticles, while the toxicity of carbon nanotubes was the highest among all tested samples; the differences in CTI of all h-BN samples practically eliminated between the ninth and eleventh day and totally disappeared on the twelfth day of the trial showing a total rehabilitation of the test animals showing that they can be characterized as less toxic in comparison with carbon nanotubes.

Conclusion

The newly developed comparatively low-temperature methods utilizing microwave synthesis and annealing can provide sufficiently high crystallinity and significantly reduce the turbostratic disorder in h-BN nanoparticles and nanosheets caused by the low temperature of lattice formation and structural defects. Mechanical processing (ball milling) induces additional turbostratic disorder (probably, due to lattice deformation and generation of structural defects) which can be effectively compensated and reduced by low and medium microwave power (about 1000 W) annealing in the inert (nitrogenous) atmosphere. A novel combined method of toxicity testing including continuous behavioral observation, as well as systolic blood pressure, body temperature and blood oxygen saturation measurements increases the reliability, accuracy and safety of nanoparticles' toxicity testing for impacted animals and reduces the health and life risks for future patients. Apparently, all the above modalities can be super-additive, sub-additive, or even antagonistic and, in principle, all considered combinations should be tested in vitro and in vivo in a wide range of doses and concentrations. However, the benefits of determining a synergistic combination can many times exceed all efforts and time spent on achieving the positive result. In vitro and in-vivo testing of the above mentioned various combinations of the uncoated magnetic nanoparticles, nanoparticles coated with zinc sulfate and h-BN and their dispersions with a number of biologically active solutions should be carried out to yield synergistic combinations of anticancer and antiviral modalities.

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ა. ჭირაქაძე[*], ნ. მითაგვარია[**], დ. ჯიშიაშვილი[*], გ. პეტრიაშვილი[§], ნ. დვალი[#], ზ. შიოლაშვილი[§], ქ. ჩუბინიძე[§], ნ. მახათაძე[§], ა. ჯიშიაშვილი[§], ზ. ბუაჩიძე[#], ი. ხომერიკი[#]

[*]*საქართველოს ტექნიკური უნივერსიტეტი, საინჟინრო ფიზიკის დეპარტამენტი; ვ. ჭავჭანიძის კიბერნეტიკის ინსტიტუტი, თბილისი, საქართველო*
[**]*აკადემიის წევრი, ივანე ბერიტაშვილის ექსპერიმენტული ბიომედიცინის ცენტრი, თბილისი, საქართველო*
[§]*საქართველოს ტექნიკური უნივერსიტეტი, ვ. ჭავჭანიძის კიბერნეტიკის ინსტიტუტი, თბილისი, საქართველო*
[#]*საქართველოს ტექნიკური უნივერსიტეტი, საინჟინრო ფიზიკის დეპარტამენტი, თბილისი, საქართველო*

ჰექსაგონალური ბორის ნიტრიდის ნანონაწილაკები, ნანოფირფიტები და ნანომილაკები (BNNPs), მათი უმნიშვნელო ციტოტოქსიკურობის გამო განიხილება, როგორც არანაკლებ პერსპექტიული მასალა ბიოსამედიცინო გამოყენებისთვის, ვიდრე ნახშირბადის ნანომილაკები (CNTs) და ნანონაწილაკები (CNPs). მიკროტალღური გამოსხივების გამოყენებით ჩატარებულმა კვლევამ უზრუნველყო ორი ახალი, შედარებით დაბალტემპერატურული მეთოდის შემუშავება და ტესტირება კრისტალურობის მაღალი ხარისხის (დაბალი ტურბოსტრატულობის) მქონე ჰექსაგონალური ბორის ნიტრიდის ნანონაწილაკების და ნანოფირფიტების სინთეზისთვის, აგრეთვე მიღებული ნანომასალების ზოგადი ტოქსიკურობის შესამოწმებლად ახალი მეთოდის სრულმასშტაბიანი გამოცდა ექსპოზირებული თეთრი ვირთაგვების ქცევით და ფიზიოლოგიურ მაჩვენებლებზე გრძელვადიანი დაკვირვების გზით. ქცევითი ეფექტების, სისხლის ჟანგბადით სატურაციის, სისტოლური წნევის და სხეულის ტემპერატურის გაზომვების საფუძველზე, მარტივი ფორმულის გამოყენებით შესაძლებელი გახდა მაღალი სიზუსტის და სანდოობის შედეგების მიღება მარტივი ფორმულის გამოყენებით და სამეცნიერო კვლევაში ცხოველთა დაცვის 4R პრინციპების სრული დაცვით. მიღებული შედეგები საშუალებას გვაძლევს დავასკვნათ, რომ მიღებული ნანომასალები შეიძლება წარმატებით გამოვიყენოთ (ქიმიოთერაპიასთან, ჰიპერთერმიასთან და სხივურ თერაპიასთან კომბინაციაში), როგორც კიბოს და ვირუსული ინფექციების მკურნალობის მაღალეფექტური დამატებითი საშუალება.

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