Laser-Ablative Aqueous Synthesis and Characterization of Elemental Boron Nanoparticles for Biomedical Applications

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Abstract

Boron-based nanoformulations look very promising for biomedical applications, including photo- and boron neutron capture therapies, but the fabrication of non-toxic water-dispersible boron (B) nanoparticles (NPs), which contain the highest boron atom concentration, is difficult using currently available chemical and plasma synthesis methods. Here, we demonstrate purely aqueous synthesis of clean boron NPs by methods of femtosecond laser ablation from a solid boron target in water, thus free of any toxic organic solvents, and characterize their properties. We show that despite highly oxidizing water ambience, the laser-ablative synthesis process follows an unusual scenario leading to the formation of elemental boron NPs together with boric acid (H_3BO_3) as an oxidation by-product, which acts to stabilize the elemental boron NPs dispersion. It was found that the formed NPs are spherical and composed of crystalline core covered by a thin sub-oxide shell, while their mean size is about 50 nm. We then demonstrate the purification of boron NPs from residual boric acid in deionized water, followed by their coating with polyethylene glycol to improve colloidal stability and biocompatibility. The synthesized NPs demonstrate low toxicity. They exhibit relatively strong absorption over a broad spectral range, in the NIR window of relative tissue transparency, promising their use as contrast agents for photoacoustic imaging and sensitizers of phototherapy, in addition to their promise for neutron capture therapy. This combined potential ability of generating imaging and therapy functionalities makes laser-synthesized B NPs a very promising multifunctional agent for biomedical applications.

Introduction

Exhibiting a series of unique properties, boron and its compounds (boron nitride, boron carbide, e.g.) are widely employed in a variety of applications, including microelectronics¹, quantum dots synthesis², combustion catalysis³, photoelectrochemical hydrogen and oxygen evolution reactions⁴. Biomedicine is also considered as one of potential beneficiaries of boron-based compounds. In particular, boron isotope ^{10}B (present in naturally abundant boron) has a high cross section of neutron capture leading to the generation of α particles, which can destroy DNAs of rapidly proliferating cancer cells in order to treat tumors^{5,6}. Some boron-containing compounds, including disodium mercaptoundecahydrododecaborate (BSH) and p-borophenylalanine (BPA)^{5,7}, have already been explored in boron neutron capture therapy (BNCT) and demonstrated a promising therapeutic effect. However, since the eradication of tumor cells requires high concentration of boron-10 isotope (this concentration is estimated to be about 10^9 atoms⁷ per cell or 20...35 $\mu\text{g/g}$ ⁸), heavy doses of molecular boron compounds such as BSH and BPA should be applied to obtain a significant therapeutic effect, which leads to toxicity issues.

The use of boron-based nanoparticles (NPs) instead of molecular agents presents an appealing alternative as the NPs contain much larger number of boron atoms and thus enhance the therapeutic outcome. Here, NPs of elemental boron look especially promising as they contain the maximal concentration of boron. Moreover, when NPs are coated by polymers such as polyethylene glycol (PEG), they can have a prolonged circulation in the blood stream due to “stealth” effect and offer an additional

passive tumor targeting mechanism based on enhanced permeability and retention (EPR) effect⁹. Finally, owing to their capability of absorbing light in visible and near-infrared region, covering the window of relative biological transparency (650-900 nm), boron NPs can serve as contrast agents in photoacoustic imaging¹⁰ and sensitizers of photothermal therapy^{10,11}. However, the fabrication of boron-based nanoformulations suitable for biomedical use presents a real challenge¹². As an example, chemical synthesis pathways are typically complicated and cause the contamination of NPs by toxic reaction products, such as carbon, chlorine, etc., while the size of formed NPs often exceeds several hundreds of nm^{10,11,13,14}. On the other hand, dry fabrication methods such as laser pyrolysis do not render possible fine control of size, crystallinity and aggregation characteristics of NPs, while NPs prepared by these methods cannot easily be water-dispersed¹⁵⁻¹⁷.

Based on the natural production of nanoclusters under the action of laser radiation on a solid target, followed by their release into liquid ambient¹⁸⁻²¹, pulsed laser ablation in liquids (PLAL) has recently appeared as a novel “physical” nanofabrication route, which promises the synthesis of a variety of novel unique NPs^{22,23}. As one of advantages, PLAL can be performed in ultrapure environment (deionized water, some organics) to exclude any contamination of the NPs surface²¹, while solutions of NPs prepared by laser ablation can be stable even without ligand protection due to electrostatic repulsion effect²⁴. Another advantage is that the oxidation of formed nanomaterials can be minimized by decreasing the content of oxygen in water²⁵ or the employment of organic solutions such as acetone²⁶. Ultrashort laser ablation (femtosecond, nanosecond) showed the best efficiency among PLAL approaches in terms of control of size and stability characteristics of formed NPs, as well as the fabrication of complex nanoarchitectures^{22,23}. In particular, we previously showed that techniques of femtosecond laser ablation and fragmentation can be used to fabricate stable solutions of low-size dispersed NPs of a variety of materials, including Au^{21,24}, Si^{25,27}, TiN^{26,28}, elemental Bi²⁹ NPs. Recently, methods of laser ablation from a Fe-B alloy target were elaborated for the fabrication of composite Fe-B NPs³⁰. The experiments were carried out in acetone in the presence of ligands (polyvinyl pyrrolidone, PVP) to control size characteristics and minimize oxidation effects, which could alter material stoichiometry. The formed NPs were considered as a promising object for combined neutron boron capture therapy and magnetic resonance imaging modalities. Despite promising characteristics, the formed NPs could have been contaminated by products of acetone decomposition (typically carbon-based compounds) due to high energy of nanosecond laser radiation used in these experiments, as it was earlier reported³¹⁻³³, while the concentration of boron in alloy nanostructures was still not very high, limiting further prospects in BNCT.

Here, we describe a laser-ablative methodology to fabricate stable aqueous solutions of pure elemental boron NPs, which should provide maximal concentration of boron atoms for BNCT and avoid any toxic contamination, as recommended by current state-of-the-art. In this methodology, we do not use organics to minimize oxidation effect. Instead, we perform fs laser ablation in deionized water in the absence of ligands, which excludes any carbon contamination of NPs. We show that laser synthesis process leads to

the formation of aqueous solutions of elemental crystalline boron NPs with the mean size of about 50 nm and boric acid (H_3BO_3), which can then be easily removed from the aqueous solution. We then describe a protocol for the coating of laser-synthesized B NPs with PEG and assess the cytotoxicity of formed NPs in vitro. We believe that synthesized B NPs can be very promising for biomedical applications.

Results

Synthesis and characterization of NPs

We performed laser ablation from elemental boron (B) bulk target in aqueous solutions according to a protocol described in Methods section. Briefly, the target was fixed vertically in a quartz cuvette and filled with deionized water (Fig. 1a). A beam from a femtosecond laser was directed through one of walls of the cuvette and focused by a converging lens on the target surface, while the cuvette was constantly moved to avoid ablation from the same area. We observed a brownish coloration of the solution after 30 minutes of the irradiation onset, which could be attributed to the formation of NPs. Final solutions were brown and transparent for as-prepared solutions and dark brown for concentrated ones, as shown in Figure 1b, while their prolonged aging at room temperature did not reveal any sign of material precipitation suggesting a high colloidal stability. As shown in Figure 1c, optical extinction spectra recorded using UV/Vis spectrometer did not contain any particular features, but has a long tail extending from visible to near-infrared region. It is important that the extinction efficiency in this tail was relatively high in the window of relative biological transparency (650-900 nm), which is in agreement with previously reported optical extinction data for elemental boron NPs¹⁰, suggesting the possibility of NPs applications in photoacoustic imaging photothermal therapy.

Our tests using transmission electron microscopy (TEM) showed that solutions prepared by laser ablation from a boron target in water were mainly composed of spherical NPs (inset in Fig. 2a), while their mean diameter was 51 ± 25 nm (Fig. 2a), as followed from our statistical analysis. According to Energy-dispersive X-ray spectroscopy (EDX) analysis, the formed NPs contained a significant amount of boron with the maximal signal in the nanoparticle core (Fig. 2b), suggesting the domination of this element in the nanoparticle composition with a particular high concentration in the core. In addition, examining TEM images, we could observe a difference of signal contrasts between central and outer parts of NPs (Fig. 2c), which confirms the hypothesis on the domination of boron in the nanoparticle core. Lower contrast of images on outer nanoparticle parts can be explained by the formation of a thin layer of boron compound-based shell (boron oxide, boric acid, etc.) on the surface during the laser-ablative process or subsequent drying of NPs on a TEM grid. A detailed analysis of high-resolution TEM (HR-TEM) images showed that the formed NPs were mainly polycrystalline (Fig. 2d), while an amorphous fraction was also largely present. The calculated interplanar spacing was $4.46 \text{ \AA}$, which corresponds to the (113) plane of the elemental β -boron, suggesting the presence of a large fraction of crystalline boron in the nanoparticle composition.

Nuances of crystalline structure of formed nanoparticles were assessed by X-ray diffraction spectroscopy. For these tests, the formed colloidal solution was dried and the resulting powder was examined. Surprisingly, our experiments recorded XRD peaks, which could not be associated with the presence of crystalline NPs of elemental boron. Fig. 3a shows a typical XRD spectrum from so dried powder. As shown in the figure, the recorded XRD peaks could only be attributed to boric acid H_3BO_3 (reference ICDD 01-073-2158, lower red spectrum) and some other by-products (in much lower quantities) related to boron oxide states and contaminations during the fabrication and characterization of NPs. To clarify the composition of formed colloids, solutions of NPs were examined using inductively coupled plasma mass spectrometry (ICP-MS). These measurements confirmed that boric acid was indeed the dominating component, but elemental boron NPs was also detected. As follows from ICP-MS data, boric acid contained 5 times more boron than NPs (0.109 and 0.022 mg/ml, respectively). Understanding that boron NPs constitute only a part of formed colloidal product, we applied a purification procedure with multiple centrifugation steps in order to extract them in a pure state. Fig. 3b shows a typical XRD spectrum from purified boron NPs, as well as a relevant spectrum from a boron powder obtained by milling the used target. As shown in the figure, these NPs are indeed composed of elemental crystalline β -Boron NPs (reference ICDD 00-031-0207, lowest red spectrum). It should be noted that there was a small fraction of boron carbide impurities (reference ICDD 01-086-1121) visible on XRD spectrum of boron target powder. Additional peaks in the spectrum can be associated with boric acid residues crystallized from the solution.

Our experiments showed that after re-dispersion in deionized water purified Boron NPs rapidly agglomerated and precipitated in a form of turbid brown flakes, while as-prepared solutions were stable for months of storage at ambient conditions. A relatively high stability of colloidal NPs solutions prepared by laser ablation in liquids is generally attributed to their electrostatic stabilization as a result of NPs charging. The origin of the NPs electrical charge is not well understood, but it is widely attributed to oxidation of the ablated material in high temperature conditions during early stages after laser-target interactions or to adsorption of charged molecules from the liquid. The latter mechanism generally requires addition of some substances to liquid, which will be absorbed on the NPs surface. However, it seems that boric acid, appearing as a by-product of synthesis during laser ablation, serves as a stabilizing agent for the formed boron NPs. We believe that this by-product adsorbs on the NPs surface and forms an electrical double layer, which stabilizes the NPs solution. This hypothesis is supported by the fact that ζ -potential of NPs increased from -36 mV in the as-prepared colloid to almost neutral value in the purified colloid, additionally indicating the loss of stability after the multiple purification steps.

To further investigate the hypothesis, we performed XPS measurements in B 1s and O 1s regions of the as-prepared (unwashed) sample, which provide information on the composition of the upper surface layer of NPs. Results of this measurement are shown in Figure 4. As shown in the figure, the peak at 187.7 eV corresponds to elemental boron (B^0), whereas a peak near 192.3 eV corresponds to the oxidized boron state B^{3+} , confirming the presence of boron oxide (B_2O_3) and/or boric acid (H_3BO_3) in the upper shell of NPs³⁴⁻³⁶. We believe that the formation of boron suboxide (B_6O) in a negligible quantity is also

possible, which could explain a slight broadening of the spectrum around 188 eV. The component at 532.1 eV can be assigned to boron oxide (boric acid), which is in a good agreement with previously reported results³⁷. It should be noted that despite the formation of boron suboxide shell, the core of NPs is composed of elemental boron, which promises the use of NPs in nuclear medicine applications.

Functionalization And Biological Assessment Of Laser-synthesized Boron Nps

As follows from our analysis, the purification of NPs from boric acid residuals and re-dispersion in water led to the loss of colloidal NPs stability. The mean hydrodynamic size of NPs was much larger (92 nm) and did not show any changes within one day, whereas their incubation in phosphate buffered saline (PBS) rapidly led to a significant increase of the mean particle size, started to reach the value of 600 nm within 4 h (Fig. 5a). This indicates the colloidal instability of boron NPs due to aggregation in the buffers.

To prevent nanoparticle interaction, we coated them with polyethylene glycol containing carboxyl terminate groups. For this aim, we used a reaction of silane-PEG hydrolysis and condensation of derivatives on the surface of boron particles in alkaline conditions. After the coating, the hydrodynamic diameter of boron NPs in distilled water slightly increased from 92 ± 34 nm to 121 ± 45 nm, which confirmed the attachment of the polymer to the particle core (Fig. 5b). In addition, we observed the increase of particle colloidal stability in PBS with no signs of any aggregation (Fig. 5b). Hydrodynamic size of B-PEG particles in PBS was 128 ± 42 nm. In addition, we observed a shift of ζ -potential of boron NPs after PEGylation to more negative values, which indicated the increase of repulsion between particles (Fig. 5c). Thus, silane based PEGylation could be applied for boron NPs stabilization in physiologically relevant conditions.

To study the degradability of boron NPs in buffer solutions we applied a spectrophotometry-based approach. The extinction spectra of boron NPs (Fig. 5d) made possible a quantitative determination of particle concentration in aqueous solutions (see calibration curve at Fig. S1). It could be seen in Fig. 5e, that 5% of boron NPs (0.1 g/l) degrade in a PBS (pH=7.4) within 3 days. A rapid and significant 32% decrease in particle concentration was found in acidic citrate buffer (pH=4.5) after one day, which indicates the ability of boron NPs to be partly dissolved in the lysosomal conditions.

The cytotoxicity of B NPs was evaluated in HeLa cells using a standard MTT assay which measures the reduction of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide into an insoluble formazan product. This process occurs in the mitochondria of live cells as a result of cellular respiration. B NPs showed slightly less toxicity at concentrations between 1 and 100 $\mu\text{g/mL}$ at 24 hours than at 72 hours (Figure 6). The IC_{50} cannot be determined from this data, but at both 24 and 72 hours even when treated with concentrations of 500 $\mu\text{g/mL}$ only about 50% cell death was observed. This data suggests bare B NPs are relatively well tolerated by cells.

Discussion

Thus, our experiments clearly demonstrated the possibility of laser synthesis of spherical boron-based NPs in organic-free environment, which present a promising object for biomedical and other applications. Our characterization tests showed that these NPs are composed of elemental boron core (mostly crystalline), coated by a thin shell composed of boron sub-oxides. Initially generated with a significant amount of boric oxide as an oxidation by-product of laser synthesis, boron NPs were later purified from boric acid by an easy centrifugation step.

One of interesting conclusions from obtained experimental data is related to unusual physico-chemical conditions of laser synthesis, which led to the formation of elemental boron NPs. The ablation was performed in aqueous ambience, which is well compatible with biological systems and ideal for the synthesis of nanomaterials for biomedical applications. On the other hand, presenting a highly oxidizing medium, water causes a strong oxidation of most materials, which can dramatically alter their properties. This effect is especially important under high energies of laser pulses (e.g., in the case of ns laser ablation this energy can reach 1.5-2 mJ per pulse) to condition a strong material heating, but even in the case of “mild” regime of fs laser ablation, with one order of magnitude lower pulse energies and much faster nanosynthesis process^{22,23}, the oxidation phenomena are still significant. As an example, even in the case of chemically inert gold NPs the oxidation phenomena lead to the formation a slight gold oxide shell, terminated by O⁻ group under pH > 5²⁴, opening avenues for its unusual interactions, via hydrogen bonding, with OH groups of important polymers such as cyclodextrins³⁸, dextran³⁹, polyethylene glycol³⁹, proteins⁴⁰ and oligonucleotides⁴¹. and rendering possible their one-step functionalization. For other materials the oxidation phenomena are even more significant, while their contribution depends on physico-chemical characteristics of materials. Several strategies to avoid or minimize oxidation phenomena were proposed. In the case of weakly oxidizing materials such as silicon (Si), a strategy based on pumping out oxygen dissolved in water by its bubbling with an inert gas (e.g., argon)²⁵ is sufficient to exclude oxidation during a fast stage of NP core synthesis, while the upper thin oxide shell is formed during a further aging of Si NPs in water ambience⁴². In the case of stronger oxidizing materials, another strategy based on laser ablation in organic solvents (ethanol, acetone, hexane, etc.) is used, which makes possible a drastic decrease of oxidation effects. In particular, this strategy was used in our earlier works on the laser synthesis of TiN^{26,28} and Bi²⁹ NPs. Torresan et al³⁰ also used the latter straightforward strategy for the fabrication of Fe-B NPs, while polyvinyl pyrrolidone (PVP) was used to control size characteristics. However, the used highly energetic ns laser radiation could lead to a partial decomposition of acetone, as it was observed in earlier studies^{31,32}, which can lead to a partial contamination of NPs, while formed NPs required further transfer to water. Our methodology did not follow the currently existing paradigm to avoid oxidation of formed material during laser-ablative synthesis. Indeed, instead of performing ablation experiments in low-oxidizing organic environment, we did it in strongly oxidizing deionized water (without pumping out dissolved oxygen). Surprisingly, the laser-ablative process followed unusual scenario leading to the formation of elemental boron NPs together with boric acid (H₃BO₃) as an oxidation by-product. Furthermore, boric acid played an important

role as it led to the stabilization of colloidal aqueous solutions of boron NPs. It is important that B NPs could later be purified from boric acid by a simple purification step and then coated by PEG to stabilize aqueous colloidal solutions of NPs and improve their biocompatibility. We believe that such strategy requires further detailed investigation and can be applied for the synthesis of a variety of other nanomaterials.

It is important that the NPs demonstrated a low cytotoxicity profile in MTT tests, which promises their successful use in biological systems. We believe that the synthesis of NPs of pure elemental boron is an important advance in theranostic (therapy+diagnostics) modalities based on boron-based compounds. First, a strong absorption value of these NPs in the first window of biological transparency promises the employment of such NPs as sensitizers of phototherapy. In such a modality, PEG-coated B NPs will be delivered to tumor region via active targeting (see, e.g., Ref. 43) or passive accumulation due to EPR effect⁹ and then heated by photons to provide local overheating (hyperthermia) of adjacent cancer cells in order to initiate their death, similarly to how it takes place in cases of laser-synthesized TiN²⁶ and Si⁴⁴ NPs. The presence of a strong absorption also means the possibility of enabling imaging modalities based on photoacoustic tomography¹⁰. Finally, the synthesized B NPs look very appealing for applications in boron neutron capture therapy (BNCT)^{5–8}. Since the formed NPs are composed of elemental boron, they should provide the highest possible concentration of boron atoms in tumor cells to maximize the therapeutic outcome. It should be noted that in our experiments we used targets of naturally abundant boron, which contains about 20% of neutron-active ¹⁰B isotope. We suppose that the proposed laser-ablative methodology should work in the case of ¹⁰B-enriched targets to synthesize NPs of elemental ¹⁰B isotope.

Conclusion

We demonstrated the possibility to synthesize spherical pure elemental boron nanoparticles by femtosecond laser ablation in deionized water. We show that laser synthesis process leads to the formation of aqueous solutions of elemental crystalline boron NPs with the mean size of about 50 nm together with boric acid (H₃BO₃) as an oxidation by-product, which helps to stabilize the elemental boron NPs dispersion. We then demonstrate the possibility of purification of boron NPs from boric acid residuals. The proposed strategy for the fabrication of elemental metal NPs in highly oxidizing water environment, followed by the exclusion of oxidized component (H₃BO₃ in our case) looks like a novel paradigm in the laser-ablative synthesis, which can be extended to other promising nanomaterials. We then describe a protocol for the NPs coating in order to improve solution stability and biocompatibility. Finally, our tests in vitro illustrated low toxicity of laser-synthesized boron compounds. We believe that synthesized B NPs can be very promising for biomedical applications.

Methods

Synthesis of nanoparticles

Nanoparticles were synthesized by laser ablation of a hot-pressed boron target (MaTeCK Material Technologie & Kristalle GmbH, Juelich, Germany, purity 99.5%, natural isotopic composition) in deionized water (18.2 MΩ at 25 °C) at ambient conditions. A beam coming from a Yb:KGW laser (1025 nm, 480 fs, 8 kHz; Amplitude Systems, France or 1030 nm, 270 fs, Avesta, TETA 10 model, Moscow, Russia) was focused on the surface of the boron target fixed vertically in a quartz cuvette filled with 50 mL of deionized water. The energy was preliminary attenuated to the value of 350 μJ per pulse. The liquid layer thickness between the inner wall of the cuvette and the surface of the target was 3.3 mm. Due to the high energy of the interacting beam, self-focusing effects take place, therefore, the quartz cuvette was moved forward in the direction of the convex lens to focus the beam on the surface of the target and to avoid damaging of the glass wall. To prevent the ablation from the same region, the cuvette with the target was constantly moved by a translation stage. The scan area was set to 7 mm x 7 mm and the displacement speed to 2.5 mm s⁻¹. The laser ablation in water lasted for 7 h.

Nanoparticles Characterization

Boron nanoparticles size, shape and composition were characterized using transmission electron microscopy (TEM, JEOL JEM-3010, 250 kV acceleration voltage, W filament) and scanning electron microscopy (SEM, JEOL JSM-7900F, 0...30 kV) equipped with EDX detector (Brüker). For that, 10 μL of elemental boron particles solution was dropped on the copper-carbon grid (200 mesh, Oxford Instrument) and dried overnight under ambient conditions. Size distribution, fast Fourier transform (FFT) and inverse fast Fourier transform (IFFT) analysis were performed in Fiji ImageJ software. Extinction spectrum was recorded using a UV-Visible spectrophotometer (UV-2600, Shimadzu) and a rectangular quartz cuvette with an optical pathlength of 10 mm.

XRD measurements of dried elemental B NPs powder were performed in a transmission mode. The instrument is equipped with a double reflection mirror (Osmic), an image plate detector (Mar345), and a high brilliancy rotating anode (Rigaku RU-200BH, 50 kV, 50 mA). The radiation is Cu Kα ($\lambda = 1.5418 \text{ \AA}$), and the size of the beam is 0.5 x 0.5 mm². The maximum 2θ value is 65° (0.3° experimental resolution). The powder was obtained by drying a concentrated solution of B NPs in deionized water under ambient conditions. The XRD reflexes were calculated using VESTA (Visualization for Electronic and STructural Analysis) software⁴⁵ and the data files from Crystallography Open Database (COD ID files: 9011170 for β-Boron and 9014010 for Boric Acid)^{46,47}.

The XPS spectra were recorded using Physical Electronic PHI Versaprobe 5000 equipped with a hemispherical energy analyzer. The hemispherical analyzer was operated in Fixed Analyzer Transmission (FAT) Mode. A monochromic Al Kα X-ray source (1486.60 eV) was operated at 25 W and 15 kV with a beam diameter of 100.0 μm. The energy of the analyzer was operated at a pass energy of 117.5 eV for survey acquisitions and 23.50 eV for high-resolution acquisitions. The energy resolution was 0.020 eV for high resolution spectra, and 1.0 eV for survey spectra. The operating pressure of XPS was around 3.5 x 10⁻⁶ Pa. Dual charge neutralization was utilized to reduce the effects of charging on the acquired signal. Survey acquisitions were taken for binding energies from 0 - 1200 eV.

Boron concentration in nanoparticles and supernatant were measured using inductively coupled plasma mass spectrometry (ICP-MS). Nanoparticles were centrifuged at 15 000 g, 30 min, supernatant and particle residue were separated and dissolved in 10% nitric acid under the heating at 80 °C. A mass-spectrometer (NexION 2000, PerkinElmer, USA) was calibrated before the analysis using serial dilutions of sodium tetraborate (Sigma-Aldrich, USA) with known concentrations. ^{11}B peak was used for analysis.

Nanoparticles purification

To purify B NPs from the excess of boric acid formed during laser ablation, centrifugation was performed. First, the solution was centrifuged at 5000 g 5 min to separate large particles. Then, the solution was centrifuged twice at 15000 g 15 min and redispersed in deionized water at each step.

Nanoparticle coating

To increase boron nanoparticle stability in buffers they were coated with polyethylene glycol (PEG). For this aim 1 mg of particles were dissolved in 1 mL of 95% ethanol and mixed with 100 μL of ethanol containing 100 μg of Silane-PEG (5kDa) and 10 μg of Silane-PEG-carboxyl (5kDa). After that 10 μL of 30% ammonia hydroxide was added to accelerate hydrolysis of silanes. Solution of boron nanoparticles was heated at 60 °C for 2h and then washed 2 times from unreacted chemicals with water by centrifugation (5 000 g 5 min, then 15 000 g, 15 min).

Functionalized nanoparticles characterization

The hydrodynamic diameter and ζ -potential measurements were performed by dynamic and electrophoretic light scattering, respectively, using a Zetasizer Nano ZS (Malvern Instruments, UK) device. Number weighted size distributions were used for analysis. ζ -potential measurements were performed in a 10 mM NaCl solution using the Smoluchowski approximation.

Extinction spectra of aqueous colloids of nanoparticles were recorded by an Infinite M1000 PRO spectrophotometer (Tecan, Austria).

Degradation kinetics measurement

To study boron NP chemical stability in distilled water, PBS (pH = 7.4) or citrate buffer (pH = 4.5), 100 μg of particles were added to 1 ml of the corresponding medium and rigorously mixed. The extinction intensity of NPs at 500 nm was measured to analyze particle concentration at each time point. The obtained intensities were averaged ($n=3$) and normalized to the initial signal to determine the content of undissolved particles. Before each measurement samples were thoroughly sonicated and stirred.

Cells and Culture Conditions

HeLa cells (ATCC, Manassas, VA) were grown in 100 mm cell culture dishes and maintained in Advanced DMEM media, supplemented with 10% fetal bovine serum, 1% glutamax, 1% Antibiotic Antimycotic

Solution at 37°C in a humidified atmosphere containing 5% CO₂.

Cytotoxicity

Cytotoxicity of Boron NPs was determined by measuring the viability of cells by the standard MTT assay. Briefly, 2.5×10^3 HeLa cells/well were plated in 96-well plates. After incubation for 24 h, 20 µl of the desired nanoparticle concentration was added to the well containing 180 µl of media. Untreated cells were used as a control. Following a 24 or 72 h incubation period media was removed, and cells were incubated with 100 µl 500 µg/mL methyl thiazolyl tetrazolium (MTT) for 2 h at 37 °C, 5% CO₂. The MTT solution was removed, and the formazan reaction products were dissolved in DMSO overnight in the incubator. The optical density of the formazan solution was read on a microplate reader (BioTek Instruments, Inc., Winooski, VT) at 490 nm with a reference wavelength of 630 nm. Cell viability was normalized to untreated cells used as the control.

Declarations

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Author contributions statement

P.N.P and A.V.K. conceived the research. A.I.P., A.A.P., S.M.K., A.V.K. designed the experimental fabrication design, A.I.P. and A.A.P. fabricated and partially characterized the formed NPs. I.B.B., I.V.Z. and S.M.D. chemically modified and functionalized the NPs. J.C.B. and PNP conducted chemical analysis and tests in vitro, A.I.P., A.A.P., I.B.B., J.C.B., I.V.Z., A.A.P., I.N.Z., S.M.K., S.M.D., P.N.P. and A.V.K. analyzed and discussed obtained data. A.V.K. prepared the manuscript using data from co-authors. A.V.K., P.N.P. and I.N.Z. guided the project. All authors have given approval to the final version of the manuscript.

Competing Interests

The authors declare no competing interests.

References

1. Muzyka, K. *et al.* Boron-doped diamond: current progress and challenges in view of electroanalytical applications. *Anal. Methods* **11**, 397–414 (2019).
2. Ding, Y. *et al.* Efficient Full-Color Boron Nitride Quantum Dots for Thermostable Flexible Displays. *ACS Nano* **15**, 14610–14617 (2021).
3. Van Devener, B., Perez, J. P. L., Jankovich, J. & Anderson, S. L. Oxide-Free, Catalyst-Coated, Fuel-Soluble, Air-Stable Boron Nanopowder as Combined Combustion Catalyst and High Energy Density Fuel. *Energy & Fuels* **23**, 6111–6120 (2009).
4. Jung, H. J. *et al.* Preparation of TiO₂-Decorated Boron Particles by Wet Ball Milling and their Photoelectrochemical Hydrogen and Oxygen Evolution Reactions. *Mater. (Basel, Switzerland)* **9**, (2016).
5. Malouff, T. D. *et al.* Boron Neutron Capture Therapy: A Review of Clinical Applications. *Front. Oncol.* **11**, 351 (2021).
6. Barth, R. F., Coderre, J. A., Vicente, M. G. H. & Blue, T. E. Boron neutron capture therapy of cancer: current status and future prospects. *Clin. cancer Res. an Off. J. Am. Assoc. Cancer Res.* **11**, 3987–4002 (2005).
7. Salt, C., Lennox, A. J., Takagaki, M., Maguire, J. A. & Hosmane, N. S. Boron and gadolinium neutron capture therapy. *Russ. Chem. Bull.* **53**, 1871–1888 (2004).
8. Sauerwein, W. Neutron capture therapy: principles and applications. (2012).
9. Matsumura, Y., Maeda, H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. *Cancer Res.* **46**, 6387–6392 (1986).
10. Guo, T. *et al.* Boron Quantum Dots for Photoacoustic Imaging-Guided Photothermal Therapy. *ACS Appl. Mater. Interfaces* **13**, 306–311 (2021).
11. Fu, Z. *et al.* Functionalized boron nanosheets as an intelligent nanoplatform for synergistic low-temperature photothermal therapy and chemotherapy. *Nanoscale* **12**, 14739–14750 (2020).
12. Tatiya, S., Pandey, M. & Bhattacharya, S. Nanoparticles containing boron and its compounds—synthesis and applications: A review. *J. Micromanufacturing* **3**, 159–173 (2020).
13. Hao, J. *et al.* Crystalline Semiconductor Boron Quantum Dots. *ACS Appl. Mater. Interfaces* **12**, 17669–17675 (2020).
14. Ji, X. *et al.* A Novel Top-Down Synthesis of Ultrathin 2D Boron Nanosheets for Multimodal Imaging-Guided Cancer Therapy. *Adv. Mater.* **30**, 1803031 (2018).
15. Rohani, P., Kim, S. & Swihart, M. T. Boron Nanoparticles for Room-Temperature Hydrogen Generation from Water. *Adv. Energy Mater.* **6**, 1502550 (2016).
16. Hieu, N. D. *et al.* Preparation of boron nanoparticles by pulsed discharge of compacted powder. *Jpn. J. Appl. Phys.* **59**, SCCC05 (2020).
17. Singh, A. *et al.* Boron-Nanoparticle-Loaded Folic-Acid-Functionalized Liposomes to Achieve Optimum Boron Concentration for Boron Neutron Capture Therapy of Cancer. *J. Biomed. Nanotechnol.* **15**,

1714–1723 (2019).

18. Fojtik, A., Henglein, A. Laser Ablation of Films and Suspended Particles in a Solvent - Formation of Cluster and Colloid Solutions. *Ber Bunsen Phys Chem*, **97**, 252–254 (1993).
19. Neddersen, J., Chumanov, G., Cotton, T. M., Laser-Ablation of Metals - a New Method for Preparing Sers Active Colloids. *Appl Spectroscopy* **47**, 1959–1964 (1993).
20. Mafuné F., Kohno J., Takeda Y., Kondow T., Sawabe H. Formation of Gold Nanoparticles by Laser Ablation in Aqueous Solution of Surfactant *J. Phys. Chem. B* **105** 5114–5120 (2001).
21. Kabashin, A. V. & Meunier, M. Synthesis of colloidal nanoparticles during femtosecond laser ablation of gold in water. *J. Appl. Phys.* **94**, 7941 (2003).
22. Kabashin, A. V. et. al, Nanofabrication with Pulsed Lasers. *Nanoscale Res. Lett.*, **5**, 454–463 (2010).
23. Zhang, D., Gökce, B. & Barcikowski, S. Laser Synthesis and Processing of Colloids: Fundamentals and Applications. *Chem. Rev.* **117**, 3990–4103 (2017).
24. Sylvestre, J. P. et al. Surface chemistry of gold nanoparticles produced by laser ablation in aqueous media. *J. Phys. Chem. B* **108**, 16864–16869 (2004).
25. Al-Kattan, et al. Ultrapure laser-synthesized Si nanoparticles with variable oxidation state for biomedical applications. *J. Mater. Chem. B*, **4**, 7852 (2016)
26. Popov, A. A. *et al.* Laser- synthesized TiN nanoparticles as promising plasmonic alternative for biomedical applications. *Sci. Rep.* **9**, 1194 (2019).
27. Petriev, V. M., Tischenko, V. K., Mikhailovskaya, A. A., Popov, A. A., Tselikov, G., Zelepukin, I., Deyev, S.M., Kaprin, A.D., Ivanov, S., Timoshenko, V.Y., et al. Nuclear nanomedicine using Si nanoparticles as safe and effective carriers of ^{188}Re radionuclide for cancer therapy. *Sci. Rep.* **9**, 2017 (2019).
28. Zelepukin I V, Popov A A, Shipunova V O, Tikhonowski G V, Mirkasymov A B, Popova-Kuznetsova E A, Klimentov S M, Kabashin A V, Deyev S M 2020 Laser-synthesized TiN nanoparticles for biomedical applications: evaluation of safety, biodistribution and pharmacokinetics. *Mater. Sci. Eng. C.* **120** 111717.
29. Bulmahn, J. C. *et al.* Laser-Ablative Synthesis of Stable Aqueous Solutions of Elemental Bismuth Nanoparticles for Multimodal Theranostic Applications. *Nanomaterials* **10**, 1463 (2020).
30. Torresan, V. *et al.* Biocompatible Iron–Boron Nanoparticles Designed for Neutron Capture Therapy Guided by Magnetic Resonance Imaging. *Adv. Healthc. Mater.* **10**, 2001632 (2021).
31. Amendola, V., Riello, P. & Meneghetti, M. Magnetic Nanoparticles of Iron Carbide, Iron Oxide, Iron@Iron Oxide, and Metal Iron Synthesized by Laser Ablation in Organic Solvents. *J. Phys. Chem. C* **115**, 5140–5146 (2011).
32. García-Calzada, R. *et al.* Facile laser-assisted synthesis of inorganic nanoparticles covered by a carbon shell with tunable luminescence. *RSC Adv.* **5**, 50604–50610 (2015).
33. Abderrafi, K. *et al*, Laser ablation of a silicon target in chloroform: formation of multilayer graphite nanostructures. *Journal of Physics D: Applied Physics*, **46**, 135301 (2013)

34. Shin, W. G., Calder, S., Ugurlu, O. & Girshick, S. L. Production and characterization of boron nanoparticles synthesized with a thermal plasma system. *J. Nanoparticle Res.* **13**, 7187–7191 (2011).
35. Zhang, H., Du, X. & Li, G. Effect of Encapsulation of Boron Nanoparticles Using Reduced Graphene Oxide on their Oxidation Characteristics. *Propellants, Explos. Pyrotech.* **46**, 1240–1247 (2021).
36. Pivkina, A. N. *et al.* Comparative Analysis of Boron Powders Obtained by Various Methods. I. Microstructure and Oxidation Parameters during Heating. *Combust. Explos. Shock Waves* **54**, 450–460 (2018).
37. Moddeman, W. E., Burke, A. R., Bowling, W. C. & Foose, D. S. Surface oxides of boron and B12O2 as determined by XPS. *Surf. Interface Anal.* **14**, 224–232 (1989).
38. Kabashin, A. V., Meunier, M., Kingston, C. & Luong, J. H. T. Fabrication and characterization of gold nanoparticles by femtosecond laser ablation in aqueous solution of cyclodextrins. *J. Phys. Chem. B.* **107**, 4527–4531 (2003).
39. Besner, S., Kabashin, A. V., Meunier, M. & Winnik, F. M. Synthesis of size-tunable polymer-protected gold nanoparticles by femtosecond laser-based ablation and seed growth. *J. Phys. Chem. C* **113**, 9526–9531 (2009).
40. S. Petersen *et al*, Penetratin-Conjugated Gold Nanoparticles – Design of Cell-Penetrating Nanomarkers by Femtosecond Laser Ablation, *J. Phys. Chem. C* **115**, 5152 (2010)
41. Sobhan, M. A., Sreenivasan, V. K. A., Withford M. J., Goldys, E. M., Non-specific internalization of laser ablated pure gold nanoparticles in pancreatic tumor cell, *Colloids&Interfaces*, **92**, 190 (2012)
42. Kabashin, A. V. *et al*, Laser-processed nanosilicon: a multifunctional nanomaterial for energy and healthcare. *ACS Nano* **13**, 9841–9867 (2019).
43. Shipunova, V.O. *et al.* Dual regioselective targeting the same receptor in nanoparticle-mediated combination immuno/chemotherapy for enhanced image-guided cancer treatment. *ACS Nano* **14**, 12781–12795 (2020).
44. Oleshchenko V. A. *et al*, Localized infrared radiation-induced hyperthermia sensitized by laser-ablated silicon nanoparticles for phototherapy applications *Appl. Surf. Sci.*, **516**, 145661 (2020).
45. Laugier, J., Bochu, B. LMGP-Suite of Programs for the Interpretation of X-ray Experiments (2011).
46. Callmer, B. An accurate refinement of the β -rhombohedral boron structure. *Acta Crystallographica B: Structural Crystallography and Crystal Chemistry*, **33**, 1951–1954 (1977).
47. Zachariasen, W. H. The crystal lattice of boric acid, BO_3H_3 . *Zeitschrift für Kristallographie-Crystalline Materials*, **88**, 150–161 (1924).

Figures

Figure 2

High resolution electron microscopy images of laser-synthesized NPs: **(a)** Typical TEM image of NPs (inset) and corresponding size distribution. **(b)** Typical EDX map of NPs prepared by fs laser ablation in water. Blue color corresponds to boron. **(c)** Magnified view of a TEM image of NPs illustrating the presence of a core and a shell. **(d)** HR-TEM image of a polycrystalline nanostructure.

Figure 3

X-ray diffraction (XRD) measurements of NPs produced by laser ablation in deionized water: **(a)** XRD of as-prepared sample showing the presence of boric acid. **(b)** XRD spectra from samples of purified NPs and a boron powder obtained by milling the used target. The presence of elemental boron clearly visible

Figure 4

X-ray photoelectron spectroscopy (XPS) measurements in different regions: **(a)** B 1s region. **(b)** O 1s region.

Figure 5

Colloidal stability tests: **(a)** The evolution of hydrodynamic size of boron NPs incubated in distilled water and PBS. **(b)** Hydrodynamic sizes of boron and PEG-B NPs in water and PBS. **(c)** ζ -potential distributions of boron and PEG-B NPs. **(d)** Extinction spectra of boron NPs at 0.04-0.4 g/l concentrations in distilled water. **(e)** Kinetics of boron NP degradation in water, PBS (pH=7.4) and citrate buffer (pH=4.5).

Figure 6

Cytotoxicity evaluation of boron NPs.

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