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MEDICAL PHYSICS AND BIOPHYSICS

Carbon Nanodots Obtained by Microwave Synthesis: Physical Properties and Assessment of Cytotoxicity on a Model of Glioblastoma and Embryonal Kidney In Vitro

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Abstract—Carbon nanodots (CNDs) produced by liquid-based synthesis methods are an example of a biocompatible, nontoxic nanomaterial with physical properties promising for various applications. In our work, CNDs were obtained from organic precursors by express synthesis in a microwave reactor followed by purification in isopropyl alcohol and were studied by transmission electron microscopy, infrared spectroscopy, and optical absorption spectroscopy in the ultraviolet–visible–near-infrared range, as well as photoluminescence. Cytotoxicity assessment was performed on in vitro models of glioblastoma and embryonic kidney. The obtained results indicate the prospects of the used method of CND synthesis in the production of nanomaterials for biomedical luminescent diagnostics.

Keywords: nanodots, microwave synthesis, cytotoxicity, glioblastoma

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INTRODUCTION

Carbon dots (CNDs), which are predominantly carbon-based nanoparticles with characteristic sizes ranging from a few to several tens of nanometers, were first purposefully synthesized and studied in 2004. Their distinctive features, such as low toxicity and spectrum-tunable effective photoluminescence, make them promising for a wide range of applications [1]. An additional advantage is the possibility of synthesizing CNDs from cheap and readily available carbon-containing materials and even biowaste [2]. They can be used in many fields—in optical information technologies, chemical catalysis, and biomedicine.

There are various methods for synthesizing CNDs—hydrothermal, solvothermal, using a microwave reactor. The hydrothermal route involves heating an aqueous solution in a container made of inert material in an oven and allows producing a pure aqueous solution of particles without toxic solvents, which is very important for biomedicine. However, hydrothermal synthesis is very time-consuming, takes many hours, and may not be suitable for the synthesis of CDs with red luminescence [3]. Solvothermal synthesis is similar to hydrothermal synthesis, but during it, special solvents, such as DMF, are used. This method is more universal, since solvents make it possible to adjust the synthesis, but the resulting particles will be in this solvent, and purification is required for biomedical purposes [4].

A microwave reactor is similar to a hydrothermal reactor, but uses gigahertz electromagnetic radiation for heating, which allows producing large quantities of particles much more quickly, and synthesis can take only a few minutes. In addition, this method makes it possible to obtain CNDs with high luminescence quantum yield [3]. CNDs can be obtained from a wide variety of materials—orange peel, citric acid, malic acid, and various carbon-containing compounds. The resulting CNDs are promising for applications in biomedicine, including delivery of drugs, genes, hyperthermia of tumors, and destruction of bacteria [4]. Separately, it is worth noting that such nanoobjects are well suited for bioimaging owing to their low toxicity compared to quantum dots based on heavy metals.

The goal of our study was obtaining in a microwave reactor and primary characterization of CNDs that are promising for biomedical applications.

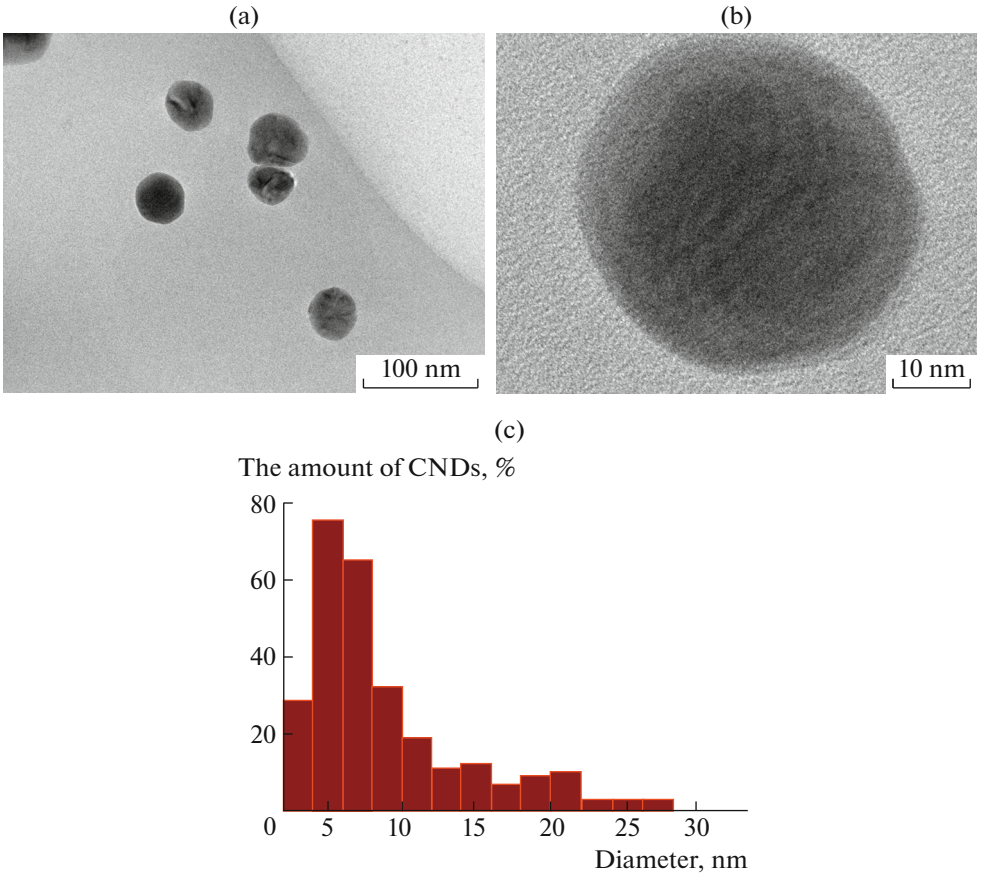

Fig. 1. (a, b) TEM images of CNDs; (c) histogram of size distribution of CNDs according to TEM data.

MATERIALS AND METHODS

CNDs were synthesized in an Anton Paar microwave reactor (Czech Republic) from 300 mg of urea and 150 mg of citric acid in 5 mL of distilled water in 10 mL glass vials. Reactor parameters: temperature of 180°C, synthesis time of 15 min, stirring speed of 1200 rpm, cooling after synthesis to 55°C. The resulting solutions were purified by adding isopropyl alcohol, followed by transferring the purified fractions into distilled water.

The structure, shape, and size of the particles were studied by high-resolution transmission electron microscopy (TEM) on a JEOL JEM-2100 instrument with resolution of 0.19 nm.

Extinction spectra were measured using a UV–VIS 752P spectrophotometer in the range of 200–900 nm with resolution of 3 nm.

Fourier transform infrared (FTIR) spectroscopy of aqueous solutions of CNDs was carried out on a Bruker IFS-66v/s instrument in the range of 500–4000 cm^{-1} and a step of 4 cm^{-1} in the geometry of multiply disrupted total internal reflection. Photoluminescence spectra upon excitation with wavelengths of 365, 405, and 532 nm were measured using a TP2000P OPTOSKY spectrometer in the range from 200 to 1100 nm with resolution of 1 nm.

The cytotoxicity of CNDs was assessed using the standard MTT method on the reduction of tetrazolium salts (Sigma, USA). Twenty-four hours after subculturing on 96-well plates, the test drugs in different solvents were added to the cells of the experimental group (6 replicates for each group). The drugs were dissolved in the culture medium at the concentration of 0.88 mg/mL and 0.45 mg/mL. Only a complete culture medium was added to control wells. Seventy-two hours after incubation with the studied drugs, cell proliferation was assessed morphologically at the qualitative level using an inverted microscope (Zeiss Axiovert, Germany). A cytotoxic MTT test reflecting the metabolic activity of cells was performed: 0.02 mL of 5% MTT reagent solution was added to the wells; after 2 h of incubation, the medium was removed from the wells and 0.2 mL of (Panreac, Spain) was added to each well. After complete dissolution of the formazan crystals, spectrophotometry of the samples was carried out on a microplate reader (Anthos 2010, Austria) at the wavelength of 495 nm. The viability was calculated using the following formula: % viable cells = De/Dc × 100%; where De is the optical density of the solution

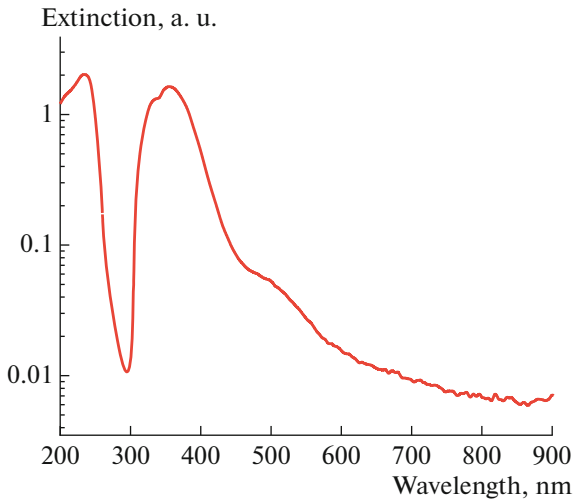

Fig. 2. Extinction spectrum of an aqueous solution of CNDs with a concentration of 0.1 mg/mL.

in the experimental group and Dc is the optical density of the solution in the control [5].

For the MTT test, transplantable cell lines of embryonic kidney HEK-293 and mouse glioblastoma M6 were used (collection of experimental tumors of the nervous system and neural tumor cell lines of the Academician A.P. Avtsyn Research Institute of Chemistry and Chemistry, Petrovskii Russian Scientific Center for Surgery, Russia, http://www.morfolhum.ru/unikalnaya-nauchnaya-ustanovka/), stored in an atmosphere of liquid nitrogen; they were thawed and subcultured first in plastic vials and then on 96-well plates in the amount of 10^4 cells per well and incubated for 24 h. Cells were cultured in Imdm gluta max medium (Gibco, USA) with or without 10% fetal calf serum (Hyclone, USA) (serum-free culture medium) and 0.1 mg/mL antibiotic penicillin + streptomycin (Gibco, USA) at t = 37.0°C, in an atmosphere of 5% carbon dioxide and 95% air.

Table 1. Percentage of metabolically active cells of embryonic kidney HEK-293 and glioblastoma M6 lines under the influence of the studied drugs and their combinations, Median (Q1; Q3), %

Observation groups	Cell lines	
	HEK-293	M6
Control	100 (82;106)	100 (94;102)
CD in complete culture medium (0.88 mg/mL)	93 (87;108)	90 (82;96)
CND in complete culture medium (0.45 mg/L)	95 (84;99)	100 (94;104)
CND in 1% F 68 solution in complete culture medium (0.65 mg/mL)	82 (77;87) $p < 0.046$	88 (74; 90) $p < 0.002$

In addition, CNDs in a pluronic solution, used to pass particles through the blood-brain barrier in the treatment of brain tumors, were added to glioblastoma cells to determine the cytotoxicity of this solution.

The experimental data were analyzed using the STATISTICA 8.1 program. The obtained indicators were compared using the nonparametric Kruskal–Wallis H test (comparison of multiple independent groups). Differences were considered significant at $p < 0.05$.

RESULTS

As a result of studying CNDs using transmission electron microscopy (Fig. 1a), a size distribution was constructed that is lognormal in nature (Fig. 1b). High-resolution TEM showed that the particles had an almost spherical shape and a layered internal structure (Fig. 1b).

The absorption bands of CNDs were determined from the extinction spectra when the resulting solutions were diluted to 0.1 mg/mL. The main absorption maxima correspond to wavelengths of 235, 360, and 510 nm (Fig. 2).

Chemical bonds in CNDs were studied using Fourier transform infrared spectroscopy (Fig. 3). Absorption bands were detected corresponding to the lines C–N (1400 cm^{-1}), C=O (1645 cm^{-1}), C–H (2811 cm^{-1}), and O–H/N–H (3360 cm^{-1}). According to [6], the presence of C–N bonds on the surface of carbon dots is the cause of the appearance of red luminescence.

The photoluminescence spectra upon laser excitation with the wavelengths of 365, 404, and 532 nm were broad bands with maxima at the wavelengths of 460, 490, and 600 nm, respectively.

The obtained results of in vitro studies indicate that CNDs with a concentration of 0.45–0.88 mg/mL do not have a statistically significant cytotoxic effect on mouse glioblastoma M6 cells and human embryonic kidney HEK 293 cells, but the addition of pluronic caused an increase in the cytotoxicity (Table 1).

DISCUSSION

Using the microwave synthesis method, CNDs were obtained whose optical absorption maxima were 250, 400, and 510 nm. The presence of an absorption band in the green region makes it possible to obtain effective luminescence with a maximum in the region of 610 nm, which, because of its proximity to the window of biological transparency of tissues, makes promising the use of such CNDs in biomedical optical diagnostics. Purification with isopropyl alcohol resulted in increased red luminescence.

The resulting CNDs had low cytotoxicity; no statistically significant effect on the proliferation of either kidney cells or tumor cells was recorded. This corresponds to published data; namely, low toxicity of

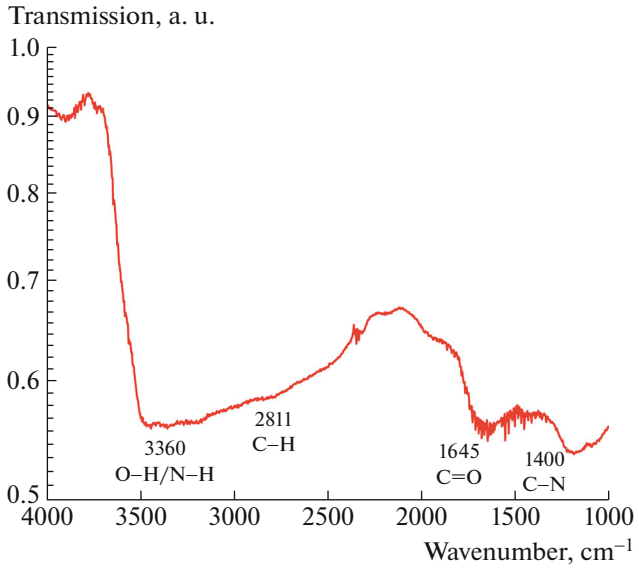

Fig. 3. IR transmission spectrum of an aqueous solution of CNDs.

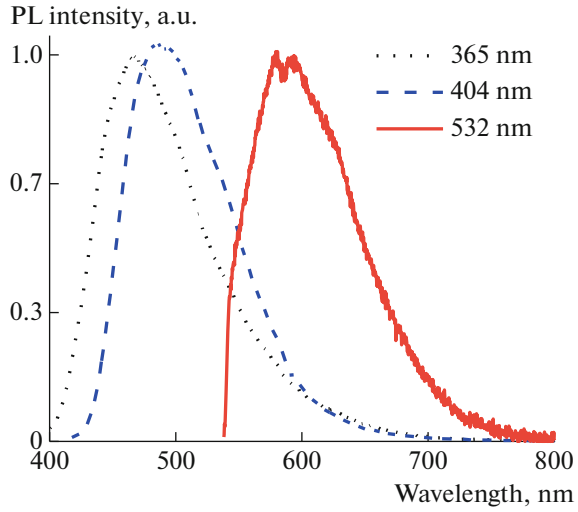

Fig. 4. Photoluminescence spectra of aqueous solutions of CNDs under laser excitation with the wavelengths of 365, 404 and 532 nm, which gave maxima at the wavelengths of 460, 490 and 600 nm, respectively.

CNDs makes them promising for biomedical applications, more attractive than the materials already used at the moment, for example, quantum dots based on heavy metals. The addition of pluronic F-68 to a CND solution increases cytotoxicity, despite the reduced concentration of CNDs in this solution, which is probably due to the possible toxicity of pluronic [7] or the process of CND endocytosis stimulated by this drug.

CONCLUSIONS

To summarize, one can say that carbon nanodots obtained from urea and citric acid by the express method of synthesis in a microwave reactor have low cytotoxicity and have intense luminescence in the red region of the spectrum, which makes them a promising nanomaterial for further study with the aim of creating nanoagents for luminescent bioimaging, as well as with further modification and functionalization for creating contrast agents for MRI and sensitizers of local hyperthermia of tumors.

FUNDING

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CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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