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GENERATION OF REACTIVE OXYGEN SPECIES BY MULTI-WALLED CARBON NANOTUBES UNDER LIGHT IRRADIATION

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Reactive oxygen species (ROS) were generated as a result of the photoexcitation of multi-walled carbon nanotubes (MWCNT) in water. ROS levels were detected using electron paramagnetic resonance spectroscopy and spin traps. Irradiation of MWCNT with visible and near infrared light in water was followed by the generation of superoxide radical anions. The effect of a self-induced ROS generation after photoirradiation of MWCNT has been investigated. Photoirradiated MWCNT may be useful for photodynamic therapy of tumors through ROS generation.

Key words: multi-walled carbon nanotubes, reactive oxygen species, light irradiation, electron paramagnetic resonance spectroscopy, photodynamic therapy of tumors.

The use of nontoxic and biocompatible materials in biology and medicine is fundamental for the development of biotechnology. Prospects in this direction are connected with the discovery of the new forms of carbon — fullerenes and nanotubes. Their unique physical and chemical properties [1, 2], intrinsical to these nanostructures on one side, and the biological activity both *in vitro* and *in vivo* on the other side, offer possibilities of their wide application in nanomedicine and nanopharmacy [3].

Apart from intensive studies on carbon nanotube (CNT) bioactivity, the received opinion on the mechanisms of their interaction with biomacromolecules (for example, with DNA) [4, 5], their abilities to penetrate through the membrane of cells and thus to be the carriers of medicines, on one side, and to be blockers of ion channels, on the other side is not well known today [6–8]. The biocompatibility and cytotoxicity of CNT, which are connected both with the effects of size, type, and functionalization of CNT as well as with the type of cells require still a detailed analysis [9–14].

It is known that CNT intensively absorb in the near infrared (NIR) light range ~(700–1100) nm, where the biological systems are transparent [3]. In this case IR irradiation leads to the destruction of malignant tumors as a result of the local heating of CNT *in vivo* [15, 16]. Another important consideration is the ability of CNT to generate the reactive oxygen species (ROS) such as singlet oxygen, superoxide anions and hydroxyl radicals as a result of the photoexcitation and the use of these nanomaterials as effective tool for the brake action of the cancer cells growth. So, in paper [17] it was studied the heat and ROS generation capabilities of single-walled CNT (SWCNT on Si(100) support; the purity and length of the SWCNT were 90 vol% and (5–20) мм, correspondingly) upon exposure to NIR light (the wavelength was 1 мм). The relative amount of ROS produced by SWCNT during NIR light irradiation has been measured indirectly using the X-ray diffraction analysis technique. Other scientists reported that multi-walled CNT (MWCNT) in aqueous suspension do not generate ROS in the presence of H_2O_2 [18], but they have observed that MWCNT exhibit a remarkable radical scavenging capacity in contact with an external source of hydroxyl or superoxide radicals [19].

The aim of this study was to evaluate in detail the conditions for the ROS generation, in particular, superoxide radical anions, using MWCNT in water by means of light irradiation with the use of methods of electron paramagnetic resonance (EPR) spectroscopy and spin traps.

Materials and methods

Chemical vapour deposition was used to produce MWCNT with high purity [12]. The synthesized MWCNT were analyzed by transmission (TEM, Fig. 1), scanning (SEM, Fig. 2) electron microscopy, and thermogravimetric analysis (TG).

From SEM and TG it was estimated that the content of MWCNT in the samples was higher than 90%. The average diameter of the MWCNT was estimated to be in a range from 8 to 12 nm.

The MWCNT contained impurities such as amorphous carbon, metal catalyst on the tip and outside of the tubes, and metal residue encased in the nanotube morphology as iron nanoparticles. The MWCNT samples were treated for 8 h with diluted HCl in order to remove impurities, mainly some metal parts outside on the tube, and afterwards they were washed several times with de-ionized water. It has been observed by HRTEM that the tubes still contain some iron clusters, which are completely capsulated by the inner tubes. The determination of the iron content by TG and atomic absorption spectroscopy revealed an iron content of less than 0.1 wt%. It is important to emphasize that metal catalyst impurities can be a powerful source for the ROS generation [20, 21].

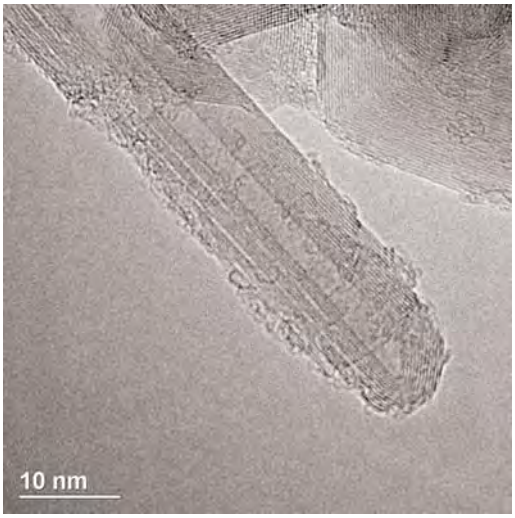

Fig. 1. **HRTEM (Philips Tecnai 20 S-TWIN HRTEM) micrograph of a typical MWCNT**

To produce a water suspension in a typical experiment, 100 mg of MWCNT was stirred in 50 ml of water under argon for several hours. Stirring was required to allow the solid MWCNT to go in the water solution. The mixture was then filtered through a membrane (pore size of 1.2 μm). The MWCNT were collected on the membrane. The filtrate has a brown color and contains small amounts of short CNT. The solubility of CNT in water is due to some surface carboxyl groups on the MWCNT [12]. Depending on the time of the water treatment the concentration of MWCNT in water was determined to be 0.01, 0.05 and 0.1 mg/ml, respectively.

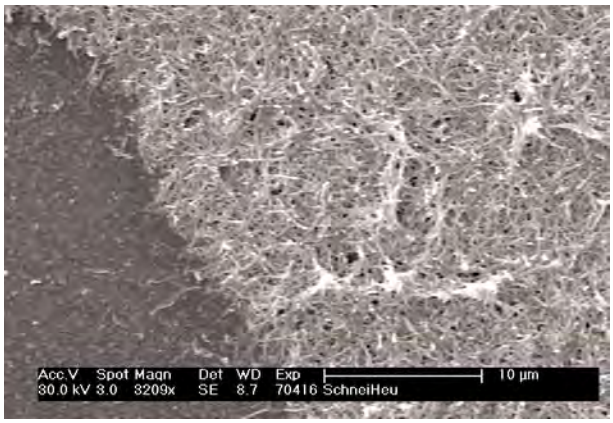

Fig. 2. **SEM (FEI XL30 LaB6 SE modus 30 kV) micrograph of a MWCNT deposit.**
The images reveal the presence of MWCNT inside the deposit. The calculated length of the CNT is (1–4) μm

In order to detect ROS the EPR and spin traps techniques were applied [22]. EPR spectra were recorded with a modified EPR spectrometer PE1307 operating in the X-band (9.15 GHz) at 100 kHz modulation frequency, 0.5 G modulation amplitude width and 40 mW microwave power. As spin harboring trap 1-hydroxy-2,2,6,6-tetramethyl-4-oxypiperedine at the concentration of $2–10^{-3}$ M was used. It possesses a high affinity to the superoxide radical anions, binds them, and turns into a nitroxyl radical that has been detected by EPR at room temperature. Its EPR spectrum reveals a triplet with the characteristics: $g = 2.005$, $A_N = 16$ G, $H_{pp} = 0.4$ G.

Irradiation of the MWCNT water suspension was carried out in a special quartz cuvette (V = 170 ml) using the «BIOPTRON» lamp (Switzerland) with a power of 80 W for 2 min. This lamp irradiates linearly polarized light with a wavelength of 400–2 000 nm. Immediately after light irradiation the EPR spectrum has been recorded.

Results and discussion

The EPR spectra of the superoxide radical anions generated by MWCNT in water (0.1 mg/ml) after their photoirradiation are given in Fig. 3.

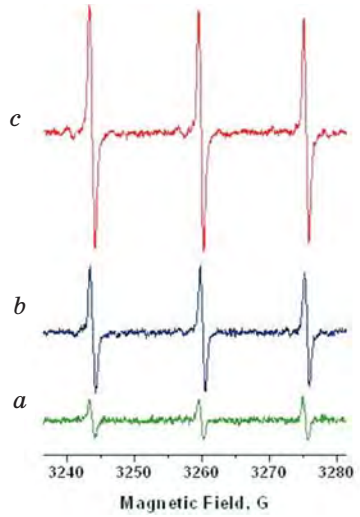

Fig. 3. **Dynamics of EPR spectra of the superoxide radical anions generated by MWCNT in water (0.1 mg/ml; T = 290 K):**
a — before photoirradiation; *b* — 2 min after finishing photoirradiation; *c* — 45 min after finishing photoirradiation

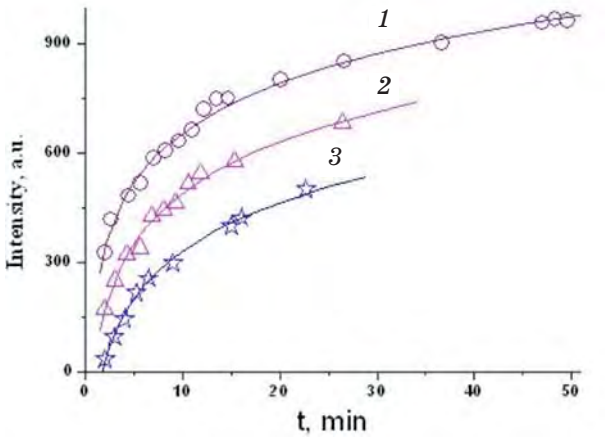

Fig. 4. **Time dependence of the concentration of the superoxide radical anions (reported as the EPR spectra intensity) generated by MWCNT in water after photoirradiation:**
1 — 0.1 mg/ml MWCNT; *2* — 0.05 mg/ml MWCNT; *3* — 0.01mg/ml MWCNT

As it can be seen, the intensity of EPR signals increases with time after finishing irradiation of MWCNT confirming therefore that the ROS concentration increases as well (Fig. 3, *b* and 3, *c*). We presume that these findings are closely related to intensive light absorption by MWCNT and a following conversion to local heat with time. Considering results [23], the light scattering of the investigated MWCNT and their aggregates (up to 20 nm in diameter) in the investigated irradiation range (400–2 000) nm does not exceed 10%. Therefore, if in the absence of MWCNT it is not possible to excite oxygen by incident radiation, then this cannot be achieved by the scattered field too. As consequence, the local heating of the investigated samples may be the reason for the ROS formation.

The increasing of the ROS concentration with time is demonstrated in Fig. 4.

Considering the obtained EPR results it was established that MWCNT generate ROS with the rate of (9.7±0.3) nMol/mg×min^{-1} independently on their concentration in water. Moreover, the MWCNT samples with the concentration 0.1 mg/ml (Fig. 4, *1*), 0.05 mg/ml (Fig. 4, *2*) and 0.01 mg/ml (Fig. 4, *3*) generate the ROS with the indicated rate during 47, 26 and 21 min, respectively, after their VIS/IR irradiation. In conclusion, a significant size's effect for MWCNT occurs, as a consequence of their aggregation in water with increasing concentration [12]. Indeed, as the number of nanotubes is increased (correspondingly, the total nanotube size/mass too), for a constant irradiation power, we expect that the heat efficiency (the range of transfer and duration) increases.

Thus, we established that MWCNT in water at low concentration (0.01–0.1 mg/ml) after short-term (2 min) VIS/IR irradiation (400–2 000 nm) can generate the ROS (superoxide radical anions) with the constant rate of (9.7±0.3) nMol/mg^{-1}·min^{-1}. Moreover, in dependence on their concentration in water, MWCNT can generate ROS with the indicated rate after finishing photoirradiation. Most likely, the local heating of investigated samples is the reason for the ROS formation.

In summary our results showed that MWCNT can be used in the nanomedecine for the regulation of the pathologic states, caused by the disturbances of the metabolism of the ROS, for example in the photodynamic therapy of malignant tumors S. V. P. is grateful to DFG for support.

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REFERENCES

1. *Dresselhaus M. S., Dresselhaus G., Eklund P. C.* Science of Fullerenes and Carbon Nanotubes: Their Properties and Applications. — Acad. Press: New York, 1996.
2. Nanomaterials Handbook / Gogotsi Yu (Ed). — CRC Press, 2006.
3. Medicinal Chemistry and Pharmacological Potential of Fullerenes and Carbon Nanotubes. Series: Carbon Materials: Chemistry and Physics. Cataldo F, Da Ros T (Eds.), Vol. 1. — Springer Netherlands, 2008.
4. *Dovbeshko G. I., Repnytska O. P., Obraztsova E. D., Shtogun Y. V.* DNA interaction with single-walled carbon nanotubes: a SEIRA study // Chem. Phys. Lett. — 2003. — V. 372. — P. 432–437.
5. *Gao H. J., Kong Y.* Simulation of DNA-nanotube interaction // Annu. Rev. Mater. Res. — 2004. — V. 34. — P. 123–150.
6. *Park K. H., Chhowalla M., Iqbal Z., Sesti F.* Single-walled carbon nanotubes are a new class of ion channel blockers // J. Biol. Chem. — 2003. — V. 278. — P. 50212–50216.
7. *Kam N. W. S., Liu Z., Dai H. J.* Functionalization of carbon nanotubes via cleavable disulfide bonds for efficient intracellular delivery of siRNA and potent gene silencing // J. Am. Chem. Soc. — 2005. — V. 127. — P. 12492–12493.
8. *Kostarelos K., Lacerda L., Pastorin G. et al.* Cellular uptake of functionalized carbon nanotubes is independent of functional group and cell type // Nat. Nanotechnol. — 2007. — V. 2. — P. 108–113.
9. *Casey A., Davoren M., Herzog E. et al.* Probing the interaction of single walled carbon nanotubes within cell culture medium as a precursor to toxicity testing // Carbon. — 2007. — V. 45. — P. 34–40.
10. *Grabinski C., Hussain S., Lafdi K. et al.* Effect of particle dimension on biocompatibility of carbon nanomaterials // Ibid. — 2007. — V. 45. — P. 2828–2835.
11. *Cui D.* Advances and prospects on biomolecules functionalized carbon nanotubes // J. Nanosci. Nanotech. — 2007. — V. 7. — P. 1298–1314.
12. *Prylutska S. V., Grynyuk I. I., Matyshevska O. P. et al.* Estimation of multi-walled carbon nanotubes toxicity *in vitro* // Physica E. — 2008. — V. 40. — P. 2565–2569.
13. *Prylutska S.V., Grynyuk I.I., Grebinyk S.M. et al.* Comparative study of biological action of fullerenes C_{60} and carbon nanotubes in thymus cells // Mat. Wiss. Werkstofftech. — 2009. — V. 40. — P. 238–241.
14. *Warheit D.B.* Long-term inhalation toxicity studies with multiwalled carbon nanotubes: Closing the gaps or initiating the debate? // Toxic. Sci. — 2009. — V. 112. — P. 273–275.
15. *Kam N. W. S., O'Connel M., Wisdom J. A., Dai H. J.* Carbon nanotubes as multifunctional biological transporters and near-infrared agents for selective cancer cell destruction // Proc. Natl. Acad. Sci. USA. — 2005. — V. 102. — P. 11600–11605.
16. *Torti S. V., Byrne F., Whelan O. et al.* Thermal ablation therapeutics based on CN_x multiwalled nanotubes // Int. J. Nanomed. — 2007. — V. 2. — P. 707–714.
17. *Lee Ch., Kim H., Cho Y., Lee W. I.* The properties of porous silicon as a therapeutic agent via the new photodynamic therapy // J. Mat. Chem. — 2007. — V. 17. — P. 2648–2653.
18. *Fenoglio I., Tomatis M., Lison D. et al.* Reactivity of carbon nanotubes: Free radical generation or scavenging activity? // Free Rad. Biol. Med. — 2006. — V. 40. — P. 1227–1233.
19. *Fenoglio I., Greco G., Tomatis M. et al.* Structural defects play a major role in the acute lung toxicity of multiwall carbon nanotubes: Physicochemical aspects // Chem. Res. Toxicol. — 2008. — V. 21. — P. 1690–1697.
20. *Manna S. K., Sarkar S., Barr J. et al.* Single-walled carbon nanotube induces oxidative stress and activates nuclear transcription factor-kappa B in human keratinocytes // Nano Lett. — 2005. — V. 5. — P. 1676–1684.
21. *Pulskamp K., Diabat S., Krug H. F.* Carbon nanotubes show no sign of acute toxicity but induce intracellular reactive oxygen species in dependence on contaminants // Toxicol. Lett. — 2007. — V. 168. — P. 58–74.
22. *Burlaka A. P., Sidorik E. P.* Reactive oxygen species and nitric oxide under tumorous process. — К.: Наук. думка, 2006.
23. *Burlaka A., Lukin S., Prylutska S. et al.* Hyperthermic effect of multi-walled carbon nanotubes stimulated with near infrared irradiation for anticancer therapy: *in vitro* studies // Exp. Oncol. — 2010. — V. 32. — P. 48–50.

ГЕНЕРАЦІЯ АКТИВНИХ ФОРМ КИСНЮ БАГАТОСТІННИМИ ВУГЛЕЦЕВИМИ НАНОТРУБКАМИ ПІД ДІЄЮ СВІТЛОВОГО ОПРОМІНЕННЯ

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З використанням методів електронного парамагнітного резонансу та спінових уловлювачів уперше зареєстровано активні форми кисню (АФК), зокрема супероксидні аніон-радикали, які генерувались опроміненими у видимій і близькій ІЧ-ділянках світла багатостінними вуглецевими нанотрубками (БВНТ), що містились у водній суспензії. Було виявлено ефект самоіндукованої генерації АФК після припинення фотоопромінення БВНТ. Отримані результати свідчать про можливість застосування фотозбуджених БВНТ у фотодинамічній терапії злоякісних пухлин.

Ключові слова: багатостінні вуглецеві нанотрубки, активні форми кисню, світлове опромінення, електронний парамагнітний резонанс, фотодинамічна терапія злоякісних пухлин.

ГЕНЕРАЦИЯ АКТИВНЫХ ФОРМ КИСЛОРОДА МНОГОСЛОЙНЫМИ УГЛЕРОДНЫМИ НАНОТРУБКАМИ ПОД ВОЗДЕЙСТВИЕМ СВЕТОВОГО ОБЛУЧЕНИЯ

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С использованием методов электронного парамагнитного резонанса и спиновых улавливателей впервые зарегистрированы активные формы кислорода (АФК), в частности супероксидные анион-радикалы, которые генерировались облученнными в видимой и ближней ИК-областях света многослойными углеродными нанотрубками (МУНТ), находящимися в водной суспензии. Был обнаружен эффект самоиндуцированной генерации АФК после прекращения фотооблучения МУНТ. Полученные результаты свидетельствуют о возможности использования фотовозбужденнных МУНТ в фотодинамической терапии злокачественных опухолей.

Ключевые слова: многослойные углеродные нанотрубки, активные формы кислорода, световое облучение, электронный парамагнитный резонанс, фотодинамическая терапия злокачественных опухолей.