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SHORT COMMUNICATIONS

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HYPERTHERMIC EFFECT OF MULTI-WALLED CARBON NANOTUBES STIMULATED WITH NEAR INFRARED IRRADIATION FOR ANTICANCER THERAPY: *IN VITRO* STUDIES

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It is proposed to use the novel paradigm of treating cancer with hyperthermic therapy using multi-walled carbon nanotubes (MWCNT) stimulated with near infrared (NIR) irradiation. *Aim:* To establish the capacity of MWCNT stimulated with NIR irradiation to destroy Erlich ascitic carcinoma (EAC) cells. *Materials and Methods:* EAC cells suspension was irradiated with NIR heating lamp with a wavelength of 0.78–1.40 mm and power density of 3.5 W/cm2 over 1.5 min in the presence of MWCNT (0.1 mg/ml). The changes in the temperature of suspension with the NIR exposure time was measured using the differential cooper-constantan thermocouple. The viability of EAC cells was evaluated by trypan blue staining. *Results:* The death of 95.2% of EAC cells in the presence of MWCNT was observed after 1.5 min of NIR light irradiation: thermal ablation temperature was ~50 °C. *Conclusions:* It was demonstrated that addition of MWCNT to EAC cell suspension results in the photo-ablative destruction of cells exposed to short time NIR irradiation.
Key Words: **multi-walled carbon nanotubes, NIR irradiation, Erlich ascitic carcinoma cells, hyperthermic therapy.**

Carbon nanotubes (CNT) have special electrical, optical and thermal properties caused by the arrangement of the carbon atoms confined in nanometer sized volumes [1]. Due to their shape (high aspect ratio since the tube diameter is much smaller than tube length), CNT can influence the electric field in their localized area, which enhances absorption of electromagnetic energy and generates rapid heating of the tube [2–3]. CNT have been used for thermal ablation [4–6]. However, the multi-walled CNT (MWCNT) behave as highly efficient dipole antennae with broad absorption spectra compared with the specific resonance absorptions of single-walled CNT (SWCNT), rendering them amenable to stimulation by a range of near infrared (NIR) energy sources. Additionally, MWCNT as the metallic tubes can be expected to absorb significantly more NIR irradiation compared with materials such as SWCNT, because, per weight, SWCNT contain both metallic and semiconducting ones.

Hyperthermia, defined as temperatures above 40 °C, is used clinically to treat a variety of malignancies [6–8]. Irreversible cell damage is incurred for temperatures above 45 °C. Therefore, clinical intraperitoneal hyperthermic therapy procedures use mild hyperthermia between 40 °C and 42 °C for peritoneal perfusion [7–8]. Thus, the novel paradigm of treating cancer with hyperthermic therapy using MWCNT stimulated with NIR irradiation [6, 9] was considered.

Chemical vapour deposition was used to produce MWCNT with high purity [10]. The synthesized MWCNT were analyzed by transmission and scanning electron microscopy (SEM) (Fig. 1), 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.

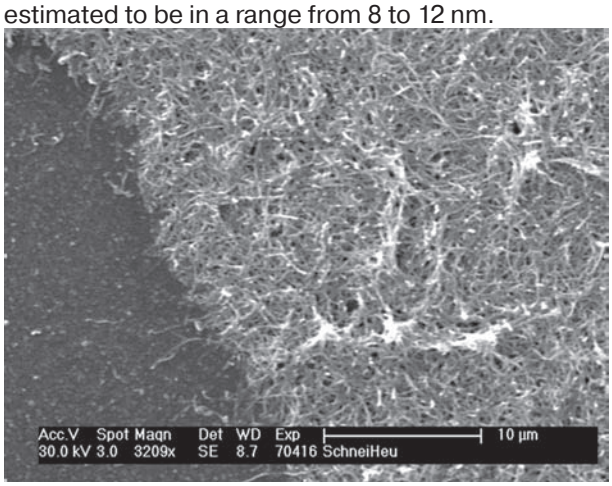

Fig. 1. SEM (FEI XL30 LaB6 SE modus 30 kV) micrograph of MWCNT deposit. The images reveal the presence of MWCNT inside the deposit. The calculated length of the MWCNT is (1–4) µm

To produce a water suspension of MWCNT 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

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Abbreviations used: CNT – carbon nanotubes; EAC – Erlich ascitic carcinoma; MWCNT – multi-walled carbon nanotubes; NIR – near infrared; SEM – scanning electron microscopy; SWCNT – single-walled CNT; TG – thermogravimetric analysis.

of 1.2 μm). The MWCNT were collected on the membrane. The filtrate has a brown color and contains small amounts of short CNT. CNT are soluble in water due to some surface carboxyl groups on the MWCNT [10]. The maximum concentration of MWCNT in water was determined to be 0.1 mg/ml. It is important to note that under this and less concentration of MWCNT in water they are not toxic for normal and/or transformed cells [10–11].

In our experiments the outbreed mice (with body weight of 20 g), maintained on standard chow diet, were used. All experiments were carried out according to the rules of local Ethic Committee.

On day 8–12th after intraperitoneal transplantation of Erlich ascitic carcinoma (EAC) to animals (1 x 10^6 cell/animal), the cancer cells were isolated. EAC cells were washed off the ascitic fluid and resuspended in RPMI 1640 medium with 8 mM $NaHCO_3$, 20 mM HEPES. EAC cells (1–2 x 10^5/ml) without additives and in the presence of MWCNT (0.1 mg/ml) were incubated for 12 h at 37 °C to irradiation. The number of viable cells was counted after staining with 0.4% trypan blue solution. The number of EAC viable cells without additives and in the presence of MWCNT without irradiation after 12 h of incubation was 100% and used as a control.

Irradiation of the MWCNT water suspension without and with EAC cells was carried out in a quartz cuvette (V = 1.5 ml) using the NIR heating lamp (IF-9900 Gold, Hasell CO, USA) with power density of 3.5 W/cm^2 for 2 min. This lamp irradiates light with a wavelength of (0.78–1.4) μm, which is transparent to the biological systems. Changes in the temperature of samples with the NIR exposure time was measured at 30 s intervals by using the differential cooper-constantan thermocouple to within ± 0.1 °C.

Fig. 2 shows the temperature of MWCNT water suspension without (curve 1) and with EAC cells (curve 2) as function of NIR light exposure time. It was observed that NIR irradiation of MWCNT water suspension (MWCNT concentration 0.1 mg/ml) by 3.5 W/cm^2 λ = (0.78–1.4) μm NIR lamp continuously for 2 min caused heating of the aqueous suspension to ~70 °C (Fig. 2, curve 1). Thus, optically stimulated electronic excitations of MWCNT rapidly transferred to molecular vibration energies and caused heating: the temperature of MWCNT increases highly and quickly with an increase in the NIR irradiation time (linear rate ~0.4 °C/s). NIR irradiation of EAC cells suspension in the absence of MWCNT did not affect the number of viable cells. The decrease of viable EAC cells number in the presence of MWCNT without irradiation was not observed. Thus, cells have a high transparency to NIR light, and neither exposure to NIR nor exposure to MWCNT alone is sufficient to induce cell death.

Varying NIR lamp exposure time (range: 1–1.5 min) revealed that the thermal ablation temperature threshold (~39–50 °C) of EAC cells suspension with MWCNT (0.1 mg/ml) could be reached (Fig. 2, curve 2) and as a result the hyperthermia effect on cancer cells was observed. For EAC cells with internalized inside MWCNT (mainly in the cytoplasm and intracellular vesicles) [12–13], which act as tiny NIR heaters/antennas (see Appendix), extensive cell death (95.2 ± 4.8%) was observed after 1.5 min irradiation under a 3.5 W/cm^2 power.

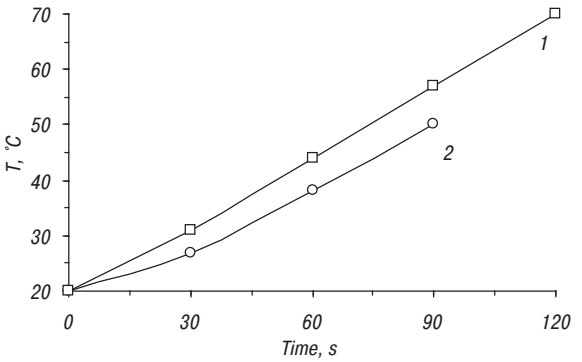

Fig. 2. The temperature of MWCNT aqueous suspension without (curve 1) and with EAC cells (curve 2) as function of NIR light exposure time

In summary, the ability of MWCNT to induce temperature increases compatible with thermal ablation at low concentration, and short NIR light exposure time suggests that MWCNT may be useful as photothermal mediators.

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APPENDIX

We consider a model of MWCNT as a cylindrical structure comprising *N* coaxial infinitesimally thin shells. We enumerate the shells in the MWCNT consecutively from 1 to *N*, beginning from the innermost shell, so that their cross-sectional radii comply with the condition $R_N > R_{N-1} > R_1$. The cross-sectional radius R_N of the outermost shell is assumed to be much smaller than the free-space wavelength λ. Accordingly the model proposed in [14], in the NIR regime an electrically thin ($R_N < \lambda$) MWCNT may be modeled as a thin homogeneous cylinder with the effective conductance per unit length given by

$$\sigma_T = \sum_{p=1}^{N} (2\pi R_p \sigma_p), \qquad (1)$$

where σ_P is the axial surface conductivity of the *p*-th shell, $p \in [1,N]$. Following [12], the conductivity σ_P is assumed to be the same as for a SWCNT with identical geometrical parameters. The effective conductance σ_T practically does not depend on the chirality of shells for MWCNT with the diameter larger than 5 nm [15].

The scattering efficiency of a MWCNT of a finite length *L* is determined by the formula

$$\eta = P_r / (P_r + P_t), \qquad (2)$$

where

$$Pr = \frac{\omega^2 |\sigma_T|^2 \pi}{4c^3} \int_0 \sin^3\theta \left| \int_{-L/2}^{L/2} e^{ikz\cos\theta} E_z^{(0)}(z) \right|^2 d\theta \quad (3)$$

is the scattered power, and

$$P_t = \frac{1}{2} Re(\sigma_T) \int_0^{\pi} \sin^3\theta \left| E_z^{(0)}(z) \right|^2 dz \quad (4)$$

is the power loss due to Ohmic dissipation. The quantity $E_z^{(0)}$ is the z component of the incident electric field, and θ is the angle with respect to z axis. The later coincides with the nanotube axis.

In the NIR regime the real part of σ_T increases with the frequency increase, that leads to the growth of the scattering efficiency. Table gives the ranges of variation of the scattering efficiencies for MWCNT with different length L and diameter D in the investigated spectral range λ = (1.4–0.78) μm.

Table. Scattering efficiency (%) for MWCNT with different length L (μm; rows), diameter D (nm; columns) and number of shells N (columns) in the spectral range λ = (1.40–0.78) mm

Scattering efficiency,%	D = 8 (N = 12)	D = 12 (N = 17)	D = 20 (N = 28)
L = I	0.30–0.70	0.70–1.5	1.2–3.5
L = 2	0.40–0.76	0.87–1.7	2.0–3.8
L = 4	0.43–0.80	0.93–1.7	2.2–4.0

From Table one can conclude that the largest scattering efficiency corresponds to MWCNT of the largest diameter and maximal length at the smallest wavelength of the investigated range and does not exceed 4%. Thus, MWCNT intensively absorb in the above NIR light range and can be lead to the destruction of malignant tumors as a result of the local heating.

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