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# Magnetofullerenosome Mediated Near-Infrared Laser Therapy of Hamster Tongue Carcinoma

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**Abstract:** This study was performed to demonstrate the usefulness of magnetofullerenosomes- a lipidic nanostructures with incorporated fullerenes $C_{60}$ in their lipid bilayers and co-encapsulated photosensitizer naphthalocyanate and magnetite nanoparticles for the oral cancer combined hyperthermia and photodynamic therapy. Tumors were induced in golden hamster tongue by 9,10-dimethyl 1-1,2-benzanthracene application. Magnetofullerenosome suspension was locally injected into the tumor-bearing tongue and the tongues were irradiated with near-infrared laser light leading to tumor heating, and photosensitizer release combined with its activation. The inhibition of the growth of tongue carcinoma group was significantly greater than in the control group. These results strongly suggest the usefulness of this novel multifunctional approach to the cancer therapy of oral region that is accessible to this treatment.

## 1. Introduction

Conventional surgical treatment of solid tumors is an effective therapy for the removal of well defined, accessible, primary tumors located within nonvital tissue regions. However, the high morbidity and invasive nature associated with surgical resection renders this therapy unsuitable for treatment of small, poorly defined metastases or other tumors embedded within vital regions. Another alternative of cancer therapy is the photodynamic therapy (PDT) which is a treatment that is based upon the differential uptake by cancerous cells of photosensitizing agents, followed by irradiation of the cells to cause a photochemical reaction generating chemically disruptive species, such as singlet oxygen [1]. These disruptive species in turn injure the cells through reaction with cell parts e.g. cellular and nuclear membranes.

Many of the pharmacological properties of conventional "free" drugs can be improved through the use of drug delivery systems. Recently we have developed fullerenosomes [2], a novel nanosystem prepared by incorporation of $C_{60}$ fullerenes into lipid bilayers of phosphatidylcholine liposomes. As we have shown [3–5], single nanosecond near-infrared laser pulse energy absorbed by $C_{60}$ leads to explosive release of encapsulated material. Our aim in this study is to show that magnetically responsive photosensitized fullerenosomes represent a novel multifunctional approach to the cancer therapy of oral region that is accessible to this treatment. This therapeutic strategy also exploits the naturally occurring deficit of NIR-absorbing chromophores in most tissues, permitting trans-

mission of NIR light (700–1,000 nm) through tissue with scattering-limited attenuation and minimal heating. Light within this spectral region has been shown to penetrate tissue at depths beyond 1 cm with no observable damage to the intervening tissue [6].

## 2. Material and Methods

### 2.1. Magnetofullerenosome Preparation

35 mg of dipalmitoyl-phosphatidylcholine and 2 mol % of distearylphosphatidyl-ethanolamine-PEG-2000 (Sigma, St. Louis, USA) were dissolved in 10 ml of a diethyl ether and chloroform mixture (1:1, v/v) in a rounded bottom flask. Required amounts of fullerene $C_{60}$ (kindly supplied by Dr. H. Michnik) were added to this mixture. Finally Bis(di-iso-butyloctadecylsiloxy)-2,3-naphthalocyanato silicon (isoBO-SiNc) prepared by chemical synthesis [7] and dextran magnetite were added to the flask and emulsified in a Labsonic 2000 sonicator bath (Branson Ultrasonics, Danbury, UK) at 100 W for 5 min at 45 C. The emulsion was then evaporated at 45 Cunder a reduced pressure (30 mm Hg) in a rotary evaporator [8] until an opaque suspension of magnetofullerenosomes with co-encapsulated isoBO-SiNc and dextran magnetite had formed. Magnetofullerenosomes were separated from non-encapsulated $C_{60}$, dextran magnetite, and isoBO-SiNc by centrifugation (at 10,000g) and resuspended in phosphate buffered saline (PBS).

### 2.2. Treatment Protocols

6-week-old male golden hamsters were used as experimental models. During the study, the animals were given pellets and tap water ad libitum. The carcinogenic treatment was performed by the method of Salley [9]. The treatment was started at 8 weeks of age. The mucosa of the right edge of the tongue was abraded with a dental cleanser three times a week, and a 0.75 % 9,10-dimethyl-1,2-benzanthracene acetone solution (Sigma, USA) was applied with a drawing brush. The carcinogenic treatment was continued until a cancer was grossly confirmed (20–25 weeks), and the animals were used in the experiments when the long diameter of the tumor reached 8 mm or more. This kind of oral cancer develops from the normal covering epithelium through the stages of epithelial hyperplasia and epithelial dysplasia and that this process closely resembles the carcinogenic process of human oral squamous cell carcinoma from a precancerous lesion.

For each group the effectiveness of the treatment was evaluated by comparing the rate of tumor growth. Tumors were measured daily by a calliper assuming a hemi ellipsoidal structure for the tumor nodule and measuring the two perpendicular axes $D_1$ and $D_2$ and the height $D_3$. Individual tumor volumes were calculated according to formula:

$$V = \quad /6(D_1 \quad D_2 \quad D_3).$$

Magnetofullerenosomes (100 μl) were infused interstitially into the tumor centre for 2–3 min using a 30G needle and a dental aesthetic cartridge after the blood flow of the tumor tissue was blocked temporarily by wrapping with a silk and he blood flow was restored immediately after infusion. Control tumor sites received a saline infusion. 10 min after infusion the

tongue was pulled and tumors were exposed to near-infrared laser light (820 nm, 4 W/cm$^2$, 5 mm spot diameter). Temperature was monitored using IR-thermometer.

## 3. Results and Discussion

The tumor volume in the control group (10 animals) was $167 \pm 56$ m$^3$ (mean ± SD) before treatment and $772 \pm 145$ mm$^3$ after treatment (after 100 days), showing almost five-fold increase. On the contrary, the tumor volume in the infrared laser irradiated group (10 animals) was $178 \pm 64$ mm$^3$ before treatment and $324 \pm 101$ mm$^3$ after treatment indicating strong anti-tumor effect. Significant differences were observed in treated group after treatment compared with the control group by Student's *t*-test ($P < 0.01$). Three tumor-bearing animals which belonged to the laser irradiated group, were alive on day 250 of the study, when the observation ended. These our first results demonstrated usefulness of nanotechnology for cancer therapy.

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