Enhanced Cell Electro-permeabilisation Combined with Phototherapy to Target-Killing Cancer Cells by Using Multi Functional Carbon Nanotubes

Pei-Chi Lee[*], Ming-Jium Shieh[**]

[*]Institute of Biomedical Engineering, College of Medicine and College of Engineering, National Taiwan University, No. 1, Section 1, Jen-Ai Road, Taipei 100, Taiwan, d98548001@ntu.edu.tw
[**]Institute of Biomedical Engineering, College of Medicine and College of Engineering, National Taiwan University, No. 1, Section 1, Jen-Ai Road, Taipei 100, Taiwan, soloman@ntu.edu.tw

ABSTRACT

Carbon nanotubes (CNTs) have been proposed and actively explored as multifunctional innovative carriers for drug delivery. This study presents a new modality for cell electro-permeabilisation based on the use of CNTs and external static electric fields to achieve highly drug delivery efficiency. Besides, CNTs are unique materials that absorb infrared (IR) radiation in the special spectral window where body tissues are most transparent. Absorbed IR promotes molecular oscillation leading to efficient heating of the surrounding environment. By using these characteristics of CNTs, we conjugated photosensitizers on chemically functionalized single-wall carbon nanotubes (f-SWNTs) to combine photodynamic therapy and hyperthermia for killing cancer cells specifically and effectively. Thus, CNT complex drug delivery is promising for improving the efficacy of drug delivery and treatment to decrease side effects for future cancer therapy.

Keywords: *carbon nanotubes*, *electro-permeabilisation*, *photodynamic therapy*, *hyperthermia*

1 INTRODUCTION

Rencently, SWNTs become the novel drug carriers in tumor targeted drug delivery systems have already been developed by several investigators. Owing to their several unique properties, such as electronic properties, strong opitical absorbance and thermal conductivity. We tried to comebine all of their physical properties to applied to electroporation and phototherapy, *i.e.*, SWNTs can not just only to be the carriers of drug but can also enhance the drug delivery efficiency and to be possessed of the ability to kill the cancer.

According to their high aspect ratio since the tube diameter is much smaller than tube length, they have virtue of the electric field enhancement at their tips. An electro-permeabilisation method based on CNTs has been reported for electroporation[1]. This technique exploits the metallic behaviour of CNTs and specifically the "lightening rod effect" of CNTs to create localized high-field regions at the CNT tips. [2] [3]

VNc is a promising photosensitizer for photothermal therapy because of its high optical absorption coefficient at 808nm. CNTs can also absorb light in the near-infrared region(700~1100nm), and can cause cell death by a localized photothermal effect[4] [5]. We loaded VNc onto SWNTs to make SWNTs effective for passive delivery.

2 MATERIALS AND METHODS

2.1 Synthesis of SWNT

Oxidative Shortening of SWNT 100 mg of pristine SWNT of a mixture of concentrated H_2SO_4/HNO_3 (3/1, v/v) and stirred for 24 hours. The resultant suspension was then diluted with 250 mL of water, and the SWMTs were collected on a 100-nm-pore membrane filter and washed with deionized water. The obtained SWNTs were further redispersed in water, centrifuged at 10000*g* for 20min to remove the water immiscible carbon nanotubes or other residuals, lyophilized to dryness at room temperature.

oxSWNT-PEG The resulting particles were dissolved in water and sonicated for 3 h. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysulfosuccinimide (Sulfo-NHS) were added to the nanotubes. This was followed by treatment with poly(ethylene glycol) bis(3-aminopropyl) terminated (Mn ~1500) and 72 h room temperature stirring.

oxSWNT-PEG-VNc oxSWNT-PEG (10mg) were dissolved in DMF and sonicated for 1 h. The VNc was added to it and the resulting blackish green solution was further sonicated for 30 min. This was followed by room temperature stirring for 48 h.The solid was washed with ethanol with repeated centrifugation until the solution was free of any green color. The resulting oxSWNT-PEG-VNc was freeze-dried.

2.2 Cell culture

Both HT29 human colorectal cancer cells and Hela cells was maintained in a humidified 5% CO_2 incubator at 37 ℃ in DMEM (GIBCO BRL, Gaithersburg, MD, USA) supplemented with 10% heat-activated fetal bovine serum (FBS) and 1% antibiotics (Antibiotic–Antimycotic).

2.3 Electroporation

We used the pipet-type electroporator (MP-100, Invitrogen) and Neon™ 100µl kit (Invitrogen) for cell permeabilisation was as follows. Cells were seeded on T75 flask in the culture medium for 1 day before the assay. For cell permeabilisation in

100µl, cells were trypsinised, centrifuged for 5 min at 1500rpm and re-suspended in pulsing medium at a concentration of 10^6cells/ml. The pulsing medium was PBS added with CNT solution (0.01% of Pluronic F127 and 20 µg/ml of oxSWNT-PEG-VNc). Voltage pulses were 50-1300 V, the pulse duration was 20-40 ms, the number of pulses was 2-100. After EP, the cell were pipetting with medium and seeding to 24 well. After the cells incubated for 24h, we used laser to irradiate the cells.

2.4 Phototherapy *in Vitro*

After the cells incubated for 24h, the culture medium was replaced by only DMEM. A laser with a wavelength of 808 nm (power, 1.3 W) was used to irradiate the cellls for 3min. After irradiation, we incubated the cells for 3h. The cell viability was estimated by using an MTT assay.

3 RESULTS

3.1 Cell viability of HT29 cells when expose to different voltage of EP

We used PBS added with CNT solution as pulsing midium compared with control (pulsing midium only with PBS) at different voltage. After EP, the cell were pipetting with medium and seeding to 24 well. After the cells incubated for 24h, the cell viability was estimated by using an MTT assay. High voltage obviously decreased cell viability to 71.70% and 80.12%.

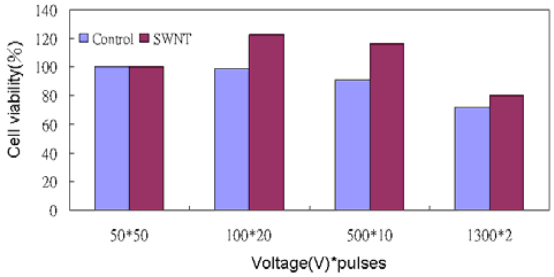

Figure 1: Electroporation treatment of HT29 cells. The viability of HT29 cells was estimated by using the MTT assay. The viabilities of the cells with and without CNT as pulsing midium at different pulsing voltage were indicated as blue and purple bars, respectively. The pulse duration was 40ms.

3.2 Intracellular uptake

In order to decrease the pulse voltage by using CNT as nanoelectrode arround the cell environment to increse cell viability, we compared pulsing medium with and without CNT. At high voltage 1005V, CNT electrode seemed no obvious effect of cellular uptake(Fig 2c and d), but at the lower voltage 50V, the image showed the red signals by using pulsing medium with CNT(Fig 2b) was much more than just using PBS(Fig 2a). We determined that we can low the pulsie voltage by using CNT as a nanoelectrode to

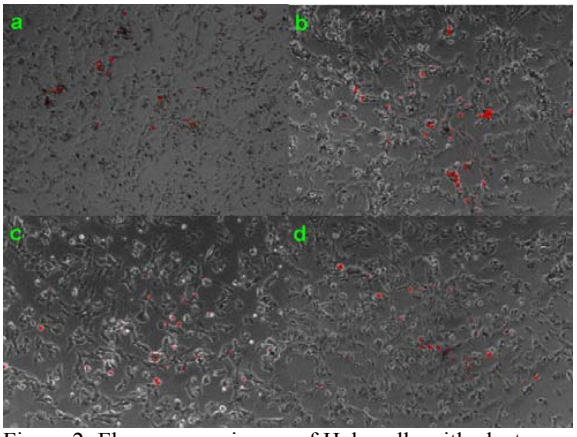

Figure 2: Fluorescence image of Hela cells with electroporation treatment after 24h. (a)(c) Pulsing medium with only PBS. After EP, cells were seeding into the DMEM mixed with CNT soluion(0.01% of Pluronic F127 and 20 µg/ml of oxSWNT-PEG-VNc). (b)(d) Pulsing medium:PBS with CNT soluion. (a)(b)EP parameter: 50V*40ms*100. (c)(d) EP parameter: 1005V*35ms*2 (voltage*duration*number).

enhance the celluar uptake of the drug (Fig 3). The flow cytometry measurements (Fig. 3) revealed that 92.39% of the cells took up oxSWNT-PEG-VNc with EP by using pulse voltage at 50V.

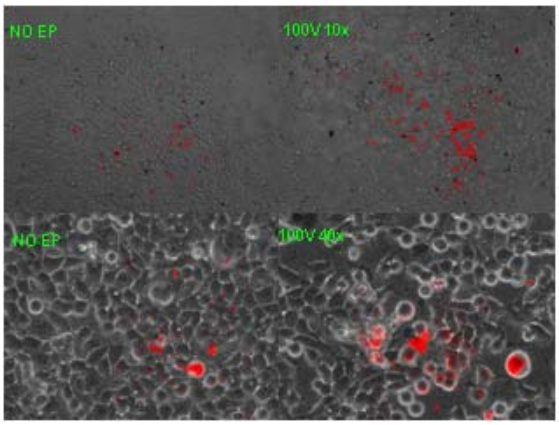

Figure 3: Fluorescence image of HT29 cells with electroporation treatment after 24h. All pulsing medium for EP was PBS with CNT solution.

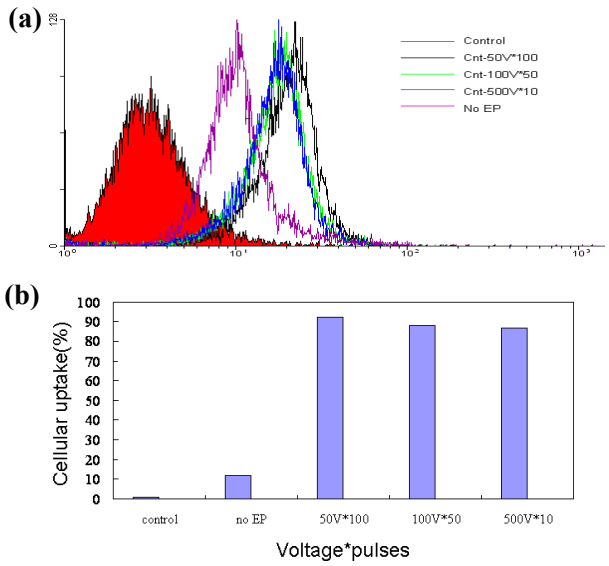

Figure 4: Flow cytometry of the HT29 cells with electroporation treatment after 24h. Pulsing medium was PBS with CNT solution. (a) black line with red area, control; solid black line, 50V*100(voltage*pulse number); green line, 100V*50; blue line, 500V*10; purple line, no EP. The Pulse duration was 40ms. (b) Quantitate of flow cytometry. control: 0.87%, no EP: 11.59%, 50V*100: 92.39%, 100V*50: 87.73%, 500V*10: 86.43%.

4 CONCLUSIONS

We develop a oxSWNT-PEG-VNc double electroporation and phototherapy system using the unique characteristics of SWNT. SWNTs showed the promising ability to be a nanoelectrode for using in electroporation treatment to enhance the drug delivery efficiency.

REFERENCES

[1]Michael Stacey, Christopher Osgood, Bhargava Subhash Kalluri1, Wei Cao, Hani Elsayed-Ali and Tarek Abdel-Fattah, "Nanosecond pulse electrical fields used in conjunction with multi-wall carbon nanotubes as a potential tumor treatment", Biomed. Mater. 6 (2011) 011002

[2] D. Cai, D. Blai, F.J. Dufort, M.R. Gumina, Z. Huang, G. Hong, D. Wagner, D. Canahan, K.Kempa, Z.F. Ren, T.C. Chiles, "Interaction between carbon nanotubes and mammaliancells characterization byflowcytometry and application", Nanotechnology 19 (2008)345102

[3] V.Raffa, G. Ciofani, O. Vittorio, V. Pensabene, A. Cuschieri, " Carbon nanotube-enhanced cell electropermeabilisation ", *Bioelectrochemistry*, (2009)

[4] Minfang Zhang, Tatsuya Murakami, Kumiko Ajima, Kunihiro Tsuchida, Atula S. D. Sandanayaka, Osamu Ito, Sumio Iijima, and Masako Yudasaka, "Fabrication of ZnPc/protein nanohorns for double photodynamic and hyperthermic cancer phototherapy", PNAS, September 30, 2008, vol.105, no.39, 14773–14778

[5] Andrew Burke, Xuanfeng Ding, Ravi Singha, Robert A. Kraft, Nicole Levi-Polyachenko, Marissa Nichole Rylander,Chris Szot, Cara Buchanan, Jon Whitney, Jessica Fisher, Heather C. Hatcher, Ralph D'Agostino, Jr., Nancy D. Kock,P. M. Ajayan, David L. Carroll, Steven Akman, Frank M. Tortia,f, and Suzy V. Torti, "Long-term survival following a single treatment of kidney tumors with multiwalled carbon nanotubes and near-infrared radiation", PNAS August 4, 2009,vol.106 . no. 31,12897–12902