Effect of Carbon Nanotubes on Chinese Hamster Ovarian Cells

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ABSTRACT

We investigated the effect of various concentration of PBS buffered single-walled carbon nanotubes’ (SWNTs) solutions on Chinese Hamster Ovarian (CHO) cells at multiple time points. Optical imaging on the CHO cell morphology showed that SWNTs accumulate in various parts of the cells, and the corresponding cell morphology provided qualitative information about the state of the cells’ health. Cell-Titer-Glo Luminescence measures ATP level to give quantitative measurement of the percentage of cell survival at various SWNTs concentrations. Our results was consistent with previous findings on the acute toxicity of high concentration SWNTs. At low SWNTs concentration (0.05 mg/ml) our data showed a dramatic decrease in the cell survival after longer (2 day) SWNTs exposure. The toxicity of SWNTs even at low concentrations suggests caution in in-vivo use of SWNTs.

Keywords: carbon nanotubes, ovarian cells, toxicity, biocompatibility

1 INTRODUCTION

Carbon nanotube is a cage structure made of all carbon atoms arranged in honeycomb structure [2]. The diameter of a carbon nanotube is only a few atoms wide, which is similar to that of the DNA. This small size allows the carbon nanotube the capability to enter the cell and possibly the nucleus. Furthermore, carbon nanotubes can be functionalized with biomolecules that have specific binding with their targeted counterparts [3-6]. These traits make carbon nanotubes a promising vehicle for targeted drug delivery even at extremely small doses.

Research on applying the carbon nanotubes for ultrasensitive detection, disease diagnosis, and drug delivery is rapidly developing [1, 3-12]. While the fundamental and technological findings on carbon nanotubes show great promise, the effect of the carbon nanotubes on environment and health remains controversial [1-2, 7, 12-17]. Wide biodistribution of SWNT is well established thus the importance of studying toxicity in detail. While acute toxicity was well documented in literature, low dose toxicity is not yet well understood. We hypothesize that in an in vivo context, systemic SWNT is rapidly washed out (in the order of minutes to hours) but very low dose of SWNT remains in the serum and accumulates throughout the body. We proposed that an in vitro cell model is well suited to determine the impact of these low SWNT concentration on cell survival.

We aim to study the effect of carbon nanotubes at the cellular level using purified single-walled carbon nanotubes (SWNTs) resuspended in physiological compatible buffer such as phosphate buffered saline (PBS) and systematically study its impact on Chinese Hamster Ovarian (CHO) cells. Our experimental design takes the advantage of: (1) The ease of visualizing the SWNTs intake in real-time. (2) The well characterized morphological changes associated with cellular stress. (3) Serial quantification of cell survival with highly sensitive bioluminescence-based imaging.

2 MATERIALS AND METHODS

2.1 Carbon Nanotubes

Purified SWNTs in sterile PBS solution of pH 7 was obtained from Nano-Lab. The nanotubes were grown by chemical vapor deposition which produced median diameter of 1nm and length of 3µm. The nanotubes came COOH functionalized to allow good dispersion without surfactants. Series of acid purification and filtering removed Ni-Co-Fe catalyst particles to achieve > 97% SWNT.

Prior to experiments, nanotube solutions were sonicated for about 10 minutes before each dilution with PBS and before the administration to cells. 5 µL of nanotube solutions of various concentrations were added to 100 µL of cells for quantitative evaluation under the optical microscope and for quantitative evaluation using Cell-Titer-Glo Luminescence experiments.

2.2 Chinese Hamster Ovarian Cells

CHO cell line was obtained from the Center for Molecular Imaging Cell Research as a frozen stock and passaged in-house for at least 5 generations before experimentation. CHO cells were maintained in F-12K media containing 10% FBS.

3x10⁴ cells were plated in each 96-well plate with fresh media. SWNT was prepared in Phosphate-Buffered...
Saline (PBS) and added directly to the cells. The viability of CHO cells has been shown to correlate to their cell morphology observed under an optical microscope. In this study, we characterized the CHO cell morphology at various carbon nanotube concentrations and at various time points. Phosphate-Buffered Saline (PBS) was used because it is known that in this isotonic solution carbon nanotubes can remain stable and mammalian cells can remain healthy and viable. First, we observed that adding PBS solution had no effect on CHO cell’s morphology. Then, PBS was added to the CHO cells and this cell morphology was served as the control. To observe the cell morphology of dead CHO cells, camptothecin, a toxic chemotherapy drug, was added. To begin our experiment, various concentrations of SWNTs was added to many wells of CHO cells. The resulting morphological changes of CHO cells at each concentration gave qualitative information of how cells respond as a function of carbon nanotube concentration, and whether cells can be viable enough to continue to multiply after exposure to carbon nanotubes.

Optical live-cell imaging was done on an Olympus IX51 inverted microscope system (Zeiss, Oberkochen, Germany).

2.3 Cell Titer-Glo Luminescence

ATP content of cell was measured using the CellTiterGlo Luminescent Cell Viability Assay (Promega, Fitchburg WI) following the manufacturer’s recommended protocol. 25 µl of the reaction mixture was added to each well and mixed on a shaker for 2 min. After 30 min incubation in a 37°C incubator with 5% CO₂, the plate was read on a bioluminescence imager built in house. bioluminescence signal was then quantified using Osirix (Freeware, University of Geneva) and analyzed in Prism (Graphpad, La Jolla, CA).

3 RESULTS AND DISCUSSIONS

From our qualitative evaluation of cell, we directly observed that the intake of SWNTs by CHO cells increased with increasing SWNTs concentration after 1 day of nanotube treatment (see Fig. 1). Cells treated with intermediate concentration (5 µg/ml) of SWNTs showed increased intracellular SWNT accumulation over time while maintaining the cell morphology and cell number after 3 days compared to the control. At 5 µg/ml concentration, the SWNTs appeared to mostly accumulate in endosome of CHO cells, and without significant accumulation inside the cell nucleus. This suggested that the SWNTs can enter the CHO cells through both active endocytosis as well as passive diffusion. Such cells treated with 5 µg/ml SWNTs maintained a healthy cell morphology and was able to multiply to yield a cell number comparable to the control.

For cells treated with high concentration (50 µg/ml) of SWNTs, the cellular intake of SWNTs is rapid, and has immediate impact on cell morphology and prolonged impact on cell number. After 3-day SWNT treatment few cells had normal morphology and many were arrested during cell division (see Fig. 1).

![Figure 1: Clearly visible intracellular accumulation of SWNTs does not significantly reduce CHO cell number after 3 days at intermediate concentration (5 µg/ml). Cell number decreases significantly at high concentration (50 µg/ml).](image_url)
Figure 2: Quantitative characterization of cell viability showed a concentration dependent decrease with increasing SWCNT concentration. Cells were imaged after 1-day SWNT treatments. U-untreated, A-induced apoptotic cells.

The intensities of the Cell-Titer-Glo Luminescence, or the % survival of the CHO cells were plotted as a function of SWNTs concentrations for 1-day and 2-day after the SWNTs treatment (see Fig. 3). Cells treated with low concentration (0.05 µg/ml) of SWNTs have no effect (100% survival rate) on cell viability initially (after 1 day), but were detrimental (82% survival) after longer SWNTs exposure (2 days). For intermediate SWNTs concentrations (0.25 - 25 µg/ml) the cells generally were tolerant (>90%) of prolonged exposure to SWNTs. At high SWNTs concentration (50 µg/ml) 90% of cell survived after 1 day, but only about 56% of the cells survived after 2 days of SWNTs treatment.

Figure 3: After 1-day of SWNTs treatment (blue line), low dose of SWNTs (0.05 µg/ml) is not detrimental to the cells. At high doses, there is a significant effect on cell survival. Surprisingly, prolonged exposure (red line) even at the lowest concentration (0.05 µg/mL) significantly impacts cell survival. Generally, cells were tolerant of prolonged exposure at intermediate concentrations tested.

Combining the qualitative morphological changes from optical studies, and the quantitative metabolic changes from luminescence studies, one can assess the cytotoxicity of SWNTs to the CHO cells. It is worthwhile to note that while luminescence shows a ~90% cell survival rate after 1 day of treatment in high concentration (50 µg/ml) of SWNTs (see Fig. 3), the cell morphology and the cell number were impacted rather drastically (see Fig. 1). On the other hand, while cell morphology and cell number seemed to vary minimally from control for cells treated with low concentration (0.05 µg/ml) of SWNTs, the luminescence data showed a rather drastic decrease in survival rate over time (see Fig. 3). Our results showed that the low SWNT concentration’s toxicity to the cell warrants careful consideration in in vivo applications, especially given the reported wide biodistribution [1], as well as the potential impact to the environment.

4 CONCLUSIONS

We demonstrated the study of SWNT toxicity in cultured CHO cells using straightforward light microscopy and cell-titer-glow luminescence. Our finding was consistent with previous literature in that at high concentrations, SWNT was toxic to cells. Interestingly, we have also demonstrated that at very low concentration (0.05 µg/mL) cells viability decreased over prolonged SWNT exposure (2 day).

REFERENCES


