Magnetically Associated Carbon Nanotubes with Mammalian Cells: Flow Cytometry Characterization and Applications

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ABSTRACT

Carbon nanotube (CNT) that is synthesized by plasma enhanced chemical vapor deposition has uniform length and vertically aligned morphology. The CNT can be magnetically driven into cells because it has an embedded Ni nanoparticle. We show herein that CNT-cell complexes are formed in the presence of a magnetic field. The complexes were analyzed by flow cytometry as a quantitative method to monitor the physical interactions between CNTs and cells. We observed an increase in side-scattering signals (SSC), where the amplitude was proportional to the amount of CNTs that are associated with cells. Even after the formation of CNT-cell complexes, cell viability was not significantly decreased. The association between CNTs and cells was strong enough to be used for manipulating the complexes and thereby conducting cell separation with magnetic force. In addition, the CNT-cell complexes were also utilized to facilitate electroporation. We observed a time constant from CNT-cell complexes but not from cells alone, indicating a high level of pore-formation in cell membranes. Experimentally, we achieved the expression of Enhanced green fluorescence protein (EGFP) by using a low eletroporation voltage after CNT-coated EGFP plasmid formed complexes with cells. These results suggest that higher transfection efficiency, lower electroporation voltage, and miniaturized setup dimension of electroporation may be accomplished through the CNT strategy outlined herein.

Keywords: carbon nanotube, flow cytometry, mammalian cell, nanospearing

1 INTRODUCTION

Carbon nanotube (CNT) offers promising performance in biomolecule delivery and anti-cancer treatment. Different mechanisms have been used to mediate the interaction between CNT and cells, which are correlated to the surface chemistry of CNT. The field-mediated CNT-cell interaction has also be used to drive CNTs by magnetic force to penetrate cell membranes and shuttle DNA payloads into the cells, by a process termed nanospearing[21]. The spearing CNT are synthesized by plasma enhanced chemical vapor deposition (PECVD) process[22]. CNTs produced by PECVD have uniform and controllable morphology (*i.e.,* length, diameter, and alignment). In such kind of CNT, a metallic nanoparticle is always embedded in each CNT tip due to the special growth mechanism [122]. The as-grown magnetic nanoparticle therefore can facilitate the actuation of CNT in magnetic field. Flow cytometry was utilized to quantitatively characterize the magnetic association between CNTs and cells. The complex was also examined through the magnetically mediated cell separation. Due to the ideal one dimensional conductive structure, this kind of CNT can enhance the electric field at both ends.

2 RESULTS

2.1 CNT preparation

CNTs were grown on a silicon substrate. According to scanning electronic microscopy (SEM) of the nanotube array, the on-chip CNT amount was estimated. CNTs from a 2 × 2 cm chip were then placed into 5 ml solvent to make ~4 pM suspension.

2.2 Spearing mammalian cells and flow cytometry characterization

The nanospeared primary B cells generated distinctive SSC signal pattern. Normal viable cells have lower SSC and higher FSC in comparing to apoptotic cells or cell debris. The normal cell population can be gated in the SSC-FSC scatter plot, as shown in the control data. The FSC and SSC histograms of the gated population are displayed separately. Upon association with CNTs, a larger cellular SSC signal

was observed, while the level of FSC and percentage of normal cells remained the same as control (*i.e.,* non- speared cells).

The PLL-CNTs were used to demonstrate the SSC responses with respect to the CNT dosage up to 6.4 fmol. In parallel, the cells were stained with propidium iodide (PI) to monitor the cell viability by flow cytometry. The viable splenic B cell population in SSC-FSC plot was marked in green according to the PI positive gate in the histogram. The nanospeared cells viability remained at a similar level (~80%) despite a high SSC level that was saturated when 6.4 fmol CNTs were used for nanospearing. Of note, the CNT tended to precipitate and formed clusters at high concentration. This led to the reduction of spearing efficiency and might be the reason that SSC decreased 6.4 fmol.

2.4 CNT mediated applications by nanospearing

Following the magnetic separation of the speared cells, we obtained "dark part" and "clear part" suspension samples following the procedures in Experiment Section. The samples contained cells with and without CNTs, respectively. The SSC signals of the two parts exhibited significant difference. For the dark part, the SSC increased to $15x10^4$, while the clear part exhibited SSC under $3x10^4$. The cell counts in both parts were normalized to the total amount of cells. The cells were magnetically collected into the dark part at a percentage depending on the amount of CNTs associated with cells. For example, at 1.6 fmol, more than 90% of the cells were in complexes with CNTs. The complex structure remained stable during the process to support the physical handling of cells.

We used Bal17 cells to test nanotube-facilitated electroporation since the lymphoma cells are more amenable to transfection than the primary B cells. The time constants corresponding to cells without CNTs and CNT-associated cells were plotted vs. voltages. Clearly, the time constant decreased in CNT associated cells comparing to CNT-free Bal17 cells. The time constant of CNT associated cells at 150 V was 58 ms. The value was comparable to the time constant obtained from CNT-free cells at 230 V, which was the voltage we can obtain transfection in the CNT-free cells. The transfection was carried out by adding pEGFP plasmid to the cell suspension in the cuvette. Generally, it takes more than one day for cancerous cells to express proteins encoded by plasmids. So we checked the transfection results 48 hours after the electroporation by flow cytometry. As shown, CNT associated cells demonstrated improvement in the EGFP transfection in terms of the percentage of EGFP positive cells and the average amplitudes of EGFP signals in comparison to the control cells electroporated without the plasmid, cells.

REFERENCES

[1] D. Cai, J. M. Mataraza, Z. H. Qin, Z. Huang, J. Huang, T. C. Chiles, D. Carnahan, K. Kempa, Z. Ren, Highly efficient molecular delivery into mammalian cells using carbon nanotube spearing. *Nat. Methods*, **2005**, *2*(6), 449

[2] Z. F. Ren, Z. P. Huang, J. W. Xu, J. H. Wang, P. Bush, M. P. Siegal, P. N. Provencio, Synthesis of Large Arrays of Well-Aligned Carbon Nanotubes on Glass. *Science* **1998**, *282*, 1105

[3] Wen, J. G.; Huang, Z. P.; Wang, D. Z.; Chen, J. H.; Yang, S. X.; Ren, Z. F.; Wang, J. H.; Calvet, L. E.; Chen, J.; Klemic, J. F.; Reed, M. A. Growth and characterization of aligned carbon nanotubes from patterned nickel nanodots and uniform thin films. *J. Mat. Res.* **2001**, 3246