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Interaction between carbon nanotubes and human cell

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ABSTRACT

Carbon nanotubes (CNTs) have been attracting increasing attention for the possibilities of their widespread applications and many studies have been conducted for their development. On the other hand, the research on the influence of CNTs on human organisms is still scarce and some show concerns about genotoxicity or cytotoxicity. Therefore, in this research, we are proposing a new way of biocompatibility evaluation for CNTs by examining the relation between biocompatibility and structure of CNTs. In our cytotoxicity tests, neither chromosome abnormality nor cell cycle dysregulation occurred, evaluated by fluorescence activated cell sorting (FACS) analysis. However, significances were assessed for cell proliferation and viability for semiconducting CNTs by the paired *t*-test. It is also clear that CNTs showed adherence to cell, since efficiency of cell ablation by trypsin decreased dramatically in CNTs dishes; this phenomenon was observed especially in semiconducting CNTs. Since semiconducting CNTs do not include armchair CNTs while metallic CNTs do, it indicates that armchair CNTs have high possibility of affection toward biocompatibility. It is expected that armchair CNTs change the behavior of cells such as proliferation and viability.

1. Introduction

CNTs were discovered by Sumio Iijima in 1991 [1]. At that time, only multi-walled CNTs (MWNTs) were found, and in 1993, Sumio Iijima and Toshinari Ichihashi discovered single-walled CNTs (SWNTs) while Donald S. Bethune also discovered it severalty around the same time [2,3]. It is expected to be used in various fields, such as needles or probes [4,5], actuators [6], sensors [7,8], high power amplifier [9], emission displays (FED) [10], and moreover, for a new transportation system as a space train (elevator) [11].

On the other hand, the influence of CNTs on human organisms is still scarce. Among the limited studies, some indicate that CNTs have genotoxicity or cytotoxicity similar to asbestos [12]. In fact, a European standard known as "Restriction of the Use of Certain Hazardous Substances in Electrical and Electronic Equipment (RoHS)" came up for discussion on prohibition of the use of CNTs in all fields including medicine for the concern for their toxic nature [13]. Had it been possible to evaluate biocompatibility by chirality, there would be more advantage in the industrial field.

In order to further investigate the toxicity of CNTs, we are proposing a new way of biocompatibility evaluation after seeking the relation between biocompatibility and the structure of carbon nanotubes. Our hypothesis is that biocompatibility may differ by crystalline structures of CNTs which are attributable to the layer construction and chirality; considering the proven fact that biocompatibility differs by interaction energy toward molecules *in vivo* [14], and the energy of CNTs differs by chirality [15]. This research focuses on the biocompatibility of armchair CNTs, due to the chirality distribution in each semiconducting and metallic CNTs referring to Table 1. Metallic CNTs includes all kinds of chirality, while semiconducting CNTs do not include the chirality of armchair.

In our experiment, cytotoxicity tests of semiconducting CNTs and metallic CNTs with high purity (more than 99% of the CNTs) were examined, respectively, in order to distinguish the influence of chromosome and cell cycle dysregulation, cell proliferation and viability, and cell ablation by trypsin among the control and the above-mentioned two forms of CNTs.

2. Materials and methods

In order to examine the biocompatibility of CNTs by the difference of crystal structures, cell cultures were subjected. Materials and methods were considered for the experiments.

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Table 1
Chirality distribution of CNTs.

Chirality	Armchair	Zig-zag	Chiral
Semiconductor	×	○	○
Metal	○	○	○

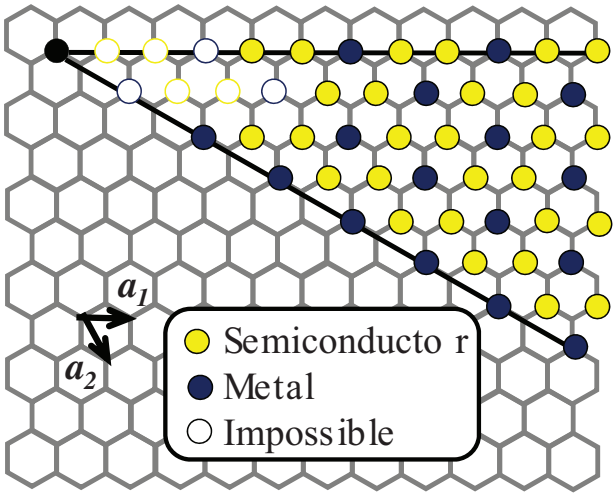

Fig. 1. Chirality of CNTs and its electrical property.

2.1. Chirality of CNTs

Carbon nanotubes (CNTs), envisioned as a rolled sheet of graphite, have many properties which can be determined by chirality. Chirality is attributing to a coordinate system shown in Fig. 1, defined in graphite sheet by two integers, (n, m), indicating the orientation to which the sheet is rolled and diameter of CNTs [16]. There are 3 types of structure according to the chirality of CNTs, referred to Fig. 2: armchair $((n, n), n = m \neq 0)$, zig-zag $((n, 0), m = 0)$, and chiral $((n, m), n > m \geq 1)$. The coordinate uniquely defines the electrical property thus CNTs have been attracting increasing attention for the possibilities of their widespread applications in the field of engineering [17]. Recently, many studies have been conducted for their mass-production on the industrial basis as well as others such as the development of array geometry control system and the application to sensors.

2.2. Selection of CNTs

Recently, there are not enough developments on separation and refinement technologies of CNTs thus it is impossible to distribute CNTs by chiralities. However, technology to distribute semiconducting CNTs and metallic CNTs were available with high purity (more than 99% of the CNTs) by Meijo Nano Carbon Co., Ltd. Therefore, semiconducting CNTs (part number: RS) and metallic CNTs (part number: BM) were selected for this research. The powders of CNTs have undetached behavior, so it was considered inappropriate to use directly for the cell culture. Additionally, dispersion liquids on offer were also unconsidered because surface acting agent polymer fluids which show cell cytotoxicity themselves were included. Since Meijo Nano Carbon Co., Ltd. have sold CNTs coated dish, we custom-built semiconducting CNTs coated dishes and metallic CNTs coated dishes as to carry out cytotoxicity tests. The density of applied CNTs was more than 3.54 ng/mm^2.

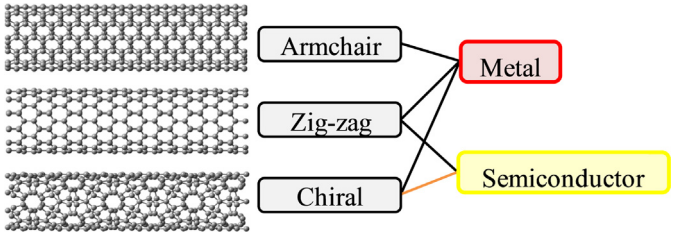

Fig. 2. Structure of CNTs.

2.3. Selection of cell

Type of cell was one of the epithelial cells, generally known as the cell which may mutate into a cancer cell, in order to evaluate biocompatibility. The type of cell chosen was HEK293T cell derived from human kidney which are one of the most widely used cultivated cell for universal cellular function research and analysis. In order to examine the influence to human organisms, it was necessary to make experiments by human cell. HEK293T cell is a clone cell behaving uniformly, so the difference in condition can be directly expressed to the cell.

2.4. Cell culture

We examined 3 types of dishes: control (gelatin coated), semiconducting CNTs coated, and metallic CNTs coated. HEK293T cells were cultivated in a humidified atmosphere in the condition of 37 °C, 5% CO_2 for 3 days until the cells were filled in the dish.

2.5. FACS analysis

FACS analysis, a quantitatively-determined analysis by measuring fluorescence which each cell emits, is short for Fluorescence Activated Cell Sorting analysis. It is statistically compared by using constant cell number. Below shows the procedure:

Hoechst33342 and propidium iodide (PI) were used for the fluorescent dye in this research. Blue phosphorogen called hoechst33342 which bind specifically with chromosomal DNA was conducted into cells to indicate chromosome and analyze cell cycle dysregulation. PI is a red phosphorogen to determine viability. It can be visualized firmly since the cells which are to die or are already dead cannot egest PI and emit fluorescence. Index "PI−" indicates the living cell rate while "PI+" shows the dead cells [18].

After dyeing the cells, FACS Calibur offered from Becton, Dickinson and Company (BD) was used for the analysis. The voltage of laser was fixed voltage in comparison. FACS radiates laser to a given number of cells drained rapidly which was stained in fluorescent antibody.

The results obtained from FACS analysis were finally evaluated by *t*-test which can show repeatability with a few samples. Each type of dish was examined 3 times in order to seek the repeatability in cell culture.

2.6. Cell proliferation assay

The following is the procedure of cell proliferation assay. First, cells were washed by phosphate buffered saline (PBS) to drag away from the dishes, then 0.05% trypsin, 0.2 mM ethylenediaminetetraacetic acid (EDTA) liquid solution was treated in 37 °C for 10 min. Trypsin is prote inase that would peel cells away from the dishes by degrading protein on the surface of cells mildly. EDTA prevents to affect trypsin from Ca and Mg ions. This will gradually assimilate the extracellular protein and suspend the adherent cell.

Finally, cells were counted by a well-known method using the hemocytometer which was complied with the protocol provided by the supplier, Abcam. It uses a counting chamber to count the cells, after deactivating trypsin by additive serum. This number shows