Cytotoxicity of Nano-materials on Human Bronchial Epithelial Cell

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ABSTRACT

This study was undertaken to address the current deficient knowledge of cellular response to nano sized particle exposure.[4] The aim of this study was to investigate the in vitro responses of human bronchial epithelial cells (BEAS-2B) after exposure to nano-materials (Ag and CNT).

BEAS-2B cell viability decreased with decreasing doses of Ag (diameters : 40~70 and 100 nm) and MWCNT (diameters : 4~6 and 10~15 nm) nano-materials. At the higher exposure (≥200 ug/ml), all materials induced significant cell damage. Nano Ag materials increased apoptosis and cytokine expression (IL-6 and IL-8) when over 100 ug/ml concentration. All MWCNTs weakly increased apoptosis and did not significantly increased cytokine expression.

In conclusion, we suggest that Ag and MWCNT nano-materials induce variable extents of cellular toxicity in a dose-dependent and size-dependent.

Keywords: silver (Ag), carbon nanotube (CNT), apoptosis, cytokine, viability

1. INTRODUCTION

In today's world, Nanoscience is experiencing a massive investment worldwide, although research on toxicological aspects of these nano size particles has just begun. To date, no clear guidelines exist to quantify the effects [1]. In particular, it is important to understand the effects of carbon nano tube (CNT) and nano sized silver (Ag) since these are the most commercial nano-material based products available.

The aim of this study was to investigate the *in vitro* responses of human bronchial epithelial cells (BEAS-2B) after exposure to Ag nano materials and multi-walled carbon nanotubes (MWCNT) [1]. The focus was on their effects on cell viability and apoptosis, as well as on the release of typical pro-inflammatory mediators such as interleukin-6 and 8 (IL-6 and IL-8). The human bronchial epithelial cell line BEAS-2B was chosen as a cellular models for possible target cells.

2. MATERIALS AND METHOD

2.1. Preparation of Nano-materials

The nano-materials used in this experiment included two types of Ag (commercial and synthetic) and two types of MWCNT (normal MWCNT and thin-MWCNT). Commercial Ag was spherical in shape and diameter is 100nm (Sigma-Aldrich, USA). The shape and diameter of synthetic Ag was spherical and 40~70nm, respectively, and was provided by Gang-teak Lee, Professor of Chemical Engineering, Yonsei University. Both types of MWCNT

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were from ILJIN NanoTech Co., Ltd. (Republic of Korea). The diameters of MWCNT and thin-MWCNT were 10~15nm and 4~6nm, respectively. The length of all MWCNTs was 10~20um.

All particles were freshly prepared in 10% FBS (fetal bovine serum)-culture medium. This mixture was sonicated for 30~60min and then further diluted with culture medium at concentrations of 0, 50, 100, 200, and 400 ug/ml.

2.2. Scanning Electron Microscopy (SEM) of Nano-materials

The MWCNTs and thin-MWCNTs were diluted in chloroform and attached to a silicon wafer. Ag nano particles were diluted in toluene, attached to the same wafer. The morphology of the particles was analyzed using scanning electron microscopy (SEM) [5].

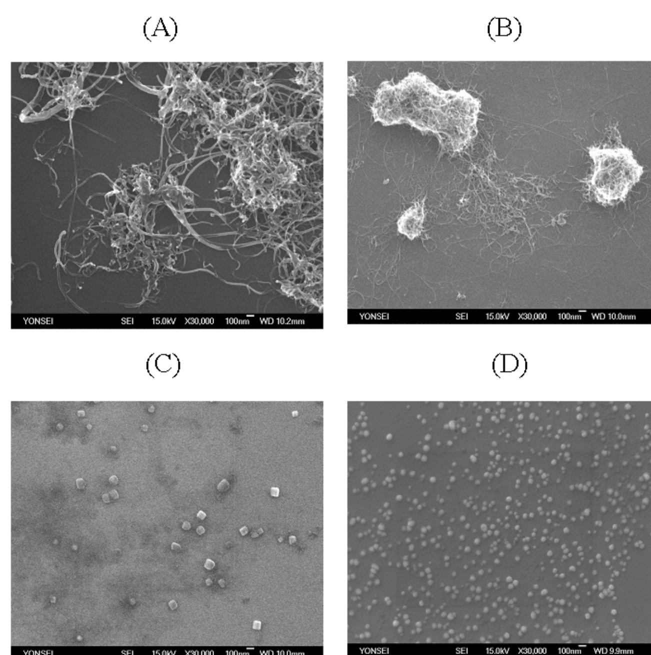

Figure 1: SEM of compounded nano-materials (x30,000). (A) MWCNT, (B) thin-MWCNT, (C) Ag (100nm), (D) Ag (40~70nm)

2.3. Cell Culture

The human bronchial epithelial cell line BEAS-2B (ATCC, CRL-9609) was purchased from the Korean Cell Line Bank (KCLB). BEAS-2B cells were grown in Minimum Essential Medium Eagle (MEM) supplemented with 10% heat inactivated FBS (WelGENE Inc., Republic of Korea) [5]. The cells were grown in a humidified incubator at 37°C (95% room air, 5% CO_2).

2.4. Cell Viability Assay

Detection sensitivity was performed using a Cell Counting Kit-8 (CCK-8, Dojindo, Japan) and was chosen because it has a higher sensitivity than other tetrazolium salts such as MTT or WST-1. CCK-8 is non-radioactive and allows for sensitive colorimetric assays for the determination of the number of viable cells in cell proliferation and cytotoxicity assays. After treatment of the cells with materials as described above, the supernatant medium was replaced by CCK-8 solution (15 $\mu\ell$/well) and incubated for 1.5 hours. The colored supernatants, without particles, were transferred into a clean 96-well plate and the absorbance measured at 450 nm. [6]

2.5. Annexin V/PI staining

Cells were analyzed for annexin V binding and propidium iodide (PI) incorporation to distinguish between apoptotic and necrotic cells. Harvested cells were washed twice in dulbecco's phosphate buffered saline (DPBS) and stained for 15 min with 5 $\mu\ell$ annexin V-FITC (BD Biosource, USA) and 10 $\mu\ell$ PI. The stained cells were analyzed via Facs Calibur analysis. The degree of apoptosis was obtained by adding numbers in the second and fourth quadrants of annexin V-positive / PI-positive and annexin V-positive / PI-negative, respectively [3].

2.6. Determination of IL-6 and IL-8

Quantitative determination of the cytokines IL-6 and IL-8 in culture medium from exposed BEAS-2B cells was performed using sandwich ELISA (OptEIA™, BD, USA). The analyses were performed according to the manufacturer's manuals. The increase in color intensity was quantified using a plate reader complete with software and the absorbance was measured at 450 nm [2].

2.7. Statistical Analysis

Values are reported as mean ± standard error of the mean (S.E.M.). Statistical analysis was performed using Student's *t*-test [1] in cases where experiments were carried out at least in triplicate.

3. RESULT AND CONCLUSION

BEAS-2B cell viability decreased in a dose dependent manner with Ag (40~70 and 100 nm) and MWCNT (4-6 and 10-15 nm) nano-materials (see Fig. 2). With exposure to Ag nano-materials at a concentration of 50 ug/ml, no or little toxicity was observed for the both 40~70 nm and 100 nm size. The Ag nano-materials were more toxic and resulted in a significant decrease in viability when 100 ug/ml. At higher exposures (≥200 ug/ml), all materials induced significant cell damage. The cell viability showed thin-MWCNT (4-6 nm) is more toxic than MWCNT (10-15 nm).

The appearance of phosphatidylserines on cell surfaces is a trademark of cell damage indicating cytotoxicity. This is also basis for the detection of apoptotic cells using dual-color analysis with annexin V-FITC/PI. With the use of an annexin V/PI staining regime, three populations of cells are distinguishable in two color flow cytometry: (1) Non-apoptotic cells: Annexin-V negative and PI negative; (2) Early apoptotic cells: Annexin-V positive and PI negative; (3) Necrotic cells or late apoptotic cells: Annexin-V positive and PI positive.

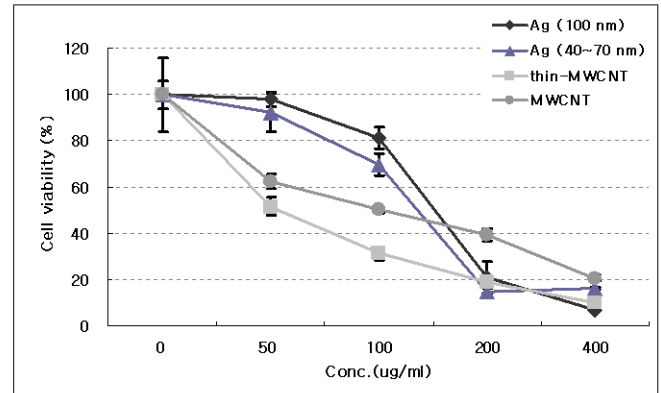

Figure 2: Effect of materials on the viability of BEAS-2B cells detected by the CCK-8 assay. Cells were treated for 24h.

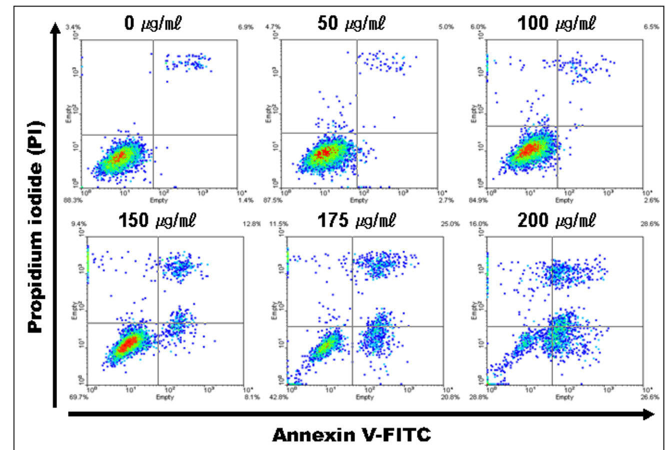

Figure 3: Annexin-V/PI double staining of BEAS-2B cells after 12h exposure with various concentrations of Ag (40~70 nm).

Figure 3 shows, the effect of Ag (40~70 nm) on the extent of apoptosis to BEAS-2B cells as measured by annexin-V/PI staining. This result indicates that Ag nano-materials increased apoptosis in cells. The MWCNTs resulted weakly increased apoptosis (data not shown).

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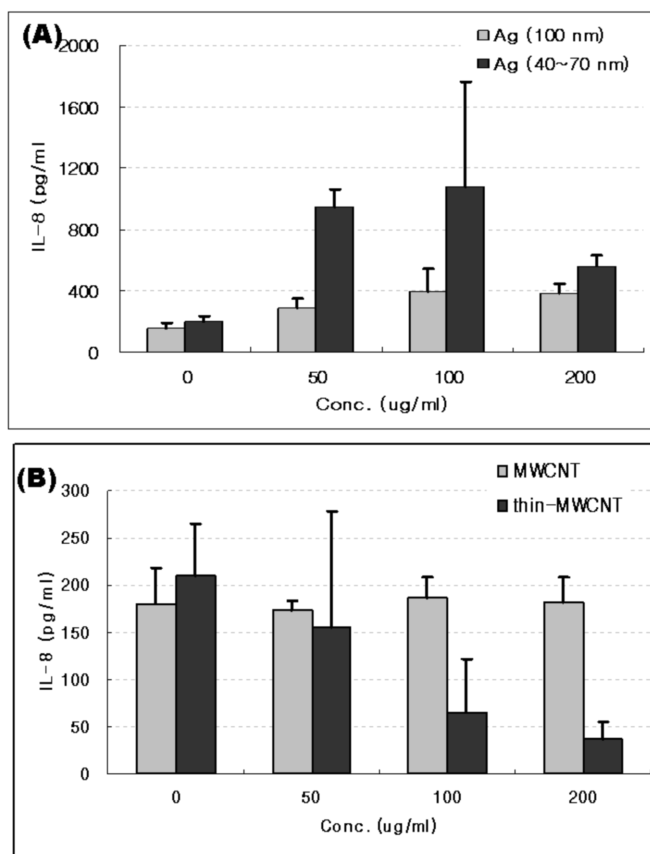

Figure 4: Effect of (A) Ag and (B) MWCNTs on IL-8 release from bronchial epithelial cells BEAS-2B. Cells were treated for 24h and the culture supernatant was analyzed for IL-8 concentration.

The potential of the materials to induce the release of pro-inflammatory chemokine IL-6 and IL-8 in human bronchial epithelial cells BEAS-2B was also investigated. A dose and size-dependent increase was observed with exposures of Ag nano-materials of up to 100 ug/ml. However, at higher exposures the levels of IL-8 decreased (see Fig. 4). Treatment with MWCNTs did not significantly increase cytokine expression. Expression of IL-6 was weak by all Ag and MWCNTs (data not shown).

In conclusion, we suggest that Ag and MWCNT nano-materials induce variable extents of cellular toxicity in a dose-dependent and size-dependent manner.

4. ACKNOWLEDGMENTS

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