TEM study of nano-carbon materials intratracheally instilled in rat lung

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Keywords: fullerene, MWCNT, EF-TEM, risk assessment, toxicity

Industrial applications of nano-materials were reported in many fields recently. However, the toxicity of these nano-materials for the human was not clear, so the toxicity test of nano-materials is important. Transmission electron microscope (TEM) was powerful technique to study the nano-materials, and used both material and biological researches. The high contrast observation of the biological cells or tissue was important, and then, the cell specimens were usually stained with the heavy elements such as U and Pb to increase the contrast of image. In the case of toxicity test of the nano-carbon materials, we must observe the biological cells containing the nano-carbon materials. However, it was difficult to observe the stained biological cell specimen with the nano-carbon materials, because the contrast from nano-carbon materials was weak and below the background of the staining heavy elements. Furthermore, the high-resolution observation is necessary for the nano-carbon materials in the biological cells. It is difficult to observe at the atomic scale resolution using the biological TEM with a high contrast objective lens.

In this study, the toxicity tests of the nano-sized fullerene particles and multi-walled carbon nanotubes (MWCNTs) were examined using the intratracheally instilled in rat lung. the zero loss TEM imaging of the rat lung tissue with fullerenes or MWCNTs is performed by using an energy-filtering TEM with the objective lens for the high-resolution imaging (Zeiss, EM922HR). The acceleration voltage was 200 kV. The electron spectroscopic zero-loss images are obtained at a loss energy of 0 eV with an energy window-width of 20 eV.

The in-vivo tests of fullerenes and MWCNTs were the intratracheal instillation of test material solution in the rat lung. Tween 80 or Triton dispersions of 0.1g/l content were used for the fullerene or MWCNTs solution, respectively. The mounts of fullerenes or MWCNTs in the solutions were 200 μ g or 1 mg. The TEM images of MWCNTs in the test solution are shown in Figure 1(a) and (b). The diameter of MWCNTs is 50 nm, and the length was ranging from 0.5 to 5 μ m. The test solution was intratracheally instilled in rat lung. The lung tissues after three days, one week, one month, and three months from the instillation were observed by TEM. The lung tissue was fixed using glutaraldehyde and osmium tetroxide solution, and then dehydrated in ethanol, and embedded in epoxy resin. Ultrathin sections were cut on a diamond knife with microtomy. The staining condition of the tissue specimen was examined.

After 1-week instillation exposure of fullerenes solution, some particles with the black contrast are observed at the cytoplasm. These particles are not observed in the nucleus or the mitochondria. According to the electron diffraction analysis of these particles, these are identified as the fullerenes. Fullerenes particles keep the fcc crystal structure. As the diameter of fullerenes in was 20 nm, fullerenes keep the particle size. Fullerenes still remained in lung tissue after 3 months instillation exposure.

W. Grogger, F. Hofer, P. Pölt (Eds.): MC2009, Vol. 3: Materials Science,
DOI: 10.3217/978-3-85125-062-6-571 , © Verlag der TU Graz 2009

TEM zero loss image of the alveolar macrophages after 3 days instillation exposure of MWCNTs solution was shown in Figure 2. Fibrous structures were observed at the cytoplasm of macrophage. According to high resolution observations, these fibrous structures are MWCNTs. MWCNTs keep the tube and the graphitic structures. MWCNTs. are also observed in the alveolar cells. MWCNTs still are observed in lung after 3 months instillation exposure.

1. This work was supported by New Energy and Industrial Technology Development Organization(NEDO) of Japan.

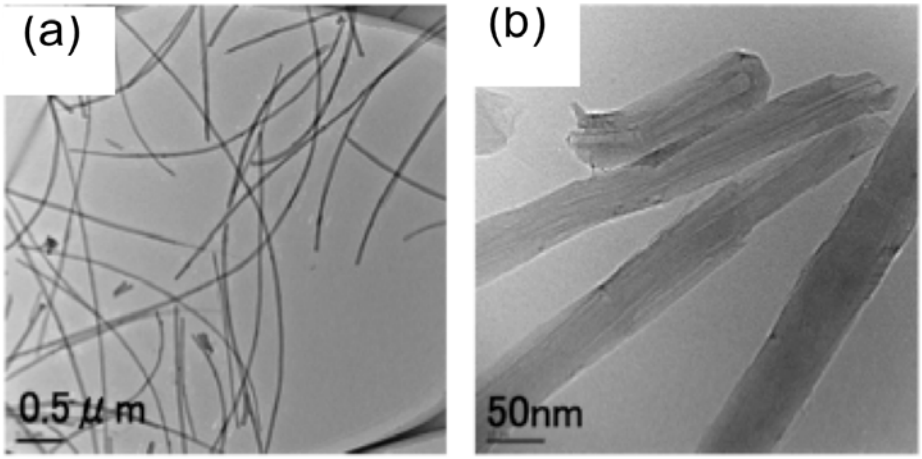

Figure 1. TEM photographs pf MWCNTs in test solution (a). Diameter of MWCNTs is 50nm, and MWCNTs have graphitic tube structure (b).

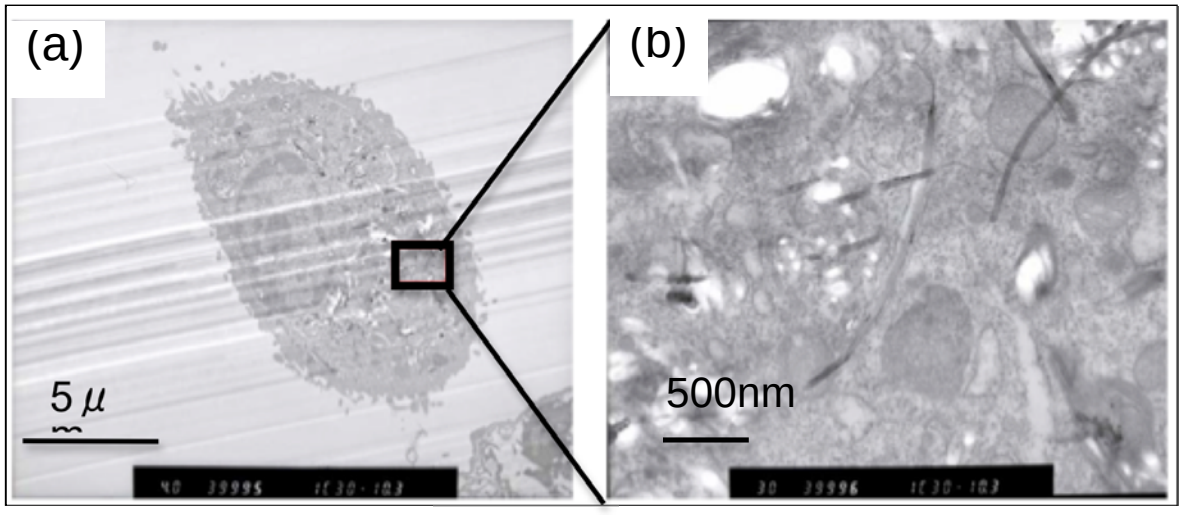

Figure 2. Zero loss TEM images of alveolar macrophage after 3 days instillation exposure (a). (b) is magnified image of area in (a).

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