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Inducing structural defects in multi-walled carbon nanotubes by biological oxidation

R.S. Chouhan[a], V. Ozguz[a], A. Qureshi[a], J. H. Niazi[a,*]

[a]*Sabanci University Nanotechnology Research and Application Center, Orta Mah. 34956 Istanbul, Turkey*

Abstract

In this study, carbon-nanotubes (CNTs) resistant soil bacteria were utilized to induce biotransformation in engineered multiwalled-carbon nanotubes (MWCNTs). It was observed that the CNTs-resistant bacteria upon interaction with engineered MWCNTs lead to their partial degradation. Raman spectra of bio-transformed-MWCNTs revealed increased intensity ratio of I_D/I_G with subsequent formation of C–O–H, C=O and COOH groups on the outer walls of nanotubes. Our results demonstrate a low-cost modification of MWCNTs by resistant bacteria to induce structural defects that potentially useful for industrial applications.

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Keywords: Carbon nanotubes, biotransformation, bacteria, nanomaterials

1. Introduction

MWCNTs are carbon-based nanomaterials (NMs) formed by rolling several graphene sheets into a cylindrical shape. Due to their unique physicochemical, optical, and mechanical properties, CNTs gained importance in to many applied fields such as composites, conductive materials, sensors, drug delivery vessels, and sorbents [1]. Although, an active area of research is centered on the biocompatibility of these NMs, very little has been done to explore the

*r.s chouhan. Tel.: +90-216-483-9879; fax: +90-216-483-9885.
E-mail address: javed@sabanciuniv.edu

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possibility of their biodegradation. Particularly, low fate of CNTs has been attributed to the availability of functional groups on the side wall of nanotubes [2, 3]. Changes in the various physicochemical properties of MWCNTs can be useful for their application in various industrial processes, where they are required to chemically modify for altered functionality. Recently, researchers have attempted to use biological means to modify such nanostructures. A very few studies showed that CNTs can be biodegraded by intra and extra cellular enzymes or by fluids phagolysosome [4-7]. Therefore, here we developed a new approach in which CNT resistant bacteria were utilized to modify structural property of engineered MWCNTs.

2. Experimental

2.1. Materials

MWCNTs used in this study had O.D. × L 7-15 nm × 0.5-10 μm (Arry, Hongkong). Almar blue, Amplex Red (N-acetyl-3,7-dihydroxyphenoxazine) and Lactate dehydrogenase (LDH) was purchased from Pierce Biotech., Inc. USA. Triton-X 100 was procured from Merck, Germany. Dimethyl Sulfoxide (DMSO) was purchased from Sigma Aldrich, USA. All other reagents used in this study were of analytical grade and filtered through 0.22 μm sterile filters. All bacterial studies and related work were carried out under sterile conditions.

2.2. Isolation and enrichment of soil bacteria and obtaining CNTs-resistant bacteria

Soil samples were collected from NMs contaminated site. The bacteria from soil samples were isolated and enriched with MWCNTs by using standard microbiological methods in mineral medium (MM) containing glucose as the only carbon source. The MM contained (per liter) 3 g KH_2PO_4, 12.8 g $Na_2HPO_4.7H_2O$, 1 g NH_4Cl, 0.5 g NaCl and supplement sources such as $MgSO_4$ (2 mM), $CaCl_2$ (0.1 mM) and glucose (varying from 0.3~0.4%) pH (6.5~7.5). For repeated enrichment and adaptation of bacteria, the MM was amended with 5 μ/mL MWCNTs. The bacteria were allowed to grow at 37 °C under constant shaking in an orbital shaker.

2.3. Interaction of engineered MWCNTs with CNTs- resistant bacteria and extraction

A 30-days of CNTs-resistant-bacteria culture was harvested by centrifugation at 9000 rpm for 10 min. The pellet containing CNTs, cells and cell-debris were washed thrice with pure ethanol, sonicated and centrifuged to separate the cells and cell-debris. The brownish MWCNTs pellet was later phase separated in a mixture of 1:1 *n*-hexane and deionized water. A black colored ring of purified MWCNTs appeared at the interface of two solvent phases which was finally collected in a separate glass vial and dried in a vacuum desiccator. Appropriate control samples were also treated the same ways as the test samples for comparison that were utilized for further characterization.

2.4. Structural characterizations

SEM images of isolated and enriched CNTs-resistant bacteria were acquired using a LEO Supra 35VP scanning electron microscope operated at 3 kV. Samples were mounted on a silicon wafer and sputter coated with a thin layer of Pd–Au before taking SEM images. Raman spectra of control (without interaction with CNT-resistant bacteria) and test (interaction with CNTs-resistant bacterial) MWCNTs samples were measured using Renishaw inVia Reflex Raman microscope and spectrometer with spectral resolution of 5 cm^{-1} using a visible excitation at 532 nm laser. Spectral range was scanned from 110 to 3690 cm^{-1} with a 30 s integration time at a laser power of 10 or 20 mW. Attenuated Total Reflectance-IR (ATR-IR) spectra of engineered MWCNTs before and after the interactions with CNTs-resistant bacteria were characterized for functional groups present on the bio-transformed MWCNTs structure. Spectra were acquired with a Nicolet iS10 FT-IR Spectrometer (Thermo Scientific, USA) with mercury cadmium telluride detector (4 cm^{-1} resolution) equipped with a diamond crystal in single reflection mode. IR spectra of sample mounted on silicon wafer were collected with an average of 100 scans with a wide spectral range from 300 – 4000 cm^{-1}.

3. Results and discussion

3.1. SEM characterization of isolated and enriched CNTs- resistant bacteria

The soil bacteria were isolated from NMs contaminated site and enriched in presence of CNTs. The CNTs-resistant bacteria showed adaptation with a high degree of survival and tolerance to MWCNTs. SEM images of isolated CNTs-resistant bacteria showed aggregated complex with bacteria surrounded by a mat of nanotube bundles (Fig. 1). The SEM examination showed that the bacteria secreted slimy byproducts that tend to for thin lining on the MWCNTs which seem to protect the cells from mechanical damage.

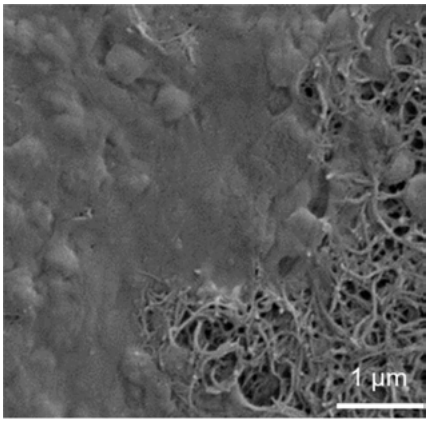

Fig. 1. SEM image showing interaction of CNTs-resistant bacteria with engineered MWCNTs.

3.2. FTIR and Raman results

FTIR and Raman spectra of engineered MWCNTs with (test) and without (control) interaction with CNTs-resistant bacteria are shown in Fig. 2a-b.

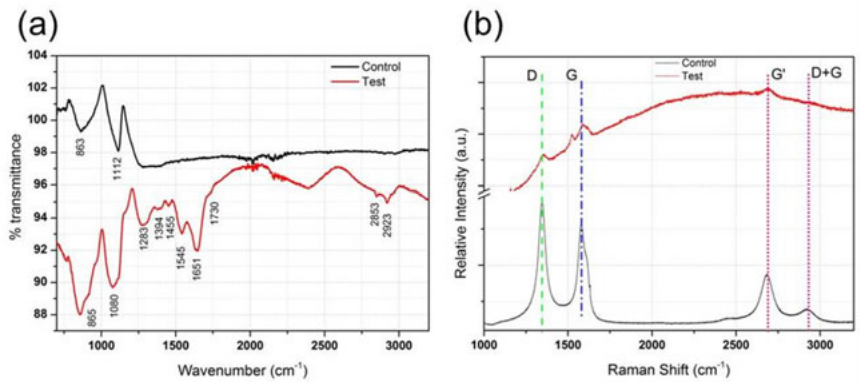

Fig. 2. (a) FTIR and (b) Raman spectra of engineered MWCNTS with and without interaction of CNTs-resistant bacteria.

FTIR spectra of test MWCNTs showed a clear distinction from its control counterpart, more prominently, the absorption bands from 1455 to 1394 cm $^{-1}$ were unique to bio-transformed test MWCNTs (Fig. 2a). Bands from

1455-1283 cm $^{-1}$ are attributed to the stretching modes of the C-O-H bonds of the carboxylic acid. FTIR spectral analysis also revealed the existence of structural modification in the outer walls of the test MWCNTs and subsequent formation of C-O-H, C=O and COOH type of functional group on the outer surface that were absent in control MWCNTs (Fig. 2a).

Raman spectra of test MWCNTs showed three major peaks that represented D, G, G', respectively for a typical MWCNT structure. Comparison of D, G, G' peaks in test MWCNTs showed a clear indication of the modification or defects taken place in bio-transformed test MWCNTs. The distinct intensity ratio of I_D/I_G from Raman spectra of test MWCNTs revealed a significant structural modification by the bacteria. Increased intensity of the D band in Raman spectra of bio-transformed test MWCNTs can be attributed to a high density of roughness (vacancies and heptagon-pentagon pairs) on the nanotube walls or defects in MW structure and thus induced surface modifications [8]. The introduction of more surface oxygen groups in atomic structure of test MWCNTs was consistent with the results of FTIR spectral analysis (Figs. 2a-b). It is postulated that these changes could be due to oxidation of bacterial enzymes at the close proximity during their interaction with engineered MWCNTs.

4. Conclusions

In this study, we reported an approach that utilized enriched natural soil bacteria resistant to CNTs for modifying the physiochemical property of engineered MWCNTs. The bacteria were initially isolated after their adaptation to CNTs by enrichment process in the laboratory. The CNTs-resistant bacteria showed adaptation with a high degree of survival and tolerance to MWCNTs. The isolated soil bacteria appeared to be highly efficient in generating deformed structural variations on the outer walls of engineered MWCNTs. Structural attributes in bio-transformed MWCNTs nanostructures showed the native hydroxyl and carbonyl groups tend to appeared upon their interaction with CNTs-resistant bacteria. These structural changes seemed to have occurred due to oxidation of CNT-resistant bacteria at close proximities and at the interface of bacteria and engineered MWCNTs. This marks a promising possibility for nanotubes to be biologically modified that find tremendous opportunities for precise modification of other NMs.

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