

Brief communication

Capture of bacteria by flexible carbon nanotubes

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Abstract

Capture of bacteria with flexible carbon nanotubes (CNTs) was done in vitro. Bundles of single-walled carbon nanotubes (SWCNTs) or multi-walled carbon nanotubes (MWCNTs) was mixed with *Streptococcus mutans*. Precipitation assays and colony-forming unit formation assays showed free *S. mutans* in the solution was significantly decreased by the addition of the CNTs. Observation of the precipitate by scanning electron microscopy showed bacterial adhesion to CNTs. It has been shown that CNTs of different diameters have significantly different effects on the precipitation efficiency, and the manners in which they capture the cells are different. We found that MWCNTs (diameter of approximately 30 nm) had the highest precipitation efficiency, which was attributable to both their adequate dispersibility and aggregation activity. From observations by scanning electron microscopy, bundles of SWCNTs and thin MWCNTs (diameter of approximately 30 nm), which were moderately flexible, were easily wound around the curved surface of *S. mutans*. Bare CNTs having high adhesive ability could be useful as biomaterials, e.g., as tools for the elimination of oral pathogens at the nano-level.

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1. Introduction

Many carbon materials exhibit excellent molecular adsorption properties, and activated carbon (AC) [1], which is widely used as an adsorbent, has a high capacity for adsorption owing to its porous structure and large surface area. The occurrence of dental caries is mainly associated with oral pathogens, and *Streptococcus mutans* is a primary cariogenic organism. Therefore, many attempts have been made to eliminate *S. mutans* from the oral cavity. One effective way is to use AC as an adsorbent, hence it is used in a wide range of oral care products, such as toothpastes and mouthwashes [2,3].

Carbon nanotubes (CNTs) have attracted considerable attention because of their unique physical properties and potential for a variety of biological applications [4]. In recent investigations, CNTs have been utilized as adsor-

bents to eliminate dyes [5]. In addition, CNTs can adsorb bacteria [6–8]; single-walled carbon nanotubes (SWCNTs) exhibit strong antimicrobial activity toward *Escherichia coli* [9,10]. However, bacterial adhesion, particularly oral bacterial adhesion, to CNTs has not yet been sufficiently investigated. If bare CNTs are found to have strong adhesive activity and winding CNTs bind oral pathogens, they may be useful as tools at the nano-level for capturing oral pathogens. In this study, we investigated oral bacterial adhesion to CNTs of different diameters and flexibility, and compared them with the widely used adsorbent AC particles. In general, SWCNTs with diameter of approximately 1 nm are known to be highly flexible, while multi-walled carbon nanotubes (MWCNTs) with diameters >100 nm are hard. Here we report that CNTs with different diameters have significantly different effects on the efficiency of *S. mutans* precipitation, that the manners in which they capture bacteria are different and that bundles of SWCNTs and MWCNTs with average diameters of 30 nm can wind around the curved surfaces of bacteria.

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2. Experimental

The SWCNTs employed were synthesized by an arc discharge method. The MWCNTs used were of two types: 30-MWCNTs (average diameter of 30 nm; produced by NanoLab Inc., Brighton, MA) and 200-MWCNTs (average diameter of 200 nm; produced by MTR Co. Ltd., OH). As a control carbon sample, a commercial activated carbon powder (AC) with an average particle size of 20 μm (Kanto Chemical Co. Inc., Tokyo, Japan) was used in this study. *S. mutans* JC2 was grown aerobically in brain heart infusion (BHI) broth at 37 °C for several days. The bacteria were harvested by centrifugation at 2500g (Kubota Centrifuge 2700), washed in phosphate-buffered saline (PBS: 20 mM $\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$, 150 mM NaCl, pH 7.4) and suspended in the same buffer to an optical density (OD) of 1.0 at 700 nm.

For a bacterial precipitation assay, 6 ml of a suspension of CNTs treated by ultrasonication in PBS was added to 3 ml of the bacterial suspension in a glass bottle. As a control, PBS solution was used in substitution for CNTs solutions (initial OD of final volume was 0.34; 1.4×10^8 colony-forming units (CFU) ml^{-1}). The solution was shaken at 200 rpm for 20 min by a universal shaker (Iwaki SHK-U3) and then centrifuged at 100g for 3 min. A 2 ml sample of the upper suspension was transferred to a quartz cell, and the OD at 700 nm was measured with an ultraviolet–visible spectrometer.

To identify the suspended carbon of the supernatant after centrifugation, aliquots of the supernatant at a carbon sample concentration of 0.66 mg ml^{-1} were dried on a slide glass. The dry substances were sputtered using

a carbon coater (Meiwa Shoji CC-40F) and then examined by scanning electron microscopy (SEM; Hitachi S4000). For a CFU formation assay of the supernatant after centrifugation, a 1 ml sample of each supernatant carbon sample at concentration of 0.66 mg ml^{-1} was used. Upon serial dilution, the diluted samples (10 μl each in triplicate) were spread evenly onto solid BHI medium plates for aerobic incubation at 37 °C for 3 days, and the colonies were then counted.

For SEM observation of the precipitate after centrifugation, the precipitate was collected on a polycarbonate filter (Advantec, 0.8 μm in pore size) and immersed in a fixative (2% glutaraldehyde in PBS) for 2 h. The samples were dehydrated in graded ethanol and dried with CO_2 in a critical point dryer (Hitachi HCP-1). The cells were sputtered using a carbon coater and then examined by SEM.

3. Results and discussion

The adhesive activities of the CNTs were assessed by a bacterial precipitation assay using *S. mutans*. The results of the precipitation assay are presented in Fig. 1a and b. When the bacterial suspensions were mixed with the CNTs, there was loss in supernatant turbidity with an increase in the amount of CNTs. The results clearly show that CNTs have adhesive ability. These results regarding *S. mutans* adhesion to CNTs are in agreement with the previously reported results regarding bacterial adhesion to CNTs in the case of *E. coli* [7–10]. Among the carbon samples, the precipitation efficiency of 30-MWCNTs was the highest, with a maximum at 0.17 mg ml^{-1} concentration. SWCNTs were less effective because they were not easily dispersed

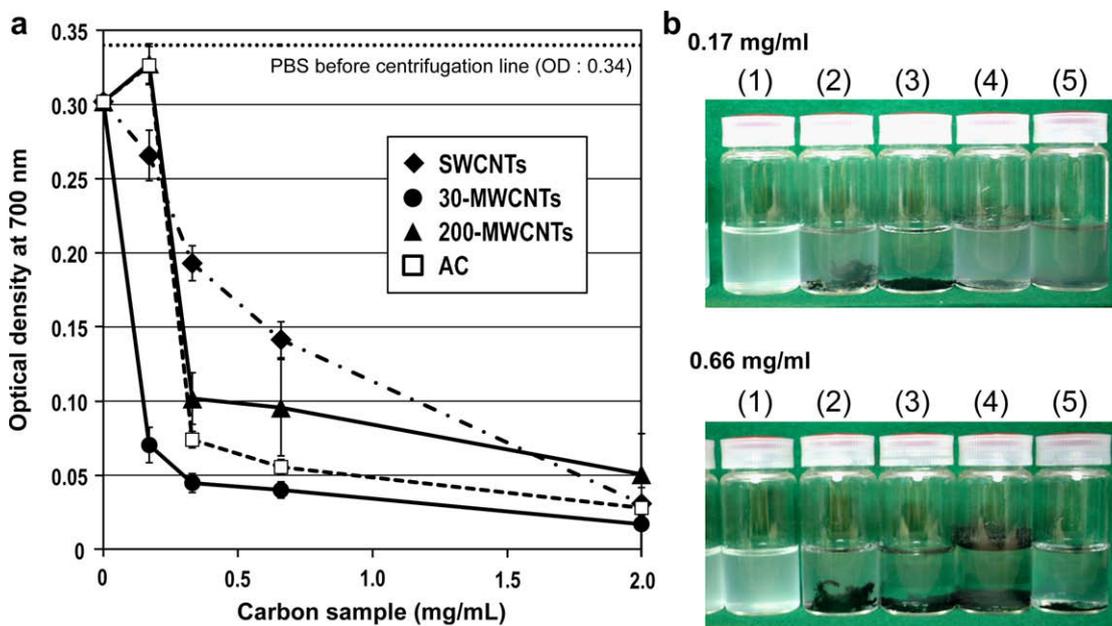


Fig. 1. (a) OD (at 700 nm) of the supernatant in precipitation assay with carbon samples. Results are presented as means \pm SE of three experiments. The upper dotted line is the OD value (0.34) of PBS before centrifugation (control). (b) Photographs of *S. mutans* mixed with carbon samples: PBS (b-1); SWCNTs (b-2); 30-MWCNTs (b-3); 200-MWCNTs (b-4); AC (b-5) after centrifugation.

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