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Author(s)	Akasaka, Tsukasa; Nakata, Keiko; Uo, Motohiro; Watari, Fumio
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Modification of the dentin surface by using carbon nanotubes

Tsukasa Akasaka *, Keiko Nakata, Motohiro Uo and Fumio Watari

Department of Biomedical, Dental Materials and Engineering, Graduate School of Dental Medicine,

Hokkaido University, Kita 13 Nishi 7, Kita-ku, Sapporo 060-8586, Japan

*Corresponding author :

Tsukasa Akasaka

Department of Biomedical, Dental Materials and Engineering, Graduate School of Dental Medicine,

Hokkaido University, Kita 13 Nishi 7, Kita-ku, Sapporo 060-8586, Japan

Tel.: +81 11 706 4251; Fax: +81 11 706 4251; E-mail: akasaka@den.hokudai.ac.jp.

Abstract. Recent studies have shown that carbon nanotubes (CNTs) can be used as biomedical materials because of their unique properties. CNTs effect nucleation of hydroxyapatite, because of which considerable interest has been generated regarding the use of CNTs in dentistry. However, there are only a few reports on the use of CNTs as dental materials. In this study, we investigated the changes induced in the surfaces of tooth slices by the application of a coating of CNTs by observing CNT-coated tooth slices both macroscopically as well as under a scanning electron microscope. Further, we investigated the effect of CNT coating on the tensile bond strength of dentin adhesives. CNTs adhered easily to the tooth surfaces when tooth slices were suspended in a CNT-dispersed solution. Interestingly, it was observed that CNTs selectively adhered to the surfaces of dentin and cementum, possibly by adhering to their exposed collagen fibers. In addition, the CNT coating did not affect the tensile bond strength of dentin adhesives. These results indicate that coating of the teeth with CNTs can be a possible application of CNTs as dental materials.

Keywords: Carbon nanotubes, coating, tooth slice, dentin, enamel, collagen, tensile bond strength

1. Introduction

Carbon nanotubes (CNTs) are attracting considerable attention because of their unique physical properties and various potential applications. Recent studies have shown that CNTs can be used as biomedical materials owing to their unique properties, since they can (i) improve the strength of composite materials [1] and implants [2], (ii) increase cell adhesion and proliferation [3], (iii) effect nucleation of hydroxyapatite [4], and (iv) provide protection against bacteria [5,6]. These properties of CNTs have generated interest regarding their use in dentistry. However, very few studies have assessed the applicability of CNTs in dentistry.

There is a continuing need for the development of better materials and methods for bonding composites and preventive and restorative resins to the enamel, dentin, and root surfaces of teeth [7]. Since recurrent caries after restorative treatment are frequent, the therapeutic effects of direct filling materials are being extensively investigated. Resin composites are not able to prevent recurrent caries, and several trials have been conducted to develop composites with antibacterial properties. Dental materials with methacryloyloxydodecylpyridium bromide incorporated into them exert antibacterial effects [8]. Further, fluoride-releasing composites possess both antibacterial and remineralizing properties, which are attributed to the release of fluoride ions [9,10].

In the present study, we investigated the changes induced in the surfaces of tooth slices by coating them with CNTs and the effect of this coating on the microtensile bond strength of dentin adhesives. We observed that CNTs adhered easily to the tooth slice surfaces when the slices were suspended in a CNT-dispersed solution. Interestingly, it was observed that CNTs selectively adhered to the surfaces of dentin and cementum, possibly by adhering to their exposed collagen fibers. Thus, we inferred that CNTs could strongly interact with collagen, but they did not interact with hydroxyapatite. In addition, CNT coating did not affect the tensile bond strength of dentin adhesives.

2. Materials and methods

2.1. Coating of tooth slices with CNTs

Multi-walled carbon nanotubes (MWNTs; average diameter, 200 nm), which were synthesized using the arc-discharge method, were obtained from MTR Ltd. (Ohio, USA). The raw MWNTs were refluxed in a 6 N HCl solution, washed thoroughly with deionized water, and then dried completely. The dominant impurity in MWNTs is amorphous carbon (<15%).

The tooth slices to be coated with CNTs were prepared by the following method. Human molars stored in isotonic saline at 4°C were cut under running water by using a diamond saw (Refine Saw; Refine Tec Ltd., Tokyo, Japan). The surfaces of the tooth slices were etched with 35% phosphoric acid for 1 min and rinsed well with deionized water. CNTs were dispersed at a concentration of 10 mg/L in deionized water by ultrasonication for 30 min, and the tooth slices were suspended in the CNT-dispersed solution. After 1 h of mixing at 500 rpm, the tooth slices were removed from the solution and were washed 3 times with deionized water to remove the unadsorbed CNTs.

For observation under a scanning electron microscope (SEM; S-4000; Hitachi, Japan), the tooth slices were fixed in a 2.5% glutaraldehyde solution and then dehydrated by critical-point drying at 37°C.

2.2. Interaction of CNTs with collagen

The interaction between CNTs and collagen was examined using an SEM. CNTs were dispersed at a concentration of 5 mg/L in 10 mL of deionized water by ultrasonication for 30 min. One milligram of collagen fibers (from bovine Achilles tendon; Nacalai Tesque Inc., Kyoto, Japan) was added to the

CNT-dispersed solution to yield a final concentration of 0.01% solution by weight; this solution was then ultrasonicated for 10 min. After 30 min of incubation, a small drop of the sample solution was placed onto a glass slide and dried. This slide was observed under the SEM after it was treated as mentioned above.

The interaction between CNTs and collagen was examined using a fluorescence confocal laser scanning microscope (CLSM). CNTs were dispersed at a concentration of 50 mg/L in 0.5 mL of 10 mM phosphate-buffered saline (PBS, pH 7.2) by ultrasonication for 30 min. One milliliter of fluorescein isothiocyanate-labeled type-I collagen (FITC-collagen; 1 mg/mL; Cosmo Bio Co., Ltd. Tokyo, Japan) was added to the CNT-dispersed solution to yield a final concentration of a 0.01% solution by weight; this solution was then ultrasonicated for 15 min. After 30 min of incubation, the mixture was centrifuged at 1,000 *g* for 5 min. The aggregated CNTs were then carefully washed 3 times with PBS and 2 times with deionized water to remove the unadsorbed collagen. A small drop of the sample solution was placed on a glass slide. This slide was then observed under a Carl Zeiss LSM 410 Axiovert microscope (Carl Zeiss, Oberkochen, Germany) in transmitted-light mode and fluorescence modes with a 20× objective and at a zoom factor of 4.8. FITC was excited by a 488-nm argon beam, and emission was recorded between 515 and 565 nm.

2.3. Microtensile bond strength test

To estimate the effect of CNT coating on tensile bond strength, we performed a microtensile bond strength test.

Fig. 1 shows a schematic diagram of specimen preparation for microtensile bond strength test. First, the crown of a tooth was cut horizontal to the tooth axis under running water using a diamond saw. The exposed surface of the tooth was abraded with silicon carbide paper up to # 600 grit, and the tooth was then perpendicularly divided into 2 parts. The dentin was etched with K-etchant gel (Kuraray Medical, Tokyo, Japan) for 30 s and thoroughly washed with water. Powdered CNTs were directly applied to the surface of the etched dentin, after which the dentin was washed with water to remove the unadsorbed CNTs. Nontreated etched dentin was used as a control for this test. Next, the surface of the CNT-coated dentin was immediately applied to a dentin adhesive system (Clearfil Liner Bond II Σ; Kuraray Medical). A composite resin (CR; Clearfil AP-X; Kuraray Medical) was applied to the entire surface of the bonded dentin. Each material was used according to the manufacturer's instructions except the K-etchant gel. Each increment was light cured (JETLITE 3000; J. Morita Manufacturing Corp., Kyoto, Japan) for 40 s. Finally, the specimens were sectioned perpendicular to the dentin adhesive-tooth interface by using a slow-speed diamond saw to yield rectangular sticks with a width of 1 mm × 1 mm. The prepared specimens were used in the microtensile bond strength test.

The microtensile bond strength test was performed according to the method proposed by Sano *et al.* [11]. The specimens were loaded in a universal testing machine (EZ test; Shimadzu, Tokyo, Japan) at a cross-head speed of 1 mm/min. The values are represented as means and standard deviations of the bond strengths. Student's *t* test was used to assess the statistical significance of the results. All statistical analyses were performed at a confidence level of 95% by using the Microsoft Excel software.

3. Results and discussion

3.1. Coating of tooth slices with CNTs

To coat the surfaces of the tooth slices with CNTs, the tooth slices were immersed into a CNT-dispersed solution. Macroscopic examination revealed that the surface of the nontreated tooth slice remained white (Fig. 2a). In contrast, the dentin surface of the CNT-coated tooth slice had become gray, although the color of the enamel had not changed (Fig. 2b and 2c). The color of the dentin surface may have changed due to the selective attachment of CNTs to the dentin. These findings indicate that the dentin of teeth can be easily coated with CNTs by suspending the teeth in a CNT-dispersed solution.

For morphological examination of the CNTs attached to the surfaces of the tooth slices, the slices were observed under an SEM. The dentin, enamel, and cementum of the CNT-coated tooth slices after are shown in Fig. 3a, 3b, and 3c, respectively. We observed that the CNTs attached to the surfaces of dentin and cementum but not to the surface of enamel. These results suggest that CNTs attach selectively to the surfaces of dentin and cementum in teeth. The lower row of Fig. 3 shows the CNT-coated dentin surface at various levels of SEM magnification. CNTs adhere to the surface of the dentin but cannot penetrate into the dentin tubules (Fig. 3d and 3e). Fig. 3f shows that on observation under high magnification levels, CNTs appeared to adhere to the collagen fibers exposed from the etched dentin. Dentin and cementum are known to be composed of minerals, an organic matrix, and water. A major part of the organic matrix is believed to contain collagen [12,13]. After demineralization by acid etching, many collagen fibers are exposed from the surfaces of dentin and cementum. CNTs could adhere to the surfaces of the dentin and cementum in teeth by interacting with the exposed collagen fibers. On the other hand, the enamel is known to be composed mainly of minerals; the organic matrix is only a small component of the enamel (approximately 1%) [12]. These results suggest that CNTs interact with the organic matrix, which mainly contains collagen, and that they do not interact with minerals like hydroxyapatite.

3.2. Interaction of CNTs with collagen

In order to directly observe the interaction between CNTs and collagen, we performed collagen adsorption tests. For observation under an SEM, CNTs were mixed with a solution containing swollen collagen fibers. An aliquot of the solution was dried on a glass slide, which was observed under an SEM. The black and white fibers in Fig. 4a indicate collagen fibers and CNTs, respectively. The images obtained revealed that the CNTs were localized on the collagen fibers. This localization implies that the CNTs interact strongly and blend with swollen collagen fibers. For observation under a fluorescence CLSM, CNTs were mixed with soluble FITC-collagen. The interaction between CNTs and soluble FITC-collagen was confirmed at a microscopic level by the observation of small aggregates of CNTs scattered on the slide, which were faintly visible under the microscope. Transmitted light and fluorescence images of these aggregates after centrifugation are shown in Fig. 4b and 4c, respectively. The images show that the CNT aggregates were coated with FITC-collagen, and a dense FITC-collagen localization was observed around the aggregated CNTs. This localization implies that CNTs interact strongly with soluble collagen.

The abovementioned findings are in agreement with those of previous studies, which have reported a strong interaction between CNTs and collagen fibers in an aqueous medium [14–16]. However, the exact nature of these interactions was not clear. In general, CNTs have the ability to adsorb nonspecifically various proteins [17]. Proteins in solution are known to adsorb CNTs via hydrophobic and π–π interactions [18]. Therefore, CNTs probably interact with collagen via the same interactions. These results support our findings that CNTs interact with the collagen fibers exposed from the dentin and cementum in teeth.

3.3. Microtensile bond strength test

Fig. 1 shows a schematic diagram of specimen preparation for microtensile bond strength test. The tensile bond strengths of the specimens are shown in Fig. 5. The bond strengths of the dentin adhesive in the CNT-coated and nontreated (control) specimens were 38.3 ± 13.9 MPa and 42.4 ± 11.7 MPa, respectively. The bond strength of dentin adhesive in the CNT-coated specimen was similar to that of the adhesive in the nontreated specimen. No significant difference was observed in the bond strengths of the between CNT-coated and nontreated specimens ($p > 0.05$).

The bonding mechanism is believed to be dependent not only on the collagen fibers but also on the roughness of the surface, extent of penetration of the bonding resin into the collagen matrix, and possible chemical interactions at the resin-dentin interface. In addition, the penetration of the resin tags into the dentin tubules and intertubular anastomoses are important [19].

The bond strength of the CNT-collagen interface was probably weak because collagen comprises only soft materials. In addition, CNTs could not penetrate the dentin tubules (Fig. 3d and 3e); they formed a thin layer on the network of collagen fibers. Thus, CNTs did not affect the bond strength even though the required amount of CNT coating was applied. These findings raise the possibility that CNTs can exert beneficial effects such as nucleation of hydroxyapatite and protection against dental bacteria without decreasing the composite strength of the restorative materials.

4. Conclusions

In this study, we investigated the changes induced in the surface of tooth slices by the application of CNT coating. The CNTs adhered easily to the surface of the tooth slice. Interestingly, it was observed that CNTs could selectively coat the surfaces of dentin and cementum and appeared to adhere to the collagen fibers exposed from dentin and cementum. In addition, CNT coating did not affect the tensile bond strength of the dentin adhesives. These results indicate that coating of the teeth with CNTs could be a possible application of CNTs as dental materials.

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Figure captions:

Fig. 1. Schematic diagram of specimen preparation for use in microtensile bond strength test.

Fig. 2. Photographs of tooth slices coated with CNTs.

(a) Nontreated tooth slice (control), (b) transverse view of CNT-coated tooth slice, and (c) sagittal view of CNT-coated tooth slice.

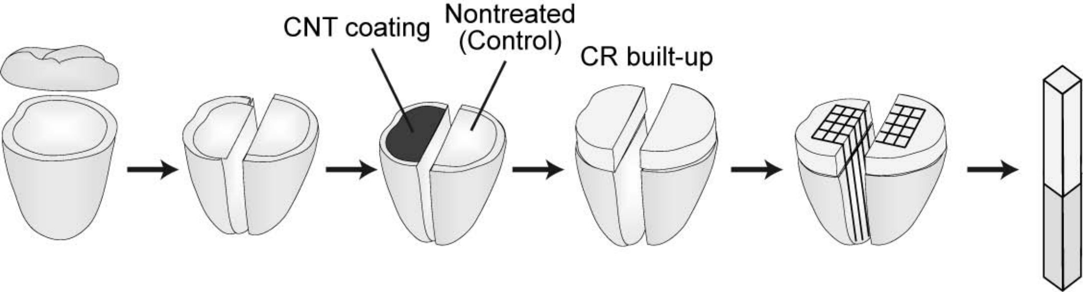
Fig. 3. Upper row: SEM images of the surface of (a) dentin, (b) enamel, and (c) cementum of a tooth after coating it with CNT. Lower row (d–f): SEM images of the surface of CNT-coated dentin observed at various levels of magnification. White arrow indicates CNTs.

Fig. 4. Characterization of the interaction between CNTs and collagen.

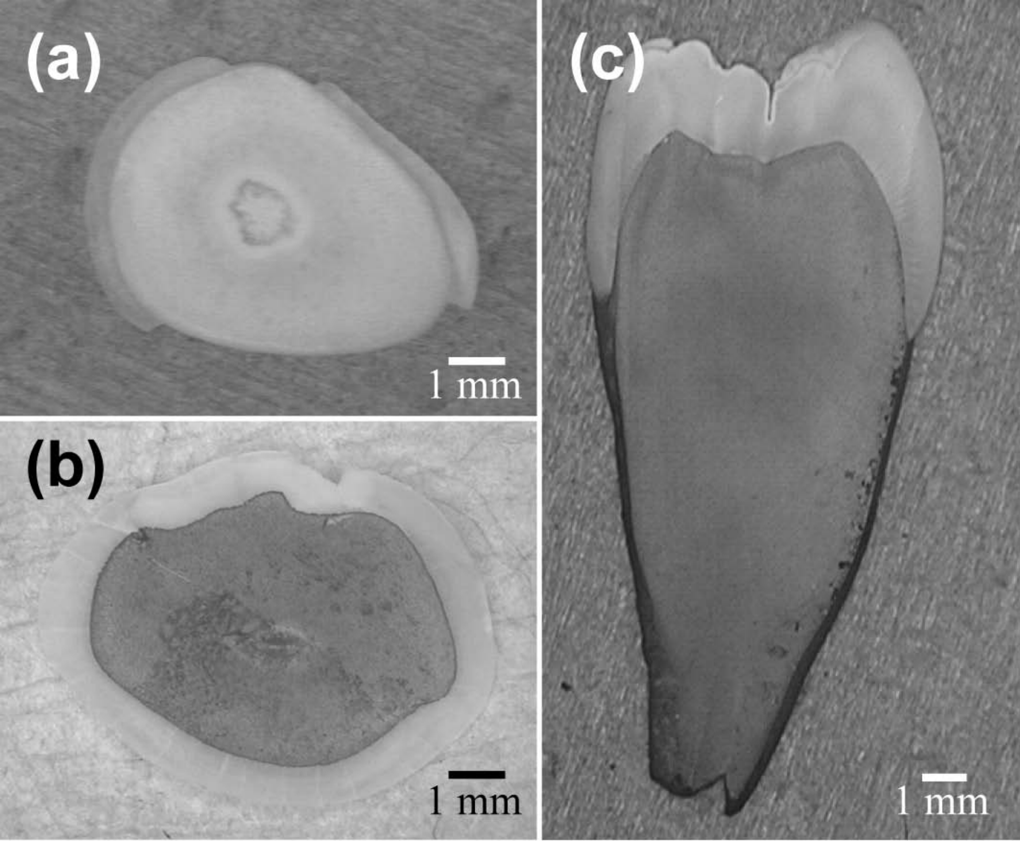
(a) SEM images of swollen collagen fibers with adsorbed CNTs. White and black fibers indicate CNTs and collagen fibers, respectively. CLSM images of CNT aggregates adsorbed on soluble FITC-collagen observed from (b) transmittance channel and (c) FITC channel.

Fig. 5. Microtensile bond strength of dentin adhesive after the application of CNT-coating.

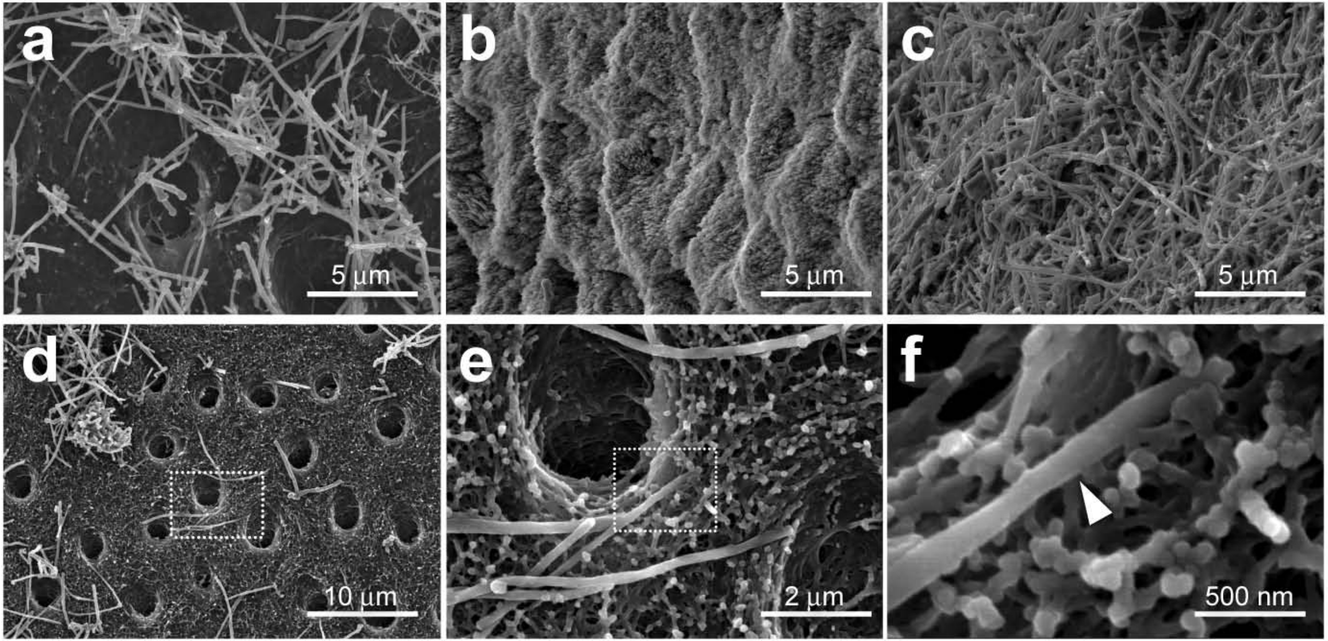
CNT coated specimens ($n = 15$) and nontreated specimens (control, $n = 11$)

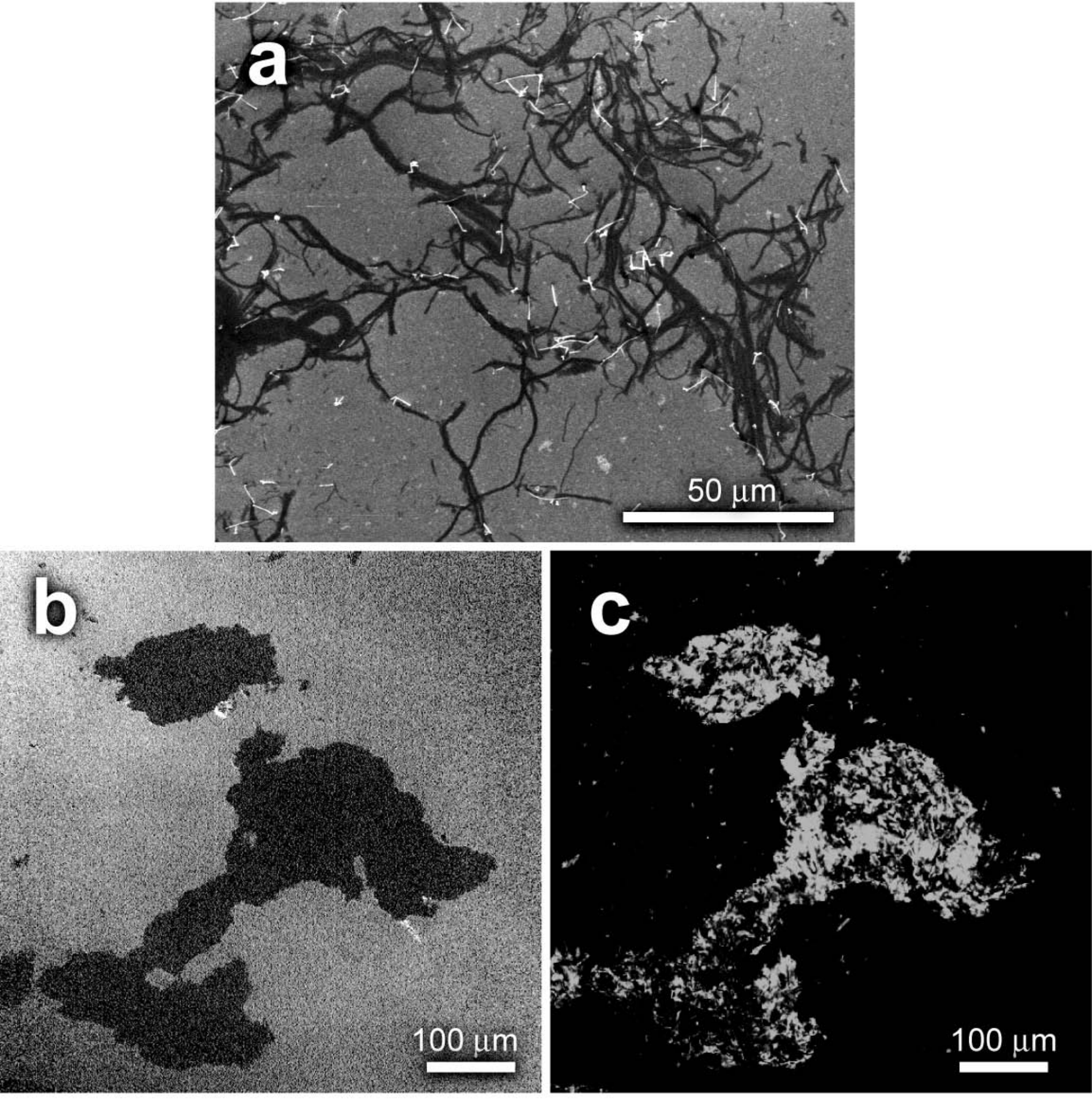


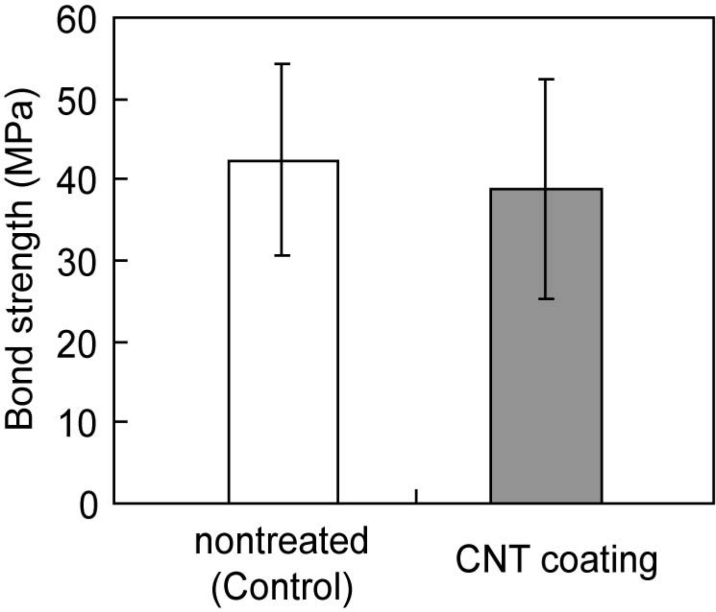
CNT coating
Nontreated
(Control)
CR built-up



(a)
1 mm
(b)
1 mm
(c)
1 mm


a
5 µm
b
5 µm
c
5 µm
d
10 µm
e
2 µm
f
500 nm


a
50 μm
b
100 μm
c
100 μm


Bond strength (MPa)
60
50
40
30
20
10
0
nontreated
(Control)
CNT coating