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Multiwalled carbon nanotube coating on titanium

Michiko Terada [a,*], Shigeaki Abe [b], Tsukasa Akasaka [b], Motohiro Uo [b], Yoshimasa Kitagawa [a] and Fumio Watari [b]

[a] Oral Diagnosis and Oral Medicine, Department of Oral Pathobiological Science, Graduate School of Dental Medicine, Hokkaido University
[b] Department of Biomedical, Dental Materials and Engineering, Division of Oral Health Science, Graduate School of Dental Medicine, Hokkaido University, Sapporo 060-8586, Japan

(*Corresponding author)
Address: Kita-13, Nishi-7, Kitaku, Sapporo 060-8586, Japan
Phone/fax: +81-11-706-4280

Abstract. Carbon nanotubes (CNTs) have excellent chemical durability, mechanical strength, and electrical properties. Therefore, there is interest in CNTs for not only electrical and mechanical applications, but also biological and medical applications. We coated titanium, a common material for dental implants, with multiwalled carbon nanotubes (MWCNTs). First, titanium was aminated and covered with collagen. Then, the carboxylated MWCNTs were coated onto the collagen attached to the titanium plate. The collagen-coated titanium plate had a homogeneous MWCNT coating, which showed strong attachment to the titanium surface as a thin layer. The surface roughness was significantly increased with the MWCNT coating. MC3T3-E1 cells were cultured on the MWCNT-coated Ti plate, and showed good cell proliferation and strong cell adhesion. Therefore, the MWCNT coating for titanium could be useful for improvement of cell adhesion on titanium implants.
Key words: multiwalled carbon nanotubes (MWCNTs), collagen, titanium, cell adhesion, surface treatment

1. Introduction

There is a great deal of research interest in periodontal ligament-attached dental implants in the study of dental implants. Ordinary dental implants surface do not have the periodontal ligament which plays an important role in shock absorption and in sensing mastication force. To combine the periodontal ligament and dental implant, surface modifications of the implant materials, *e.g.*, titanium or hydroxyapatite, have been studied [1,2]. The surface texture and properties of the dental implant affect the strong bonding and stable proliferation of periodontal ligament cells.

On the other hand, carbon nanotubes (CNTs) have excellent chemical durability, mechanical strength, and electrical properties, and therefore they are of interest in not only electrical and mechanical applications but also for biological and medical applications. At present, applications of CNTs for cell culture [3–7], drug delivery systems [8], implant materials [9], and osteogenesis [10] have been reported. Good cell affinity and proliferation had been reported for carbon nanofibers [3], single-walled carbon nanotubes (SWCNTs) [4,6], and multi-walled carbon nanotubes (MWCNTs) [5,7]. Especially strong adhesion of cells to CNTs has been reported previously [5–6].

Previously, the authors prepared MWCNT-coated cell culture dishes. Good cell proliferation and strong cell adhesion were observed on the surfaces of collagen-coated dishes with a homogeneous coating of MWCNTs [7]. This cell adhesion feature of CNTs would be applicable for the periodontal ligament combination on dental implants.

In this study, we applied the above MWCNT coating technique to titanium, which is a commonly used material for dental implants, to improve cell adhesion to the dental implant surface.

2. Materials and methods

2.1. Specimen preparation

The titanium plate (99.5%, 1 mm in thickness; Nilaco Co. Ltd., Tokyo, Japan) was polished and cut into pieces of 16×5 mm. A number of the polished Ti plates were treated with 10 w/v% of 3-aminopropyltriethoxysilane (Tokyo Chemical Industry, Tokyo, Japan) in toluene solution at 80°C for 12 h. The aminated Ti plates were soaked in atelocollagen solution (0.1 w/v%; Koken, Tokyo, Japan) at 4°C for 3 h. They were then rinsed with deionized water, and desiccated at room temperature; these were designated as “collagen-coated Ti plates.” MWCNTs (several μm to several ten μm in length and 20–30 nm in diameter; CNT Co. Ltd., Incheon, Korea) were purified by oxidation at 500°C for 90 min and treated in concentrated hydrochloric acid to remove the impurities, e.g. hydrocarbon, amorphous carbon and metallic nanoparticles. The purified MWCNTs were carboxylated to improve their dispersion in aqueous solution by the methods of Peng et al. [11]. The carboxylated MWCNTs were dispersed in sodium cholate (1 w/v%) aqueous solution [12] to a final concentration of 100

ppm with sonication for 90 min. The obtained MWCNT suspension (2 ml/dish) was poured onto the above collagen-coated Ti plate and kept at room temperature for 3 h. It was then rinsed with deionized water and dried. These three different types of Ti plate were employed for the following cell culture experiments. Hereafter, collagen-coated Ti plates treated with the MWCNT suspension are referred to as "MWCNT-coated Ti plates."

2.2. SEM Observation and surface roughness measurement

The surface structure of polished, collagen-coated, and MWCNT-coated Ti plates was estimated by scanning electron microscopy (SEM) (S-4000; Hitachi, Tokyo, Japan). The surface roughness was estimated using a surface roughness meter (Surfcom 130A; Tokyo Seimitsu, Tokyo, Japan).

For observation of cross-sections of MWCNT- and collagen-coated layers, a cover glass was used as a substitute for the Ti plate. The MWCNT- and collagen-coated cover glass was cracked carefully and the cross-section was observed by SEM to estimate the thickness and condition of collagen on the MWCNT-coated Ti plate.

2.3. Cell proliferation and adhesion estimation

Mouse osteoblast-like MC3T3-E1 cells were seeded onto three types of Ti plate at a cell density of 8×10^3 cells/plate. The cells were cultured in α-MEM (Gibco, Grand Island, NY) with 10% FBS (Biowest, Miami, FL) and PSN Antibiotic Mixture (Gibco) at 37°C in a humidified atmosphere of 5% CO_2 for 24, 48, and 72 h. The cell morphology and population were then observed by SEM, and cell proliferation on the Ti plates was estimated.

Cell adhesion was estimated by treatment using diluted trypsin-EDTA solution (Gibco), which is generally used to detach cells in subculture. The MC3T3-E1 cells were cultured until they reached confluence on three types of Ti plate and treated with 0.02% trypsin-EDTA solution. The decrease in number of attached cells with treatment time was evaluated by SEM.

3. Results

3.1. SEM images and surface roughness

Figure 1 shows SEM images of the polished, collagen-coated, and MWCNT-coated Ti plate surfaces. The Ti plate (Fig. 1-A) showed an almost flat surface with some small grooves. The collagen-coated Ti plate surface showed some small aggregates of collagen (Fig. 1-B). As shown in Fig. 1-C, on the MWCNT-coated Ti plate surface the MWCNTs formed a homogeneous covering over the collagen-coated Ti plate surface without aggregation. Figure 1-D shows a cross-section of a cover glass coated with collagen and MWCNTs using the same method as used for Ti plate coating. The collagen coating on the substrate surface had a

thickness of 150–300 nm, on top of which the MWCNTs were coated as a thin layer (several ten μm).

Figure 2 shows the surface profiles of the three types of Ti plate. The polished and collagen-coated Ti plates showed similar surface roughness, while the MWCNT-coated Ti plates showed a rougher profile. The surface SEM images (Fig. 1) indicated that the MWCNTs generated several ten nanometer-scale roughness, which was a diameter of MWCNTs, on the coated Ti plates. The mean surface roughness (Ra) values of these Ti plates are shown in Fig. 3. The estimated Ra of MWCNT-coated Ti plates was 0.13 ± 0.01 μm, which was significantly greater than those of polished (Ra = 0.05 ± 0.01 μm) and collagen-coated Ti plates (Ra = 0.05 ± 0.01 μm) (n=3, $P<0.05$, *t*-test). Thus, the MWCNT coating introduced several ten nanometer-scales to sub-micrometer-scale roughness on the titanium surface.

3.2. Cell proliferation and adhesion

Figure 4 shows SEM images of the cultured E1 cells on polished, collagen-coated, and MWCNT-coated Ti plates. The cells on the polished and collagen-coated Ti plates were spread out on the plates and became confluent after 72 h of cultivation. However, the cytoplasm of the cells on MWCNT-coated Ti plates was less spread out. Figure 5 shows SEM images of the filopodia of E1 cells on MWCNT-coated Ti plates. The filopodia were observed the ends of which appeared to make contact with MWCNTs.

Figure 6 shows the cell proliferation on polished, collagen-coated, and MWCNT-coated Ti plates. The all cells were detached by the trypsin-EDTA treatment and counted by the cytometry. The cell number on the each Ti plates was normalized by the cell number after 24 hours incubation. The cell number on each Ti plate increased constantly with incubation time, and the collagen-coated Ti plate showed the highest rate of cell proliferation. MWCNT-coated Ti plate showed slightly lower proliferation than the other Ti plates. However, the cells on MWCNT-coated Ti plates also proliferated constantly until reaching confluence.

Figure 7 shows SEM images of residual cells on the collagen- and MWCNT-coated Ti plate surface with trypsin-EDTA treatment for 10 min. The cells on the collagen-coated Ti plates were perfectly detached with trypsin-EDTA treatment. However, many cells remained attached to the MWCNT-coated Ti plate surface after 10 min of treatment. The retained cell percentage was about 9%. Many filopodia protruding from cells seemed to adhere to the surface of MWCNT-coated Ti plates, which would assist in strong cell adhesion.

4. Discussion

CNTs have a fibrous structure several to several tens of nanometers in length. Strong cell adhesion onto CNTs has been reported [5–7]. The reason for this strong cell adhesion was suggested to be mechanical binding between the cell surface or filopodia and protein

absorption on CNTs [13]. Therefore, CNTs would be candidate materials for surface treatment of implants.

Previously, we prepared MWCNT-coated cell culture dishes using carboxylated MWCNTs/sodium cholate solution. The MWCNTs adhered strongly to the surface of collagen-coated dishes due to the strong interaction between CNTs and collagen. Good cell proliferation and quite strong adhesion were obtained on the surface of MWCNT-coated dishes. In the present study, we applied the previous method for titanium coating with MWCNTs.

First, the titanium surface was aminated by covalent bonding with the collagen. The MWCNTs were dispersed on the collagen-coated titanium surface and homogeneous coating of MWCNTs on the titanium was achieved. The thickness of the collagen layer was estimated to be about 150–300 nm and MWCNTs were attached to the collagen as a thin layer (Fig. 1D). The surface roughness was significantly increased with MWCNT coating. Coated MWCNTs and the collagen layer did not detach during the ordinary cell culture procedures. McDonald *et al.* reported mechanical binding between single-walled carbon nanotubes caused by their entanglement [4]. MWCNTs would interact strongly with the collagen by the same mechanism, and the collagen was covalently bonded onto the aminated titanium surface. Therefore, titanium plates tightly coated with MWCNTs were successfully prepared. The cell numbers on MWCNT-coated Ti plates increased constantly with incubation time, with a rate of proliferation slightly lower than those of the polished and collagen-coated Ti plates. Titanium and collagen are well known to be biocompatible materials, and thus the rate of cell proliferation on MWCNT-coated Ti plates was not particularly low. Cells remained attached to the MWCNT-coated Ti plates after trypsin-EDTA treatment, which is commonly used for detachment of cells from the substrate. These observations indicated strong cell adhesion on the MWCNT-coated surface. The strong cell adhesion on MWCNT-coated Ti plate would slightly inhibit the cell locomotion while the cell division. Then, the cell proliferation on MWCNT-coated dish would be slightly suppressed comparing to the other plates.

The effects of the texture and chemical properties of the implant surface on cell affinity and bone regeneration have been studied [14,15]. Keller *et al.* [14] reported that sandblasted and acid-etched titanium surfaces showed improved osteoblast cell attachment. They concluded that the increases in surface roughness and surface area by these treatments contributed to the absorption of extracellular matrix components and cell attachment on the titanium surface. The MWCNT-coated Ti plates prepared in the present study had several ten nanometer-scales to sub-micrometer-scale roughness (Ra) due to the attached thin layer of MWCNTs. In addition, carboxylated MWCNTs would have absorption properties for some types of protein compared to untreated MWCNTs [13]. The MWCNT coating on the titanium surface would assist protein absorption and cell adhesion. Aoki *et al.* and the authors previously reported strong cell adhesion on MWCNTs [5–7]. In these previous studies, the strong cell adhesion was suggested to be due to the mechanical binding between MWCNTs and the cell surface

and filopodia. The cell adhesion of the present MWCNT-coated Ti plates would be due to the same mechanism.

5. Conclusions

The MWCNTs were homogeneously coated on the collagen-coated titanium plates. The coated MWCNTs were attached strongly on the collagen-coated surface as a thin layer. The mean surface roughness (Ra) of MWCNT-coated plates, 0.13 μm, was significantly increased in comparison with the value of 0.05 μm for polished and collagen-coated Ti plates. The cell proliferation on MWCNT-coated Ti plates was slightly lower than those of the polished and collagen-coated Ti plates, but the cells showed constant proliferation. The cell adhesion on the MWCNT-coated Ti plate was stronger than on the other plates. Therefore, the MWCNT coating on the titanium was suggested to be useful improving cell attachment on titanium implants.

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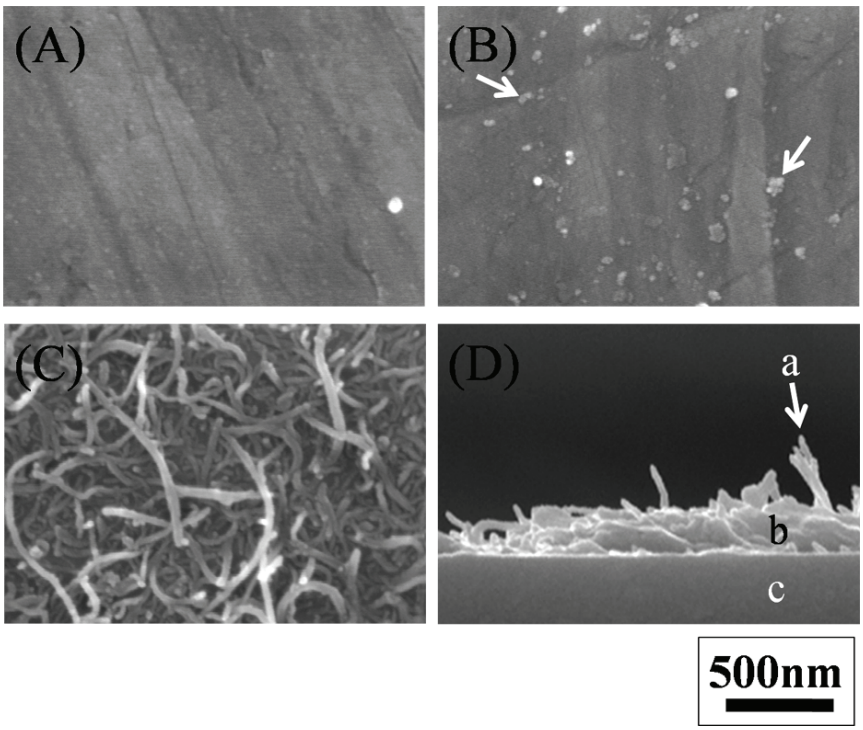

Fig. 1 SEM images of the three types of plate surface

A: polished Ti plate; B: collagen-coated Ti plate (arrow: aggregation of collagen); C: MWCNT-coated Ti plate; D: SEM images of cross-section of the cover glass treated with collagen (a: MWCNTs; b: collagen; c: cover glass).

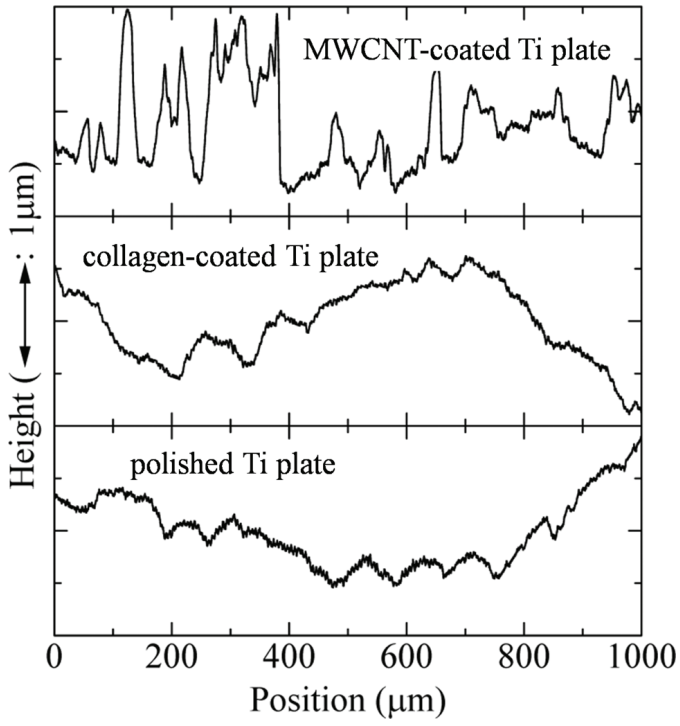

Fig. 2 Surface roughness of the three types of plates

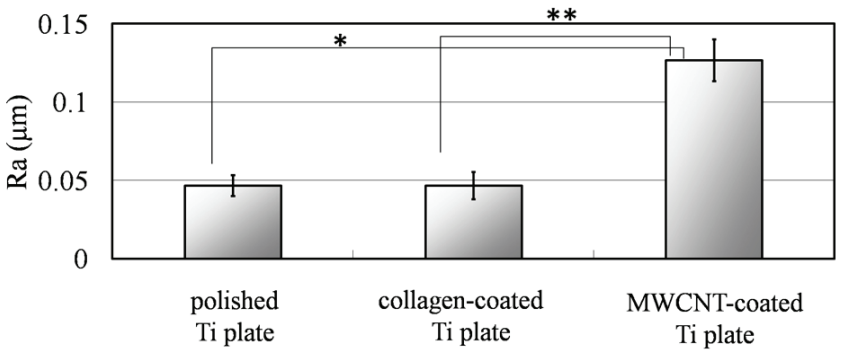

Fig. 3 Ra of polished, collagen-coated, and MWCNT-coated Ti plate surfaces

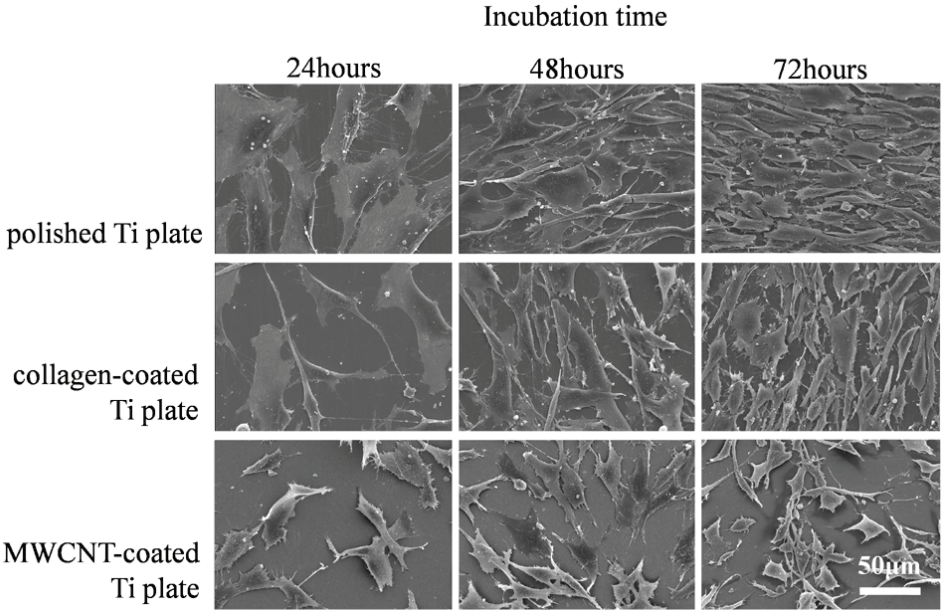

Fig. 4 SEM image of MC3T3-E1 cells on the surfaces of three types of Ti plate at various incubation times

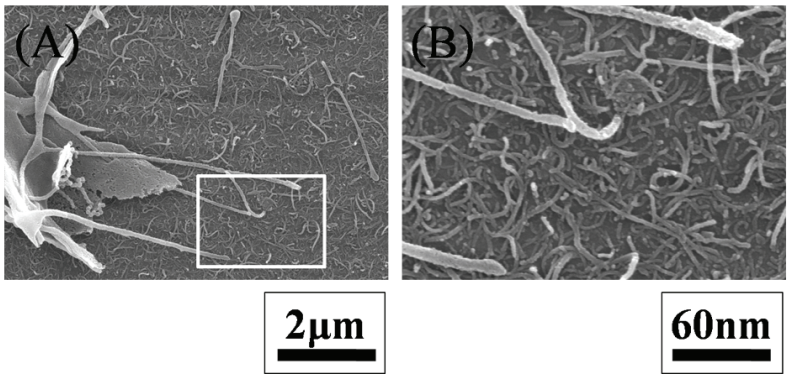

Fig. 5 SEM images of the filopodia of MC3T3-E1 cells on the surface of the MWCNT-coated Ti plate at 24 hours after incubation

A: low magnification view; B: enlargement of the square in A.

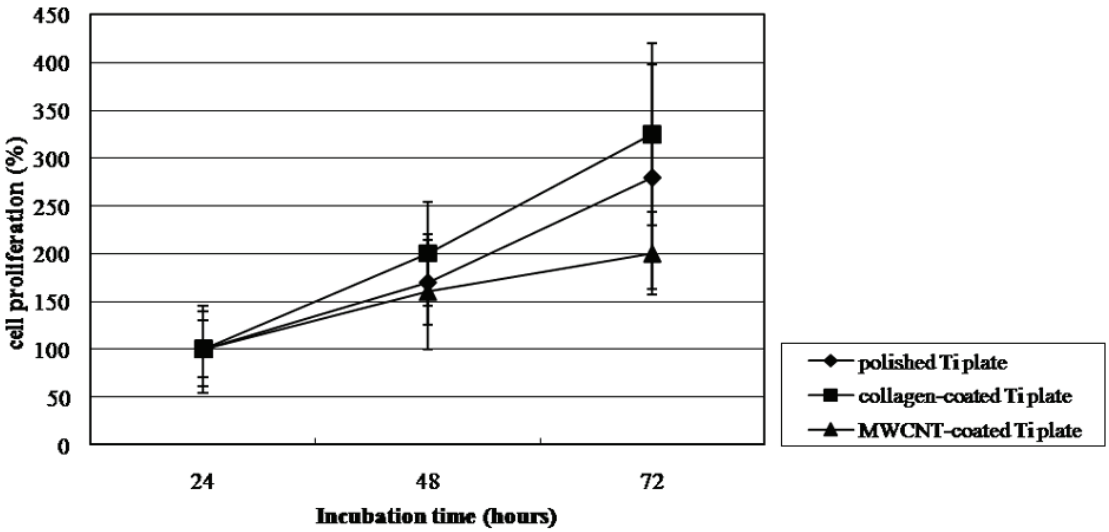

Fig. 6 Quantification of MC3T3-E1 cell growth on polished, collagen-coated and MWCNT-coated Ti plates at various incubation times

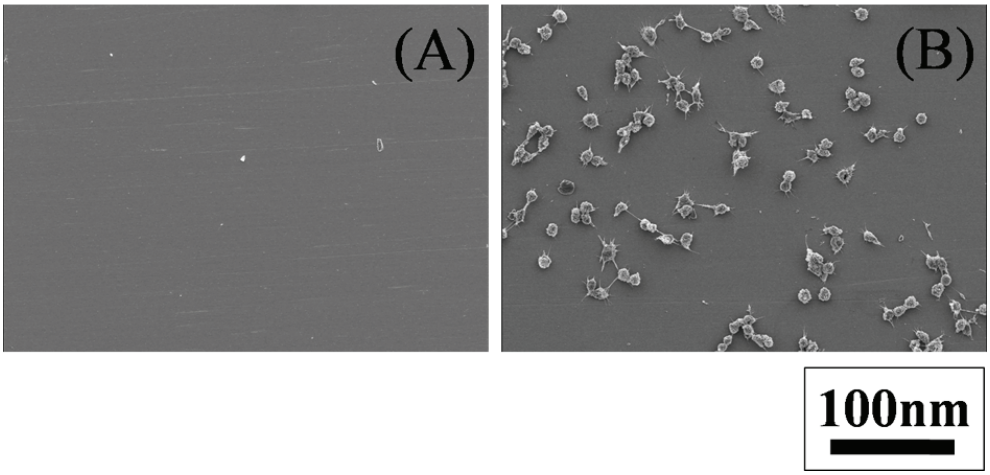

Fig. 7 SEM images of MC3T3-E1 cells on collagen- and MWCNT-coated Ti plate with 0.02% trypsin-EDTA for 10 min