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Research Article

Deposition of Apatite on Carbon Nanofibers in Simulated Body Fluid

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Carbon nanofibers (CNFs) were soaked in 1.5 simulated body fluid (1.5 SBF) in which inorganic ion concentrations are 1.5 times as high as those in the standard SBF. The influence of the CNFs content in 1.5 SBF and pretreatment of the CNFs on the biomimetical deposition of apatite were investigated. The spherical bone-like apatite particles were deposited on the pristine CNFs soaked in 1.5 SBF. Amount of deposited apatite per a unit of CNFs increased with a decrease in the CNFs content in 1.5 SBF, and it decreased markedly when the CNFs were pretreated with concentrated sulfuric acid/nitric acid (3 : 1 v/v) mixture for longer periods. Such results suggest that too many nucleation sites of apatite, which were functional groups, such as carboxyl and hydroxyl groups, existed on the CNFs in the 1.5 SBF, and most embryos formed on the sites could not grow to critical nuclei and furthermore did not grow to apatite.

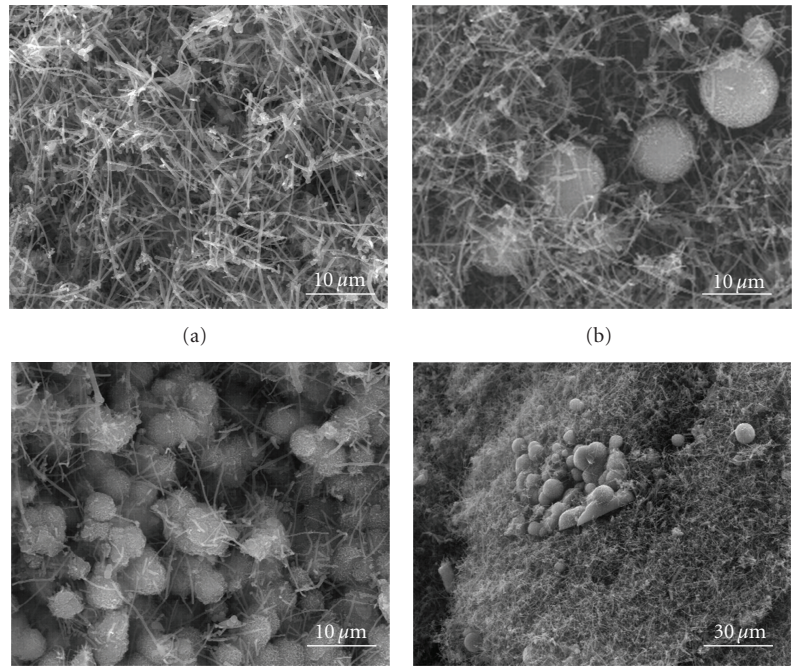
1. Introduction

Carbon nanotubes (CNTs) have exceptional mechanical [1–4], thermal [2, 5], and electrical properties [6, 7] and are recognized as incredible nanomaterials. Due to such superior properties, CNTs are considered to be very useful reinforcements or additives in various materials, such as plastics, metals, and ceramics, to improve properties of the materials and introduce novel functionalities.

In the medical field, particularly orthopedics, CNTs are anticipated to be of use as reinforcements of various biomaterials. For instance, the mechanical strength and fracture toughness of artificial bone made of hydroxyapatite ceramics will be enhanced by combining with CNTs. In addition, CNTs have good bone-tissue compatibility, accelerate bone formation in response to rhBMP-2 [8], and promote the proliferation of osteoblastic cells [9, 10]. Therefore, CNTs are expected to be uses as scaffolds to promote and guide bone-tissue regeneration.

On the other hand, it is known that apatite is biomimetically formed on the CNTs in simulated body fluid (SBF) and other solutions containing calcium and phosphate ions [11–16]. Functional groups, such as carboxyl group (−COOH), on CNTs act as nucleation sites of apatite, which induce the deposition of apatite. However, their reported apatite crystals were different in the morphology, and the deposition processes of apatite are not clear sufficiently.

In this study, in order to make apatite deposit uniformly on carbon nanofibers (CNFs) which are a kind of multi-walled carbon nanotube, the CNFs were soaked in 1.5 SBF in which inorganic ion concentrations are 1.5 times as high as those in the standard SBF. And the influence of the CNFs



(a) (b) (c) (d)

Figure 1: SEM photographs of pristine CNFs soaked in 1.5 SBF for (a) 3 days, (b) 5 days, and (c) and (d) 7 days. (a), (b), and (c) Disentangled CNFs. (d) Entangled CNFs.

content in 1.5 SBF and pretreatment of the CNFs on the biomimetic deposition of apatite were investigated, and the deposition processes were discussed in this paper.

2. Experimental Procedure

2.1. Materials and Pretreatment. CNFs used in this study were vapor-grown carbon fibers (VGCFs, diameter: 150 nm, length: 10–20 μm, Showa Denko, Japan). The pretreatment of the CNFs was carried out as follows: 1 g of the CNFs was refluxed for 24 h in 100 mL of concentrated nitric acid or phosphoric acid solutions (which are shown as CNFs pretreated with nitric acid or phosphoric acid), was sonicated in 100 mL of concentrated sulfuric acid/nitric acid (3 : 1 v/v) mixture for 3, 6, and 24 h (which are shown as CNFs pretreated with mixed acid for 3, 6, and 24 h), or was dispersed in 100 mL of 1 M NaOH, 1 M $CaCl_2$, or 25% ammonia solutions by ultrasonic (which are shown as CNFs pretreated with NaOH, $CaCl_2$, or NH_3 solutions). The CNFs pretreated with NaOH, $CaCl_2$, or NH_3 solutions were furthermore soaked in their solutions for 24 h. The pretreated CNFs were filtrated, washed with distilled water, and dried at 60°C. They were observed using a scanning electron microscope (SEM) and analyzed using a laser Raman spectrophotometer and a Fourier transmission infrared (FT-IR) spectrophotometer.

2.2. Deposition of Apatite on CNFs in 1.5 SBF. 0.0025–0.1 g of the pristine CNFs and the pretreated CNFs were dispersed in 100 mL of 1.5 SBF (Na^+ 213.0, K^+ 7.5, Mg^{2+} 2.3, Ca^{2+} 3.8, Cl^- 221.7, HCO_3^- 6.3, HPO_4^{2-} 1.5, and SO_4^{2-} 0.8 mM/L) at pH 7.25 by ultrasonic and then were soaked in the swinging 1.5 SBF at 37°C for predetermined periods of 1 day to 14 days.

After soaking in 1.5 SBF for predetermined periods, the CNFs were filtrated, washed with distilled water, and dried at 60°C. The dried CNFs were observed using an SEM and a transmission electron microscopy (TEM) and analyzed using an X-ray diffraction (XRD) analyzer and an FT-IR spectrophotometer. And they were fired at 1000°C for 1 h in air. By the firing, the CNFs were completely burned, and the deposits formed during soaking in 1.5 SBF remained as the leavings. Therefore, amount of the deposits was determined by measuring the weights of the specimens before and after the firing.

3. Results and Discussion

3.1. Deposition of Apatite on Pristine CNFs. 0.01 g of the pristine CNFs was soaked in 100 mL of 1.5 SBF for 1 day to 7 days. SEM photographs of the CNTs are shown in Figure 1. The spherical particles, which consisted of many leaf-like crystals, were observed on the CNFs soaked in 1.5 SBF for 5 days (Figure 1(b)), and they increased in the amount after 2 days (Figure 1(c)). The XRD pattern and FT-IR spectrum of pristine CNFs soaked in 1.5 SBF for 7 days are shown in Figure 2. The small and broad diffraction peak appeared at around 32° in the XRD pattern, which did not result from the CNFs and could be identified as hydroxyapatite. The absorption peaks at around 1050, 950, 600, and 550 cm^{-1} in the FT-IR spectrum originated in PO_4^{3-} of the hydroxyapatite, while those at around 1450 and 900 cm^{-1} originated in CO_3^{2-}. These results indicate that

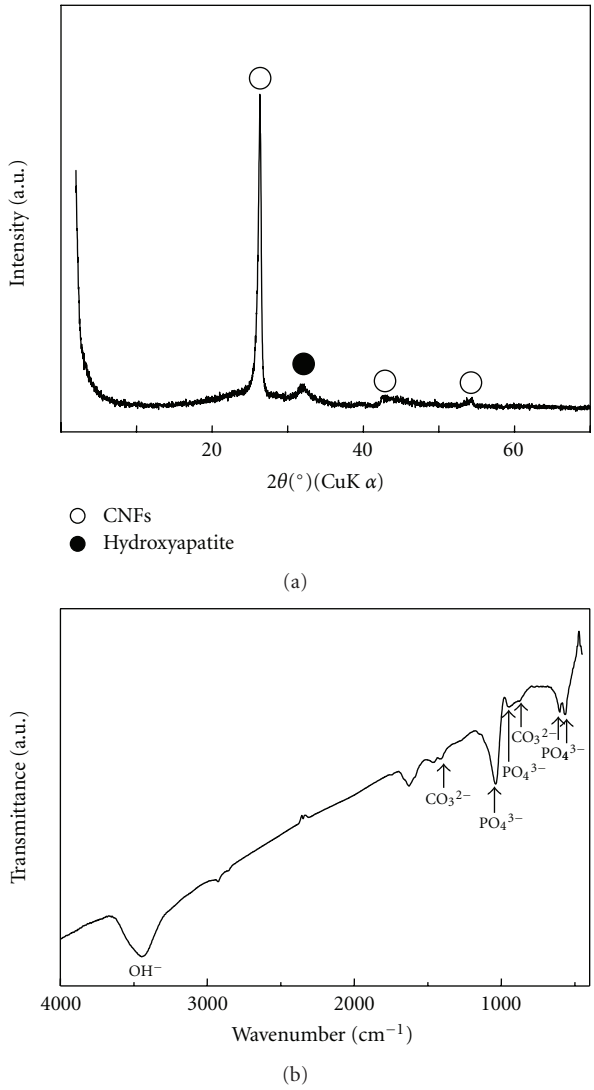


Figure 2: (a) XRD pattern and (b) FT-IR spectrum of pristine CNFs soaked in 1.5 SBF for 7 days.

the spherical particles deposited on the CNFs were bone-like apatite.

The leaf-like morphology of the deposited apatite was different from that of apatite deposited on other multiwalled CNTs soaked in revised-SBF [13] and SBF [14], such as needle-like and cubic morphology, while it was the typical morphology of apatite formed on bioactive ceramics in SBF [17–19]. The apatite particles were deposited on the surface of the disentangled CNFs and the outside of the entangled CNFs but not on the inside of the entangled CNFs (Figure 1(d)). Such deposition of apatite on the entangled CNFs was similar to that of apatite on silica gel in SBF [20]. Apatite is formed on the outside of the silica gel but not in pores of the silica gel. These results suggest that in order to make apatite deposit uniformly on the CNFs, the CNFs should be dispersed uniformly in 1.5 SBF.

TEM photographs of the pristine CNFs soaked in 1.5 SBF for 1 day are shown in Figure 3. A few spherical apatite particles with size of 1.0–1.5 μm were observed on the CNFs,

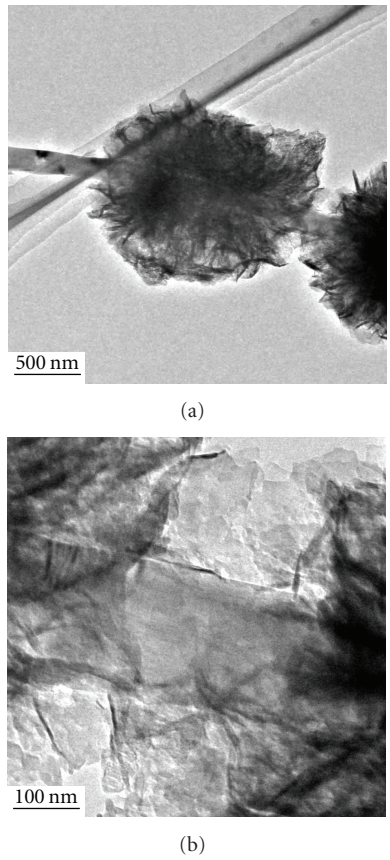


Figure 3: TEM photographs of pristine CNFs soaked in 1.5 SBF for 1 day. (a) Low magnification and (b) High magnification.

which suggests that their nuclei were formed in certain sites on the CNFs and grew radially. They were closely adhered to the CNFs and were not dropped from the CNFs even by ultrasonic. It is reported that functional groups, such as carboxyl group, on surface of CNTs act as nucleation sites of apatite [11–16]. Also it is well known that carboxyl and hydroxyl (−OH) groups act as the nucleation sites of apatite in biomimicking solutions such as 1.5 SBF [20–22]. For instance, raw silk fibers containing sericin rich in carboxyl groups can induce deposition of spherical apatite particles on their surfaces without release of Ca^{2+} ions in the solution, while normal silk fibers containing fibroin poor in carboxyl groups can not induce apatite deposition [22]. Similarly, the nucleation sites of apatite in this study might be functional groups, such as carboxyl and hydroxyl groups, which existed on the pristine CNFs, and the deposition mechanism of apatite on the CNTs should be similar to that of apatite on the raw silk fibers. However, carboxyl and hydroxyl groups on the CNFs were not detected clearly by FT-IR analysis in this study.

0.1–0.0025 g (0.01–0.00025 g/L) of the pristine CNFs was soaked in 100 mL of 1.5 SBF for 7 days. SEM photographs of the CNFs are shown in Figure 4. When the CNFs content in 1.5 SBF was higher, fewer spherical apatite particles were deposited, and their sizes were larger. When the CNFs content in 1.5 SBF was lower, more spherical apatite particles

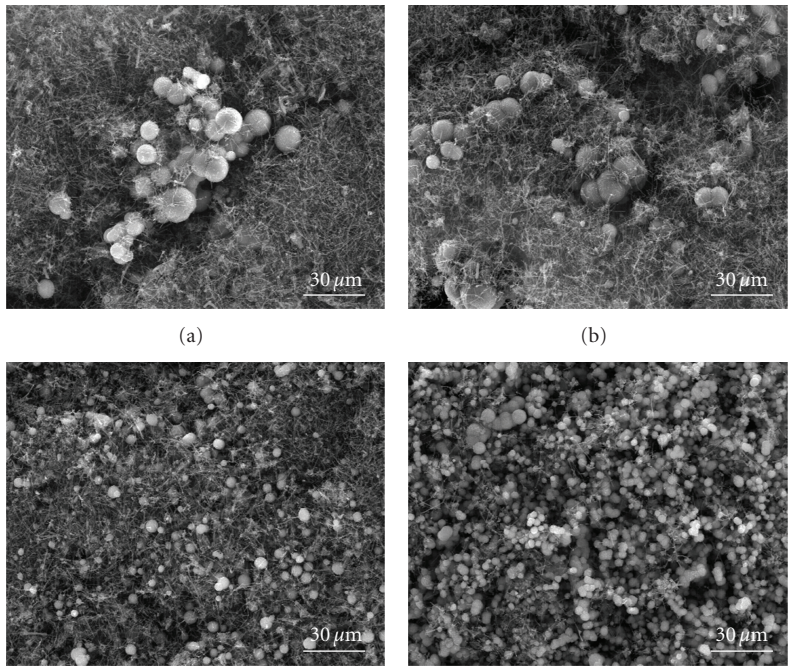


Figure 4: SEM photographs of pristine CNFs soaked in 1.5 SBF for 7 days. CNFs content in the 1.5 SBF was (a) 0.01 g/L, (b) 0.005 g/L, (c) 0.001 g/L, and (d) 0.00025 g/L.

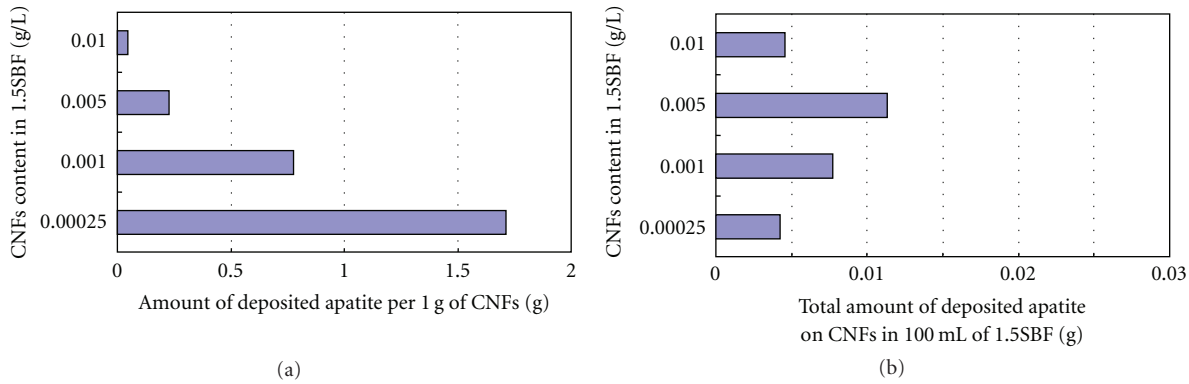


Figure 5: (a) Amount of deposited apatite per 1 g of CNFs and (b) total amount of deposited apatite on CNFs after soaking in 100 mL of 1.5 SBF for 7 days.

were deposited, and their sizes were smaller. Amount of deposited apatite per 1 g of CNFs and total amount of deposited apatite on the CNFs in the 1.5 SBF are shown in Figure 5. It is obvious that amount of deposited apatite per a unit of CNFs increased with a decrease in the CNFs content (Figure 5(a)). On the other hand, if all phosphorus in 1.5 SBF is spent on the formation of hydroxyapatite of which chemical formula is $Ca_{10}(PO_4)_6(OH)_2$, about 0.0025 g of hydroxyapatite is deposited in 100 mL of 1.5 SBF. However, total amount of the deposited apatite on the CNFs in 100 mL of 1.5 SBF was 0.004–0.011 g (Figure 5(b)).

Schematic deposition processes of apatite on the CNFs in 1.5 SBF are shown in Figure 6. When the CNFs content in 1.5 SBF was higher (Figure 6(a)), there were many nucleation sites of apatite, such as carboxyl group, in the 1.5 SBF, and many embryos of apatite nuclei were formed in many nucleation sites. However, most embryos could not grow to the critical nuclei which can grow to apatite crystals and dissolved into 1.5 SBF again. Consequently, amount of deposited apatite per a unit of CNFs became lower as the CNFs content in 1.5 SBF increased. A few embryos formed in particular nucleation sites on the CNFs could grow to the critical nuclei by chance and furthermore grew to spherical apatite particles with larger sizes. On the other hand, when the CNFs content was lower (Figure 6(b)), the number of nucleation sites was limited in the 1.5 SBF. In this case, because the embryos were formed in a small number of nucleation sites, they could grow to the critical nuclei and

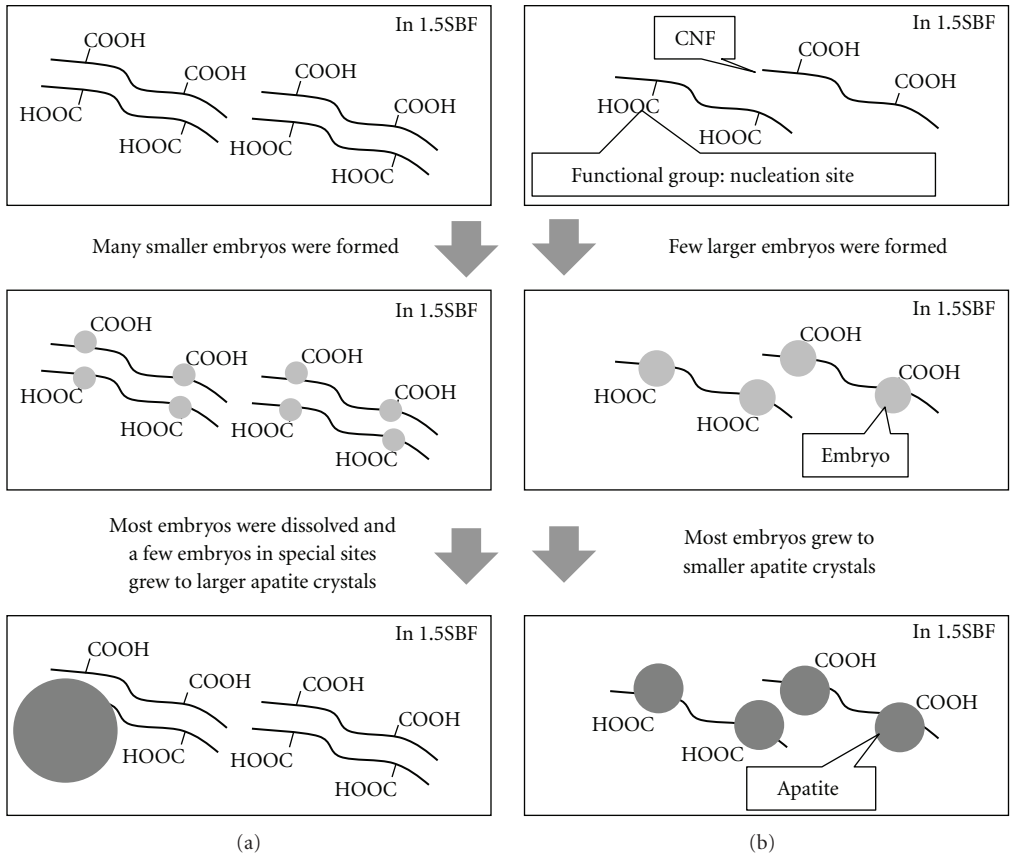


Figure 6: Schematic deposition processes of apatite in the case of (a) higher CNFs content in 1.5 SBF and (b) lower CNFs content in 1.5 SBF.

furthermore grew to apatite crystals. Consequently, more spherical apatite particles with smaller sizes were deposited uniformly on a CNF. The above results indicate that the CNFs content in 1.5 SBF should be lower to make apatite deposit uniformly on CNFs.

3.2. Influence of Pretreatment of CNFs on Deposition of Apatite. SEM photographs of the pristine CNFs and the pretreated CNFs with mixed acid for 6 h, NaOH solution and $CaCl_2$ solution after soaking in 1.5 SBF for 7 days are shown in Figure 7. The apatite particles were not observed on the CNFs pretreated with mixed acid for 6 h, while many spherical apatite particles were deposited on the CNFs pretreated with NaOH and $CaCl_2$ solutions. Amount of apatite deposited on the CNTs was influenced obviously by the pretreatment, as shown in Figure 8. It is known that functional groups, such as carboxyl, hydroxyl, and carbonyl (>C=O) groups, are formed on the CNTs by acid treatment [23–25]. As the CNFs were pretreated with mixed acid for longer periods, the intensity of D-band (defect mode) in the Raman spectra of the CNFs became stronger as shown in Figure 9, which suggests that carboxyl, hydroxyl, and carbonyl groups on the CNFs were increased by the pretreatment. The CNFs pretreated with mixed acid for 3 h increased the amount of deposited apatite, comparing with the pristine CNFs, because the carboxyl and hydroxyl groups which act as the nucleation sites of apatite became more appropriate amount by the pretreatment. However, by pretreating with mixed acid for 6 h and 24 h, too many carboxyl and hydroxyl groups might be formed on the CNFs. And so the embryos of apatite nuclei were formed in too many nucleation sites, could not grow to the critical nuclei, and dissolved again in 1.5 SBF. As a result, the amount of apatite deposited on the CNFs pretreated with mixed acid for 6 h and 24 h was decreased markedly.

The intensities of D-band in the Raman spectra of the CNFs pretreated with nitric and phosphoric acids were almost the same with the intensity of D-band of the pristine CNFs (Figure 9). However, compared with the pristine CNFs, the CNFs pretreated with nitric acid decreased the amount of deposited apatite, while the CNFs pretreated with phosphoric acid increased it. These results suggest that the surface state of the CNFs pretreated with acid depended on the type of acid used, and the functional groups on the pristine CNFs, such as carboxyl, hydroxyl, and carbonyl groups, were not increased on the CNTs by pretreating with nitric and phosphoric acids, and such functional groups might be replaced with other functional groups. In addition, 0.14 mg of phosphorus was detected in 1 g of the CNFs pretreated with phosphoric acid by analysis using an atomic absorption spectrophotometer. This suggests that the functional groups on the pristine CNFs were replaced with phosphate groups ($-PO_4H_2$). The apatite growth rate of

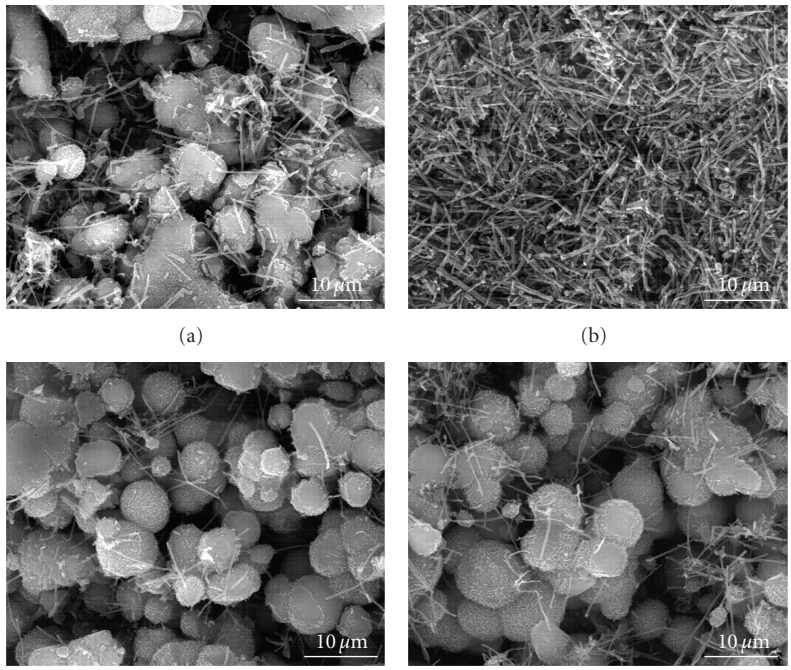


Figure 7: SEM photographs of (a) pristine CNFs and pretreated CNFs with (b) mixed acid for 6 h, (c) NaOH solution, and (d) $CaCl_2$ solution after soaking in 1.5 SBF for 7 days.

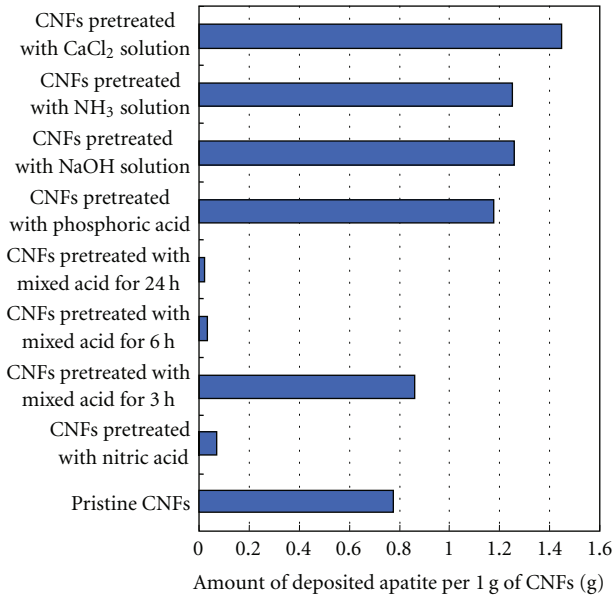


Figure 8: Influence of pretreatment of CNFs on amount of deposited apatite after soaking in 1.5 SBF for 7 days.

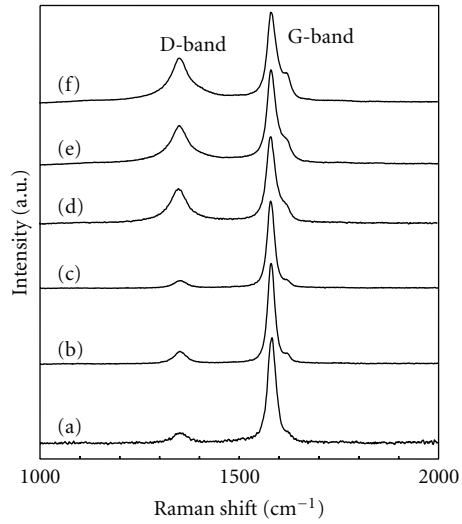


Figure 9: Raman spectra of (a) pristine CNFs, CNFs pretreated with (b) nitric acid, (c) phosphoric acid, (d) mixed acid for 3 h, (e) mixed acid for 6 h, and (f) mixed acid for 24 h.

phosphate group is higher than that of carboxyl group, and other functional groups and the phosphate groups induce a large amount of apatite on the CNFs [21].

The deposition of apatite is accelerated on raw silk fibers pretreated with $CaCl_2$ solution [22]. This is explained as follows: the pretreatment with $CaCl_2$ solution can convert the carboxyl groups into complexes such as $-COOCa^+$ and $(-COO)_2Ca$, which make it easy for the deposition of apatite to occur in a biomimicking solution. By the same mechanism, the CNFs pretreated with $CaCl_2$ solution increased the amount of deposited apatite much more, compared with the pristine CNFs. Similarly, the pretreatment with NaOH and ammonia solutions might convert carboxyl groups on the CNFs into $-COONa$ and $-COONH_3$, respectively. In

addition, 0.039 mg of sodium was detected in 1 g of the CNFs pretreated with NaOH solution by analysis using an atomic absorption spectrophotometer. The complexes such as $-COONa$ and $-COONH_3$ increased the amount of deposited apatite much more as well as complexes such as $-COOCa^+$ and $(-COO)_2Ca$.

4. Conclusions

The spherical bone-like apatite particles, which consisted of many leaf-like crystals, were deposited on the pristine CNFs soaked in 1.5 SBF. As the CNFs content in 1.5 SBF increased, amount of deposited apatite per a unit of CNFs decreased, and the sizes of the apatite particles became larger. When the CNFs content in 1.5 SBF was higher, there were more nucleation sites of apatite on the CNFs in the 1.5 SBF, and the more embryos formed in such sites could not grow to the critical nuclei. Consequently, a few embryos formed in particular nucleation sites on the CNFs could grow to the critical nuclei and furthermore grew to apatite particles with larger sizes. On the other hand, when the CNFs content was lower, the number of nucleation sites was limited in the 1.5 SBF, and most embryos formed in such sites could grow to the critical nuclei. Consequently, many apatite crystals with smaller sizes were deposited uniformly on a CNF.

Amount of deposited apatite decreased markedly when the CNFs were pretreated with mixed acid for longer periods. Since too many carboxyl and hydroxyl groups were formed by pretreating with mixed acid, the too many embryos of apatite nuclei were formed and could not grow to the critical nuclei and furthermore apatite crystals. On the other hand, by pretreating with phosphoric acids, the functional groups on the pristine CNFs might be replaced with phosphate groups ($-PO_4H_2$), which induced a large amount of apatite on the CNFs. The pretreatment with $CaCl_2$, NaOH, or ammonia solutions might convert the carboxyl groups on the CNFs into complexes such as $-COOCa^+$, $(-COO)_2Ca$, $-COONa$, and $-COONH_3$, which induced the deposition of a large amount of apatite.

It is concluded that to make apatite deposit uniformly on the CNFs biomimetically in a short time, a small amount of the CNFs pretreated by phosphoric acids, $CaCl_2$, NaOH, or ammonia solutions should be dispersed uniformly in 1.5 SBF.

Acknowledgments

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