

# A Bone Mimic Based on the Self-Assembly of Hydroxyapatite on Chemically Functionalized Single-Walled Carbon Nanotubes

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We report the use of chemically functionalized single-walled carbon nanotubes (SWNTs) as a scaffold for the growth of artificial bone material. The mineralization of SWNTs functionalized with phosphonates and poly(aminobenzene sulfonic acid) (PABS) was investigated in the solution phase and as films on substrates. Microscopy studies showed that hydroxyapatite (HA) nucleated and crystallized on the surface of the functionalized SWNTs. The negatively charged functional groups on SWNTs attract the calcium cations and lead to self-assembly of HA. The thickness of the HA layers was found to be a function of the mineralization time. Mineralization of SWNT–PABS films led to well-aligned plate-shaped HA crystals and the thickness of the crystals reached 3  $\mu\text{m}$  after 14 days of mineralization. The PABS- and phosphonates-functionalized SWNTs can be applied as supporting scaffold for bone therapy.

## Introduction

Bone tissue is a natural composite material that contains collagen fibrils and hydroxyapatite (HA) crystals in a hierarchical organization. On the smallest length scale, collagen triple helices spontaneously form nanoscale bundles, which act as a template for the crystallization of HA nanocrystals. The fabrication of artificial materials that mimic the structure and properties of natural bone is a significant challenge since it involves both organic and inorganic nanophases that have a specific spatial relation. The most common approach to mineralization is to design the organic nanophase so it can control crystal nucleation and growth of the inorganic component. Artificial bone materials have been produced in which organic substrates such as poly(lactic acid), poly(L-lactide), peptide-amphiphile nanofibers, reconstituted collagen, and others have been used in the mineralization.<sup>1–10</sup>

Carbon nanotubes (CNTs) have attracted a great deal of attention because of their novel mechanical and electronic

properties. CNTs have a one-dimensional tubular structure, consisting of  $\text{sp}^2$ -hybridized carbon atoms, and they typically have a high aspect ratio with a length thousands of times greater than their nanoscale diameter. This unique structure together with the nature of the conjugated carbon–carbon bond makes CNTs one of the strongest known materials.<sup>11–14</sup> On the other hand, CNTs are made of carbon and they have density as low as graphite. Compared with other scaffold materials such as polymers and peptide fibers, the high tensile strength, excellent flexibility, and low density of CNTs make them ideal for the production of lightweight high-strength materials such as bone. The diameter of single-walled carbon nanotubes (SWNTs) ranges from 0.7 to 1.5 nm, which is close to the size of the triple helix collagen fibrils, and thus they are excellent candidates for the construction of scaffolds for the nucleation and growth of HA. However, pristine SWNTs are not suitable for mineralization reactions because they do not contain functional groups that can attract calcium cations and thereby nucleate and initiate the crystallization of HA; for this reason we have designed a series of functionalized SWNTs specifically tailored to induce mineralization reactions.

In this paper we report the first mineralization of chemically functionalized SWNTs with HA. We examined the effect of a variety of functionalities, including the carboxylic acid group ( $-\text{COOH}$ ), the phosphonate group (diethyl methylene phosphonate SWNT ester and diethyl benzyl phosphonate SWNT amide), and the sulfonic acid group [poly(aminobenzene sulfonic acid), PABS]. These functionalities were introduced by chemical reaction and are shown

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to improve the solubility of the SWNTs in water, and to exert a strong effect on the mineralization of the SWNTs with HA.

### Experimental Section

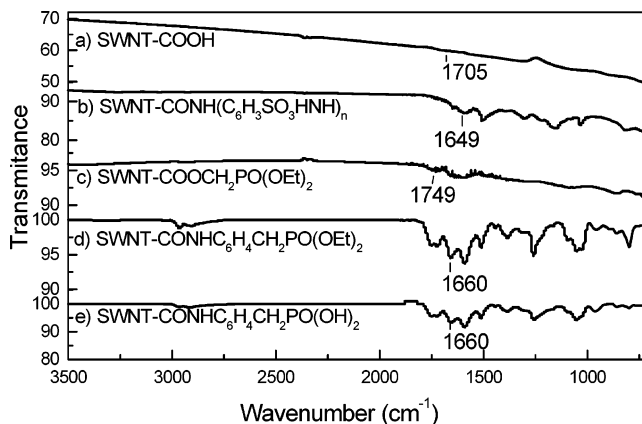
Nitric acid treated electric arc produced SWNTs (SWNT-COOH and P3-SWNT) were obtained from Carbon Solution Inc (carbonsolution.com). Other chemicals were purchased from Aldrich and used without further treatment. Mid-IR spectra were measured on a Nicolet Magna-IR 560 ESP spectrometer. Scanning electron microscopy (SEM) images were taken on a Philips SEM XL-30 microscope.

**SWNT-PABS Graft Copolymer [SWNT-CONH-(C<sub>6</sub>H<sub>3</sub>SO<sub>3</sub>HNH)<sub>n</sub>].** The SWNT-COOH (12 mg) was sonicated in 20 mL of DMF for 30 min to give a homogeneous suspension. Oxalyl chloride (0.4 mL) was added dropwise to the SWNT suspension at 0 °C under N<sub>2</sub>. The mixture was stirred at 0 °C for 2 h and then at room temperature for another 2 h. Finally the temperature was raised to 70 °C and the mixture was stirred overnight to remove excess oxalyl chloride. PABS (120 mg) prepared according to the literature method<sup>15,16</sup> was dissolved in DMF and added to the SWNT suspension. The mixture was stirred at 120 °C for 5 days. After cooling to room temperature, the mixture was filtered through a 0.2- $\mu$ m pore-size membrane and washed thoroughly with DMF, ethyl alcohol, and deionized (DI) water. The black solid (57 mg) was collected on the membrane and dried under vacuum overnight.

**Diethyl Methylene Phosphonate SWNT Ester [SWNT-COOCH<sub>2</sub>PO(OEt)<sub>2</sub>].** SWNT-COOH (20 mg) was sonicated in 20 mL of DMF for 1 h to give a homogeneous suspension. The SWNT suspension was reacted with oxalyl chloride (0.8 mL), using the same procedure as described above. Diethyl (hydroxymethyl)phosphonate (0.3 mL) was added to the SWNT suspension and the mixture was stirred at 120 °C for 5 days. After cooling to room temperature, the mixture was filtered through a 0.2- $\mu$ m pore-size membrane and washed thoroughly with ethyl alcohol and DI water. The black solid was collected on the membrane and dried under vacuum overnight (16 mg, 80% yield).

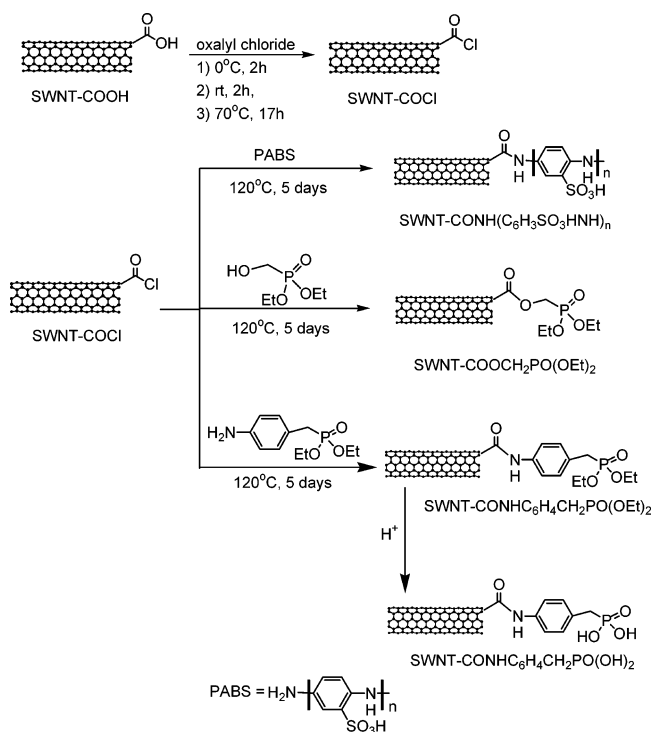
**Diethyl Benzyl Phosphonate SWNT Amide [SWNT-CONHC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>PO(OEt)<sub>2</sub>].** SWNT-COOH (40 mg) was sonicated in 40 mL of DMF for 1 h to give a homogeneous suspension. The SWNT suspension was reacted with oxalyl chloride (1.6 mL) as described before. Diethyl (4-aminobenzyl)phosphonate (400 mg) was added to the SWNT suspension and the mixture was stirred at 120 °C for 5 days. After cooling to room temperature, the mixture was filtered through a 0.2- $\mu$ m pore-size membrane and washed thoroughly with ethyl alcohol and DI water. The black solid was collected on the membrane and dried under vacuum overnight (48 mg).

**Benzyl Phosphonic Acid SWNT Amide [SWNT-CONHC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>PO(OH)<sub>2</sub>].** SWNT-CONHC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>PO(OEt)<sub>2</sub> (15 mg) was sonicated in 5 mL of DI water at room temperature for 1 h. HCl (0.05 M, 1 mL) was added slowly to the suspension of SWNT-CONHC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>PO(OEt)<sub>2</sub>, and the mixture was sonicated at room temperature for 2 h. The mixture was filtered through a 0.2- $\mu$ m pore-size membrane and washed thoroughly with DI water. The black solid was collected on the membrane and dried under vacuum overnight (13 mg, 87% yield).



**Figure 1.** Mid-IR spectra of functionalized SWNTs (film of samples on zinc selenide substrate): (a) SWNT-COOH; (b) SWNT-CONH(C<sub>6</sub>H<sub>3</sub>SO<sub>3</sub>H)<sub>n</sub>; (c) SWNT-COOCH<sub>2</sub>PO(OEt)<sub>2</sub>; (d) SWNT-CONHC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>PO(OEt)<sub>2</sub>; and (e) SWNT-CONHC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>PO(OH)<sub>2</sub>.

### Scheme 1. Chemical Functionalization of SWNTs



**Mineralization of SWNTs in Solution Phase.** A 1-mL portion of the SWNT solution (0.02 mg/mL, prepared by sonication) was dropped on a hydrophobic Teflon membrane (pore-size 0.1  $\mu$ m). CaCl<sub>2</sub> (2 mL, 10 mM) and Na<sub>2</sub>HPO<sub>4</sub> (2 mL, 5 mM) were added simultaneously to the SWNT solution on the membrane. After different periods of mineralization (30 min and 1 day), the mixture on the membrane was filtered by adding several drops of ethanol and the surface of the membrane was rinsed carefully and thoroughly with DI water. The resulting dried material on the membrane was examined with scanning electron microscopy.

**Mineralization of SWNTs on Glass Substrate.** A SWNT dispersion in water (prepared by sonication) was sprayed onto a preheated glass coverslip. After drying, the SWNT-coated glass coverslip was placed in a vial. CaCl<sub>2</sub> (2 mL, 10 mM) and Na<sub>2</sub>HPO<sub>4</sub> (2 mL, 5 mM) were added simultaneously to the vial. After different periods of mineralization (7 days and 14 days), the glass coverslip was taken out of the solution and was rinsed carefully and thoroughly with DI water. The resulting dried glass coverslip was examined with scanning electron microscopy.

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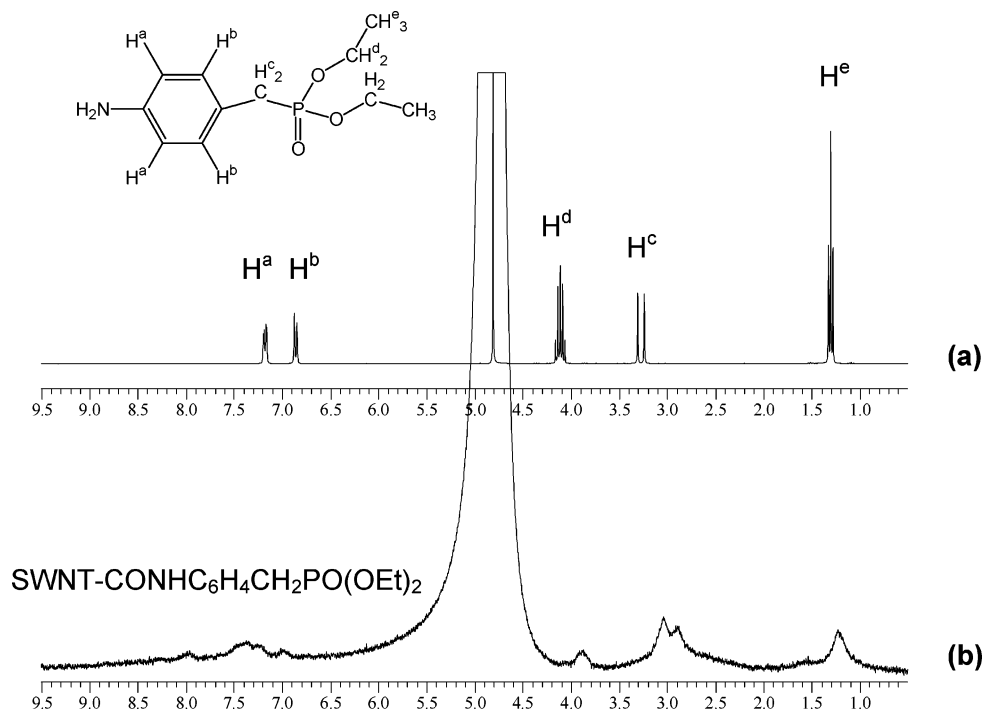


Figure 2.  $^1\text{H}$  NMR (in  $\text{D}_2\text{O}$ ) of (a) diethyl (4-aminobenzyl)phosphonate and (b)  $\text{SWNT-CONHC}_6\text{H}_4\text{CH}_2\text{PO}(\text{OEt})_2$ .

## Results and Discussion

**Chemical Functionalization.** The functionalization of the SWNTs was carried out via amidation or esterification of carboxylic acid bound SWNTs with amines or alcohols through an acyl chloride intermediate (Scheme 1). In the first step, the carboxylic acid functionalized SWNTs ( $\text{SWNT-COOH}$ ) were reacted with oxalyl chloride to form the acyl chloride intermediate,  $\text{SWNT-COCl}$ . Then the intermediate  $\text{SWNT-COCl}$  was reacted with amine- or alcohol-modified phosphonates and PABS to form the corresponding functionalized SWNTs. The mid-IR spectra of these functionalized SWNTs (Figure 1) show that these organic molecules are covalently attached to the SWNTs. The peaks centered at  $1649\text{--}1660\text{ cm}^{-1}$  in the spectra of  $\text{SWNT-CONH}(\text{C}_6\text{H}_3\text{-SO}_3\text{HNH})_n$ ,  $\text{SWNT-CONHC}_6\text{H}_4\text{CH}_2\text{PO}(\text{OEt})_2$ , and  $\text{SWNT-CONHC}_6\text{H}_4\text{CH}_2\text{PO}(\text{OH})_2$  are due to the amide carbonyl stretching vibration.  $\text{SWNT-CONHC}_6\text{H}_4\text{CH}_2\text{PO}(\text{OH})_2$  (Figure 1e) is the hydrolysis product of the benzyl phosphonate derivative and the intensity of C–H stretch is decreased in this material. The peak centered at  $1749\text{ cm}^{-1}$  in the spectra of  $\text{SWNT-COOCH}_2\text{PO}(\text{OEt})_2$  can be assigned to carbonyl stretch of the ester.

The  $^1\text{H}$  NMR spectrum (300 MHz,  $\text{D}_2\text{O}$ ) of  $\text{SWNT-CONHC}_6\text{H}_4\text{CH}_2\text{PO}(\text{OEt})_2$  shows broadened signals compared to the spectrum of diethyl (4-aminobenzyl)phosphonate (Figure 2). This phenomenon was observed in previous polymer-wrapped<sup>17</sup> and polymer-functionalized<sup>16,18,19</sup> carbon nanotubes, and has been attributed to inhomogeneities in the local magnetic field due to the magnetic anisotropy of the

nanotubes. Protons undergo upfield chemical shifts upon covalent functionalization with respect to the free diethyl (4-aminobenzyl)phosphonate as shown in Figure 2. The  $^1\text{H}$  NMR spectrum of  $\text{SWNT-COOCH}_2\text{PO}(\text{OEt})_2$  could not be obtained due to its relatively low solubility in water.

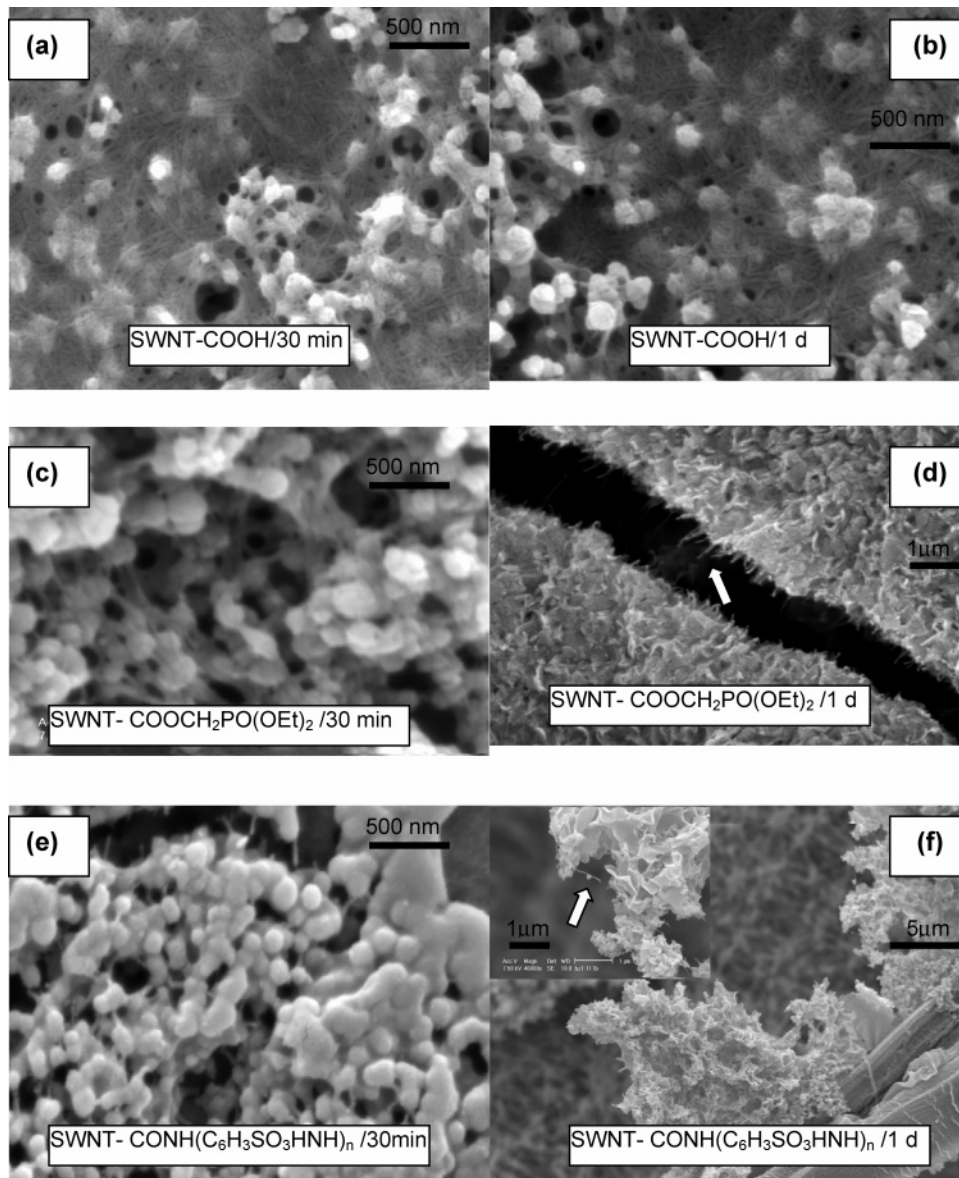
**Solution-Phase Mineralization of Chemically Functionalized SWNTs.** The phosphonate- and PABS-functionalized SWNTs are soluble in water, which allows their aqueous manipulation in the mineralization reactions. To compare the effects of the different functional groups on the mineralization reaction, the  $\text{SWNT-COOH}$  material was also investigated, but as-prepared (AP-) SWNTs were not investigated since they do not contain functionality and are difficult to process under these conditions. The aqueous solutions of functionalized SWNTs (0.02 mg/mL) were prepared by sonication of the materials in water at pH 7. The SWNT solution (1 mL, 0.02 mg/mL) was dropped on a hydrophobic Teflon membrane (pore-size  $0.1\ \mu\text{m}$ ). The  $\text{CaCl}_2$  (2 mL, 10 mM) and  $\text{Na}_2\text{HPO}_4$  (2 mL, 5 mM) solutions were added simultaneously to the SWNT solution on the membrane.

After periods of mineralization of 30 min and 1 day, the mixture on the membrane was filtered and the surface of the membrane was rinsed thoroughly with water. The dried material remaining on the membrane was examined with scanning electron microscopy (SEM, Figure 3). After 30 min of mineralization, only a small amount of calcium phosphate particles appeared on the surface of the  $\text{SWNT-COOH}$  (Figure 3a). On the other hand, a large amount of calcium phosphate particles appeared in the  $\text{SWNT-CONH}(\text{C}_6\text{H}_3\text{-SO}_3\text{HNH})_n$  and  $\text{SWNT-COOCH}_2\text{PO}(\text{OEt})_2$  samples (Figure 3c and e) and these particles closely adhered to the surface of functionalized SWNTs. Apparently, the phosphonate and sulfonate groups on the functionalized SWNTs enhanced the nucleation of HA. After 1 day of mineralization, the appearance of the  $\text{SWNT-COOH}$  sample (Figure 3b) did

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**Figure 3.** SEM images of the mineralization of chemically functionalized SWNTs for periods of 30 min and 1 day. The mineralization of SWNT-COOH for (a) 30 min and (b) 1 day showed no change after the additional time period. The mineralization of SWNT-COOCH<sub>2</sub>PO(OEt)<sub>2</sub> for (c) 30 min and (d) 1 day showed an appreciable difference in the appearance of the material. In (d), SWNTs can be seen at the broken edge of the HA (white arrow). Images of the mineralization of SWNT-CONH(C<sub>6</sub>H<sub>3</sub>SO<sub>3</sub>HNN)<sub>n</sub> for 30 min and 1 day are shown in (e) and (f), respectively. The inset in (f) shows a high-resolution image of the mineralized SWNT-CONH(C<sub>6</sub>H<sub>3</sub>SO<sub>3</sub>HNN)<sub>n</sub>, in which HA can be seen on a SWNT bundle (white arrow).

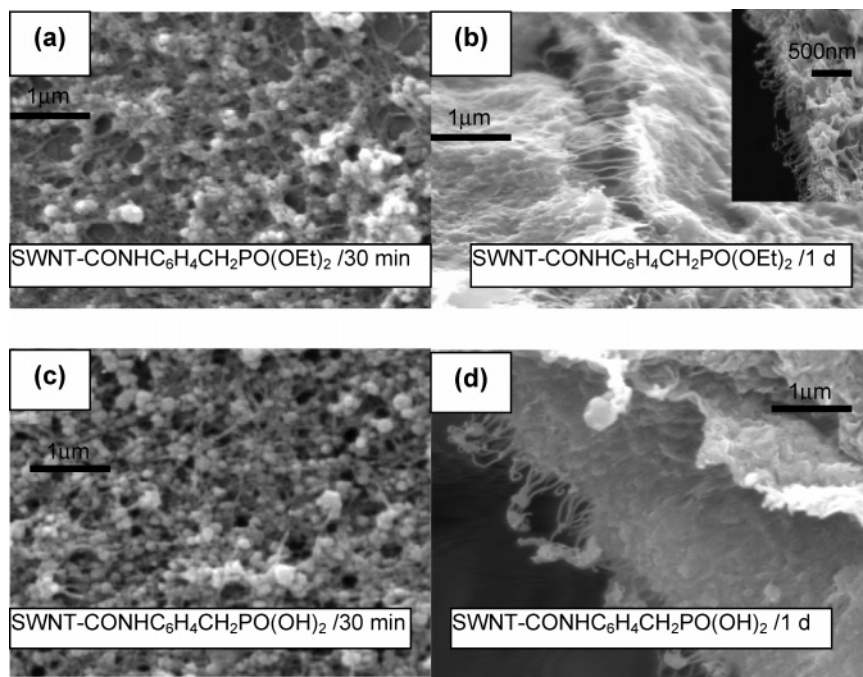
not show a significant change compared to the 30-minute mineralization sample. However, SWNT-CONH(C<sub>6</sub>H<sub>3</sub>SO<sub>3</sub>HNN)<sub>n</sub> and SWNT-COOCH<sub>2</sub>PO(OEt)<sub>2</sub> showed completely different appearances after 1 day of mineralization (Figure 3d and f). In the case of SWNT-COOCH<sub>2</sub>PO(OEt)<sub>2</sub>, a large amount of HA was formed on the SWNTs in a bulk layerlike structure (Figure 3d). At the edge of a broken layer, CNTs can be clearly observed. The material looks like a true composite in which SWNTs are reinforcing the HA matrix. For SWNT-CONH(C<sub>6</sub>H<sub>3</sub>SO<sub>3</sub>HNN)<sub>n</sub>, after 1 day of mineralization, a large amount of crystal-like HA was formed, and in a high-resolution SEM image (Figure 3f inset), it can be seen that SWNTs are reinforced by the crystal, and some pieces of small HA crystals can be found on the surface of SWNTs. An energy dispersion X-ray spectroscopy (EDS) study shows the atomic ratio is Ca/P/C/O = 10:7:17:66 for mineral HA crystal on SWNT-CONH(C<sub>6</sub>H<sub>3</sub>SO<sub>3</sub>HNN)<sub>n</sub> and is Ca/P/C/O = 7:5:20:68 for that of SWNT-COOCH<sub>2</sub>PO-

(OEt)<sub>2</sub>, and the Ca/P ratio is 1.38 and 1.36 for these materials, respectively. These values are close to the formulation of HA [Ca<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>(OH)<sub>2</sub>, Ca/P ratio = 1.6]. We assumed that all the carbon are from SWNTs, so the weight percentage of SWNTs in SWNT-CONH(C<sub>6</sub>H<sub>3</sub>SO<sub>3</sub>HNN)<sub>n</sub> mineralized material can be calculated as

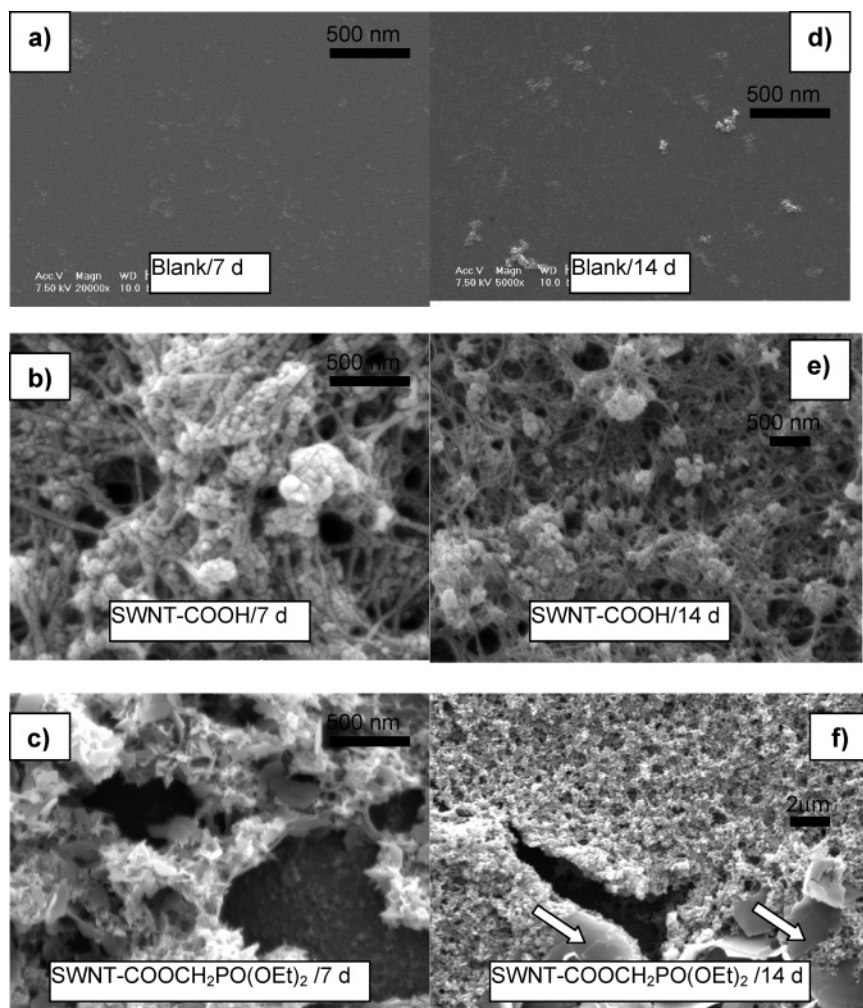
$$\frac{(12 \times 0.17)}{((40 \times 0.1) + (31 \times 0.07) + (12 \times 0.17) + (16 \times 0.66))} \times 100\% = 11\%$$

And the weight percentage of SWNTs in the SWNT-COOCH<sub>2</sub>PO(OEt)<sub>2</sub> is about 14%.

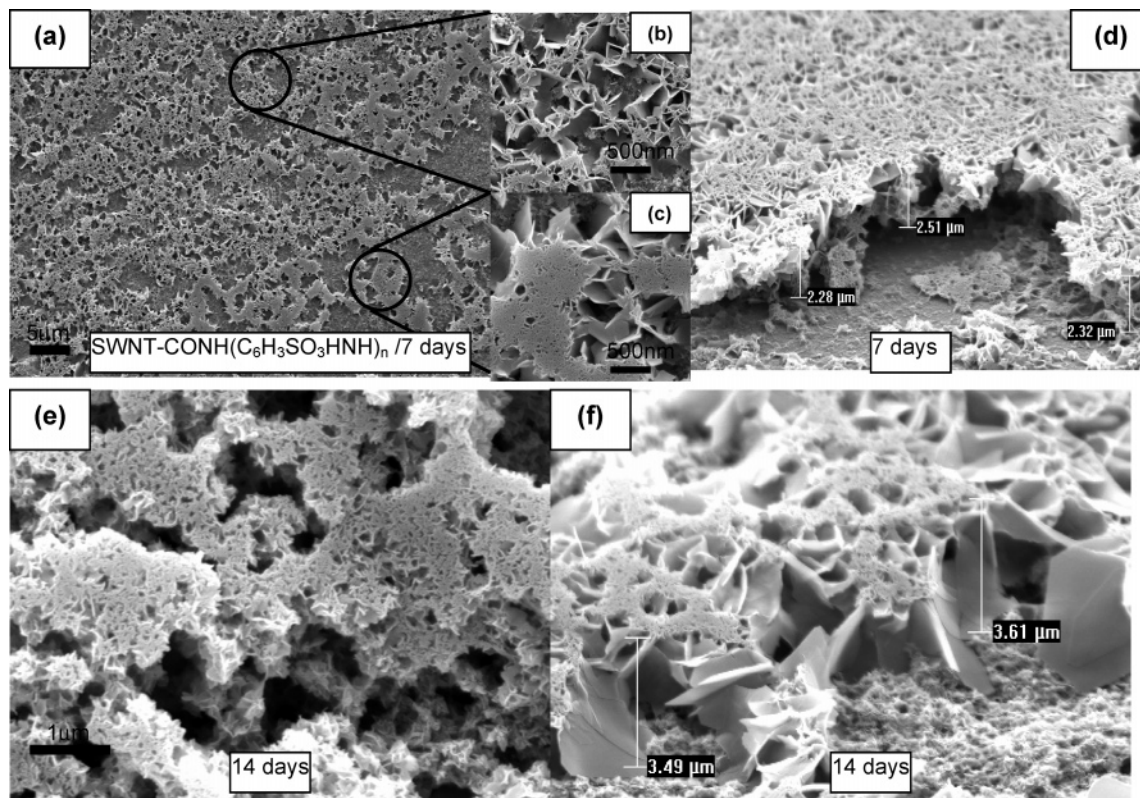
Apparently, the phosphonate and sulfonate groups on functionalized SWNTs attract the calcium cations and efficiently nucleate the HA. The functional groups are introduced at the ends and the defect sites of SWNTs, which greatly change the surface properties of SWNTs. In the three-



**Figure 4.** SEM images of SWNT-CONHC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>PO(OEt)<sub>2</sub> after mineralization for (a) 30 min, and (b) 1 day. The inset image of (b) shows well-aligned SWNTs at the edge of the materials. SEM images of SWNT-CONHC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>PO(OH)<sub>2</sub> after mineralization for (c) 30 min, and (d) 1 day.

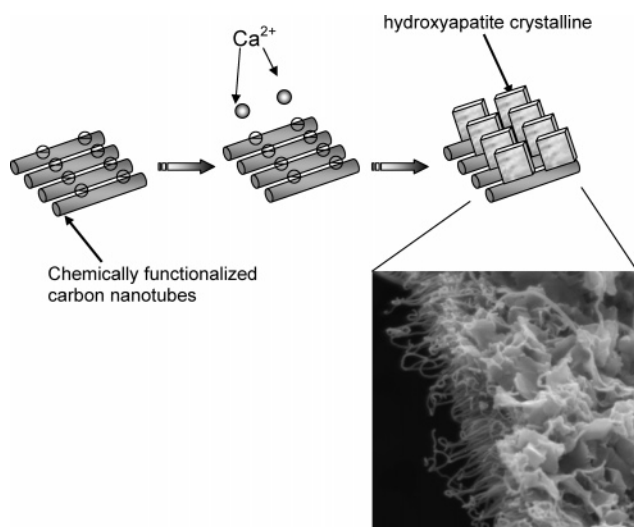


**Figure 5.** SEM images of functionalized SWNTs on glass coverslips mineralized for 7 days and 14 days. The blank glass coverslips did not show any mineralization after (a) 7 days, or (d) 14 days. The mineralization of SWNT-COOH for (b) 7 days, and (e) 14 days, did not show any difference. The mineralization of SWNT-COOCH<sub>2</sub>PO(OEt)<sub>2</sub> for (c) 7 days, and (f) 14 days. After 14 days of mineralization, some large pieces of platelike HA crystals could be found in the sample (f, white arrows).



**Figure 6.** SEM images of SWNT-CONH(C<sub>6</sub>H<sub>3</sub>SO<sub>3</sub>HNN)<sub>n</sub> mineralized for (a–d) 7 days, and (e–f) 14 days. A large region of HA crystallized on SWNT-CONH(C<sub>6</sub>H<sub>3</sub>SO<sub>3</sub>HNN)<sub>n</sub> is shown in (a). High-resolution images of platelike HA crystals are shown in (b) and (c). (d) Shows that the thickness of HA crystal is about 2.4 μm. (e) Shows the image of HA crystal on SWNT-CONH(C<sub>6</sub>H<sub>3</sub>SO<sub>3</sub>HNN)<sub>n</sub> mineralized for 14 days. (f) Shows that the thickness of HA crystal is about 3.5 μm.

**Scheme 2. Schematic Diagram of the Relations between Carbon Nanotubes and HA Crystals in the Mineralized Bundle**



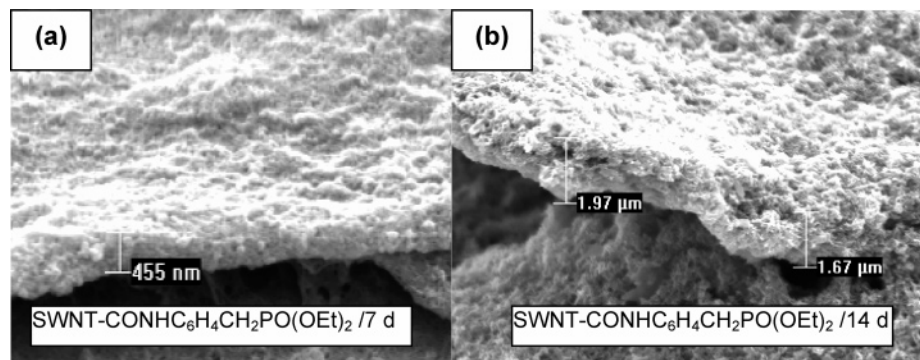
dimensional solution phase, HA crystallized on the surface of the functionalized SWNTs and caused their precipitation to form layerlike bulk materials. This is confirmed by the appearance of the solutions of SWNTs after reaction. After sonication to prepare the dispersions, SWNT-COOH, SWNT-CONH(C<sub>6</sub>H<sub>3</sub>SO<sub>3</sub>HNN)<sub>n</sub>, and SWNT-COOCH<sub>2</sub>PO(OEt)<sub>2</sub> were brown transparent solutions. After 1 day of mineralization, the solutions that originally contained SWNT-CONH(C<sub>6</sub>H<sub>3</sub>SO<sub>3</sub>HNN)<sub>n</sub> and SWNT-COOCH<sub>2</sub>PO(OEt)<sub>2</sub> were transparent and colorless with gray precipitates at the bottom

of the vial, while the SWNT-COOH solution remained brown.

The same mineralization treatment was applied to SWNT-CONHC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>PO(OEt)<sub>2</sub> and its hydrolysis product SWNT-CONHC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>PO(OH)<sub>2</sub>. The SEM images after 30 min of mineralization of SWNT-CONHC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>PO(OEt)<sub>2</sub> and SWNT-CONHC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>PO(OH)<sub>2</sub> (Figure 4a and c) are similar to that of SWNT-COOCH<sub>2</sub>PO(OEt)<sub>2</sub>. However, the appearance of the mineralization products of SWNT-CONHC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>PO(OEt)<sub>2</sub> and SWNT-CONHC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>PO(OH)<sub>2</sub> did not show much difference. The hydrolyzed phosphonate SWNT-CONHC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>PO(OH)<sub>2</sub> should carry more negative charges and thus might serve to attract more calcium cations than SWNT-CONHC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>PO(OEt)<sub>2</sub> that was in an ester form. One possible reason could be some of the ester groups on SWNT-CONHC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>PO(OEt)<sub>2</sub> have already been converted into acid groups during the basic workup procedure. This is reflected in the mid-IR spectrum of SWNT-CONHC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>PO(OEt)<sub>2</sub>, in which the intensity of C-H stretch of ethyl groups is not very strong. Nevertheless, both SWNT-CONHC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>PO(OEt)<sub>2</sub> and SWNT-CONHC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>PO(OH)<sub>2</sub> showed positive mineralization after 1 day (Figure 4b and d).

**Thin Film Mineralization of Chemically Functionalized SWNTs.** The mineralization of functionalized SWNTs was further investigated by spraying a solution of SWNTs onto glass coverslips to form a film, in order to test their effectiveness as a substratum to control the nucleation of bone.

The coverslips coated with SWNT-COOH, SWNT-CONH(C<sub>6</sub>H<sub>3</sub>SO<sub>3</sub>HNN)<sub>n</sub>, SWNT-COOCH<sub>2</sub>PO(OEt)<sub>2</sub>, and



**Figure 7.** SEM images of SWNT-CONHC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>PO(OEt)<sub>2</sub> mineralized for (a) 7 days, and (b) 14 days.

SWNT-CONHC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>PO(OEt)<sub>2</sub>, respectively, were placed in vials with solutions of CaCl<sub>2</sub> and Na<sub>2</sub>HPO<sub>4</sub>. The coverslips were kept in the solution at room temperature for different periods of time (7 days and 14 days). Then the coverslip was removed from the vials and rinsed carefully with water. After drying, the coverslip was imaged by SEM. The SEM images (Figure 5b and e) of SWNT-COOH after 7 days and 14 days mineralization are similar. The mineralization of SWNT-COOCH<sub>2</sub>PO(OEt)<sub>2</sub> for 7 days and 14 days still generated composite-like material (Figure 5c and f). In the case of 14 days of mineralization, a small amount of platelike HA crystals was found in the sample. In the control experiment, glass coverslips were treated in the same way in the absence of functional SWNTs, and no mineral deposit was found on these coverslips (Figure 5a and d).

After 7 days of mineralization, a large amount of plate-shaped HA polycrystalline mineral covered the surface throughout the glass coverslips coated with SWNT-CONH-(C<sub>6</sub>H<sub>3</sub>SO<sub>3</sub>HNH)<sub>n</sub> (Figure 6a). Interestingly, in some regions, the plate-shaped HA polycrystalline mineral was in very high density and compacted together to form mature HA crystals (Figure 6c). According to the side-view image (Figure 6d), these plate-shaped HA crystals were in vertical alignment. Since the SWNTs were horizontally deposited on the surface of the coverslips after coating, these vertical plate-shaped HA crystals apparently grow perpendicular to the axis of the SWNTs (Scheme 2). These results indicate that the orientation of the crystalline nuclei and the subsequent crystal growth are to some degree controlled by SWNTs.

After 14 days of mineralization, more regions with highly compacted HA crystals could be found on the SWNT-CONH(C<sub>6</sub>H<sub>3</sub>SO<sub>3</sub>HNH)<sub>n</sub> coated coverslip (Figure 6e). Moreover, the average thickness of the HA crystals after 14 days mineralization on the SWNT-CONH(C<sub>6</sub>H<sub>3</sub>SO<sub>3</sub>HNH)<sub>n</sub> coverslip was about 3.5 μm (Figure 6f), and higher than that from 7 days of mineralization (about 2.4 μm, Figure 6d). The morphology of HA crystals on SWNT-CONHC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>PO(OEt)<sub>2</sub> were very close to that of HA on SWNT-COOCH<sub>2</sub>PO(OEt)<sub>2</sub> (Figure 7). As in the case of SWNT-CONH(C<sub>6</sub>H<sub>3</sub>SO<sub>3</sub>HNH)<sub>n</sub>, the 14-days mineralization generated

thicker HA crystal (1.8 μm) than that obtained after 7 days of mineralization (455 nm).

The functionalized SWNTs were designed to expose negatively charged surfaces because it is reported that this promotes mineralization by establishing local ion supersaturation.<sup>5</sup> The SEM images show that phosphonate- and sulfonate-functionalized SWNTs are able to nucleate HA on their surfaces in both 3-D and 2-D experiments, whereas carboxylic-acid-terminated SWNTs were not effective. The intended function of the SWNTs is to mimic the role of collagen as the scaffold for the nucleation and growth of HA in bone. In the 2-D mineralization of SWNT-CONH-(C<sub>6</sub>H<sub>3</sub>SO<sub>3</sub>HNH)<sub>n</sub> well-aligned HA crystals were formed, which implies that the orientation of nuclei and crystal growth are not random but are controlled by the SWNTs. However, this phenomenon is not so obvious in the mineralization of other functionalized SWNTs. In the bone tissue therapy, such functionalized SWNTs could be implanted as solutions or substratum in the fracture to help growth of bone. By varying the functional groups on SWNTs it may also be possible to control the growth direction of HA. With their outstanding mechanical properties, such chemically functionalized SWNT scaffolds are ideal materials for artificial bone materials.

In conclusion, the mineralization of chemically functionalized SWNTs was investigated and HA was crystallized on the surface of SWNTs in solution phase and on thin films. In vivo, however, collagen fibrils are aligned parallel to form scaffolds for the nucleation and growth of HA. Therefore, future work will be focused on the self-assembly of functionalized SWNTs and HA, which could lead to a more complete mimic for natural bone tissue.

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