Published in final edited form as:

Mater Sci Eng C Mater Biol Appl. 2011 October 10; 31(7): 1477–1481. doi:10.1016/j.msec.2011.06.001.

A simple and rapid method to graft hydroxyapatite on carbon nanotubes

Gururaj M. Neelgund, Kehinde Olurode, and Aderemi Oki*

Department of Chemistry, Prairie View A&M University, Prairie View, TX 77446, USA

Abstract

Herein a simple and effective approach is introduced to functionalize single walled carbon nanotubes (SWCNTs) by *in-situ* grafting of hydroxyapatite (HA). The pristine SWCNTs were chemically activated through introduction of carboxylic groups on their surfaces by refluxing in the mixture of H_2SO_4 and HNO_3 . The resulting carboxylated SWCNTs were further utilized for grafting of HA. The Fourier transform infrared and Raman spectroscopic studies demonstrated the formation of HA and its grafting over SWCNTs. The phase composition of HA and existence Ca^{2+} and PO_4^{3-} ions were studied using X-ray diffraction and energy dispersive X-ray analyses, respectively. The surface morphology of functionalized SWCNTs was analyzed using scanning electron microscopy and transmission electron microscopy. Thermogravimetric analysis confirmed the existence of HA on SWCNTs by exhibiting different thermogram for pure HA and functionalized SWCNTs. Overall this method produced uniform grafting of low crystalline HA on carboxylated SWCNTs with strong interfacial bonding.

Keywords

Carbon nanotubes; Hydroxyapatite; Functionalization; *In-situ* synthesis; Characterization

1. Introduction

Carbon nanotubes (CNTs) as the quintessential nanomaterial have already compiled an impressive list of superlatives since their discovery [1]. The excellent mechanical, unique structural, electrical, thermal and chemical properties of CNTs categorized them as outstanding reinforcement material in nanocomposites [2–5]. CNTs could impart mechanical integrity to the composite at their low loadings, without diminishing its bioactivity. In addition to excellent biocompatibility, CNTs possess some bioactive properties of its own [4,6]. The extraordinary specific stiffness and strength of CNTs make it ideal material to provide the needed structural reinforcement for bone tissue scaffold [7–10].

Calcium phosphate is a key structural material of natural bones and teeth and its bio-affinity is considerably higher than that of the other biocompatible ceramics like ZrO_2 , TiO_2 , Al_2O_3 etc. Among different forms of $CaPO_4$, hydroxyapatite (HA) is important and well-known for bone substitution and reconstruction owing to its chemical composition ($Ca_{10}(PO_4)_6(OH)_2$), crystal structure and calcium to phosphate ratio (1.67) similar to apatite found in human

© 2010 Elsevier B.V. All rights reserved.

*Corresponding author: Tel: +1-936-261-3105, aroki@pvamu.edu (A. Oki).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

skeletal system [11–13]. HA has been clinically tested and accepted for orthopedic applications due to its unique bioactive and osteoconductive properties. However, the brittle nature of HA impedes its clinical applications, especially under load-bearing conditions. Therefore use of secondary constituent materials to enhance the properties of HA is required. CNTs have the potential to strengthen and toughen HA without offsetting its bioactivity, thus opening up a wide range of possible clinical uses for these materials [13].

Several methods have been reported for fabrication of CNTs-HA nanocomposites including self assembly [14], sol-gel [15], electrophoretic deposition [16], chemical precipitation [17], chemical vapor deposition (CVD) [18], spark plasma sintering (SPS) [19], freeze-granulation technique [20] etc. Xiao et al. deposited the HA on poly (ethylene glycol) functionalized multiwalled carbon nanotubes using biomineralization method [21] and Lobo et al. reported a method for the electrodeposition of hydroxyapatite films on superhydrophilic vertically aligned multiwalled carbon nanotubes [22]. White et al. demonstrated the preparation of CNTs-HA nanocomposites by both mechanical mixing and *in-situ* deposition techniques [23]. Compare to mechanical mixing, *in-situ* deposition technique provides effective coating of HA and homogeneous dispersion of CNTs in nanocomposites. However *in-situ* deposition technique requires more improvements in terms of uniform deposition and duration of reaction. In consideration of these necessities, herein we report a simple and effective single step method to functionalize single walled carbon nanotubes (SWCNTs) by *in-situ* grafting of HA. The method utilized here is simple, straight forward and rapid compared to reported methods. In addition, this method affords uniform coating of HA on carboxylated SWCNTs. The functionalized SWCNTs (*f*-SWCNTs) were characterized by Fourier transform infrared spectroscopy (FTIR), Raman spectroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray energy dispersive spectroscopy (EDS) and thermogravimetric analysis (TGA).

2. Experimental

2.1. Materials

All the reagents were purchased from Aldrich and were used without further purification unless otherwise noted. All the aqueous solutions were prepared with ultrapure water obtained from Milli-Q Plus system (Millipore).

2.2. Grafting of HA on SWCNTs

The carboxylic groups on pristine SWCNTs (*p*-SWCNTs) were introduced by refluxing them in a mixture of 1:3 (v/v) HNO₃ and H₂SO₄ under stirring at 70 °C for 24 h, followed by centrifugation and repeated washings with DI water [24,25]. The resulting carboxylated SWCNTs (SWCNTs-COOH) were dried under vacuum for 12 h and utilized for the grafting of HA. The 100 mg SWCNTs-COOH were dispersed in 25 mL DI water, mixed with 0.01mol/L aqueous solution of calcium hydroxide and stirred for 1 h under ambient conditions. The pH of the solution was then adjusted to 9 by drop wise addition of phosphoric acid under constant stirring. The HA grafted SWCNTs (*f*-SWCNTs) thus obtained was centrifuged, washed with DI water and dried under vacuum at 40 °C. The overall procedure of functionalization of SWCNTs is schematically illustrated in Scheme 1.

2.3. Characterization

FTIR spectra were recorded using Thermo-Nicolet IR 2000 spectrometer (KBr pellet). TGA were performed with a Perkin Elmer Diamond TG/DTA instrument at a heating rate of 10 °C/min. Raman spectra were recorded with Renishaw R-3000QE system in the backscattering configuration using an Argon ion laser with wavelength 785 nm. Powder

XRD patterns were recorded on Scintag X-ray diffractometer (PAD X), equipped with Cu K α photon source (45 kV, 40 mA) at scanning rate of 3°/min. SEM measurements were carried out on a JEOL JXA-8900 microscope. TEM images were obtained with a Hitachi H-8100 microscope at 200 kV. The EDS measurements were made with a ThermoNoran EDS System 6 attached to TEM.

3. Results and discussion

The grafting of HA on carboxylated SWCNTs was characterized by FTIR spectroscopy shown in Fig. 1. The spectrum of HA (Fig. 1a) displayed well resolved characteristic absorption bands of natural HA [26]. The bands at 565 cm⁻¹ and 604 cm⁻¹ were attributed to P-O bending of phosphate group and the band at 1034 cm⁻¹ was assigned to O-P-O carbonate ions of hydroxyl sites [14]. The bands at 3430 cm⁻¹ and 1640 cm⁻¹ were attributed to stretching and bending vibrations of hydroxyl (–OH) group. The band at 1459 cm⁻¹ was owing to vibrational mode of carbonate ion [27]. The spectrum of *f*-SWCNTs (Fig. 1b) displayed all the characteristic bands of HA, in addition to these a weak band at 1630 cm⁻¹ was observed owing to C-C stretching of SWCNTs. Another weak band at 1720 cm⁻¹ corresponds to C=O, which indicates the existence of carboxylic groups on the surface of SWCNTs.

To further characterize *f*-SWCNTs, X-ray diffraction analysis was performed and the patterns are shown in Fig. 2. The diffraction peaks at 26.4° and 42.6° in Fig. 2a are assigned to graphite crystallographic (0 0 2) and (1 0 0) plane of CNTs [25]. The high intensity of these peaks suggests that *p*-SWCNTs have a high graphitic structure. Two principal diffraction peaks were displayed in Fig. 2(b) at 2 θ values of 25.9° and 32°, these were attributed to (0 0 2) and (2 1 1) reflections of HA, respectively. The remaining peaks at 39.8°, 46.7°, 49.4°, 53.2° and 63.5° were assigned to (3 1 0), (2 2 2), (2 1 3), (0 0 4) and (3 0 4) reflections of HA, respectively. These values of 2 θ are in good agreement with the JCPDS file No. 09-0432. Because of the overlapping of (0 0 2) plane of HA, the characteristic (0 0 2) diffraction of SWCNTs was not distinctly observed for *f*-SWCNTs (Fig. 2b). Thus the results obtained from XRD, confirm the grafting of HA onto SWCNTs-COOH.

Raman spectra of *p*-SWCNTs and *f*-SWCNTs are presented in Figs. 3(a) and (b). The *p*-SWCNTs displayed G-band at 1587 cm⁻¹ and D-band at 1306 cm⁻¹, whereas these bands appeared at 1591 and 1310 cm⁻¹, respectively in the spectrum of *f*-SWCNTs. The appearance of G-band is signature of highly pure graphitic structure, while D-band originates from a disorder-induced mode in the graphite. Thus the intensity ratio of D to G-band (ID/IG) is the index of defect density present in SWCNTs. The ID/IG ratio calculated for *p*-SWCNTs was found to be 0.4 and for *f*-SWCNTs it was 0.81. The lower value of ID/IG for *p*-SWCNTs implies that the SWCNTs used in this experiment were composed of well-crystallized graphite. The high value of ID/IG ratio for *f*-SWCNTs indicates that the intrinsic nanotube disorder is affected by the grafting of HA [28]. Moreover, shift in the frequency value of both G and D-bands for *f*-SWCNTs towards higher wavenumber was observed, which is associated with increase in the defect density of graphite by grafting of HA.

The SEM images shown in Fig. 4 provide further evidence that confirms grafting of HA on SWCNTs. The loading of HA on the surfaces of SWCNTs is clearly detectable in Fig. 4. The images (Figs. 4b–d) taken at different regions have similar morphology, which demonstrate the homogenous grafting of HA on SWCNTs. Obviously, the grafting has a rough surface, this rough surface is helpful for good adhesion and immobilization of cells. Also it is observable from the images that SWCNTs preserved their integral shape after

grafting of HA. These phenomena were further supported by the TEM images shown in Fig. 5. Figs. 5(b)–(d) reveals deposition of HA on SWCNTs and the surface of SWCNTs was not smooth compared to carboxylated SWCNT (Fig. 5a) owing to existence of HA. Fig. 5(c) is a magnified image of Fig. 5(b), in which the HA grafted on SWCNTs is clearly detectable. It was also found that the surfaces of SWCNTs were fully covered with HA and an intimate contact exists between the SWCNTs and HA. Moreover the TEM images reveals that HA grafted on SWCNTs was of low crystalline with a thickness of about 2–3 nm, which was supported by XRD results. The elemental composition of *f*-SWCNTs was estimated by EDS analysis (Fig. 6). The intense peaks of Ca, P, and O demonstrate the deposition of HA on carboxylated SWCNTs. The ratio of Ca to P determined from the EDS was found to be 1.65, which is close to the stoichiometric value of HA (1.67) present in bone tissue [22]. In addition the peaks of Cu were also observed in EDS, these originated from the copper grid used for TEM measurements.

In order to estimate the grafting efficiency of HA on SWCNTs, TGA analysis was employed and the resulted weight loss curves are shown in Fig. 7. A total weight loss of 16.7% was observed in the range of 25–800°C for HA (Fig. 7a), which was due to removal of adsorbed water and the water released from decarboxylation of HA or condensation of HPO_4^{2-} [29]. The thermal degradation of *f*-SWCNTs was entirely different from that of HA and exhibited an overall weight loss of 70% between 25–800°C (Fig. 7b). For *f*-SWCNTs, the initial weight loss of 12.5% below 120°C was due to loss of adsorbed water and the consecutive weight loss between 130–360°C (10.6%) was resulted by the removal of water released from decarboxylation or condensation process. The subsequent weight loss of 45.9% observed between 360–580°C was mainly contributed by combustion of SWCNTs. The residual weight measured for HA at 800 °C was 83.9% and for *f*-SWCNTs it was 30.1%. Thus the conformational changes observed in the thermal degradation of HA and *f*-SWCNTs demonstrate the effective grafting of HA on SWCNTs.

4. Conclusions

In summary, a simple and rapid method for effective grafting of HA on SWCNTs has been reported. This technique is economic, easy to repeat and can be optimized for mass production. As prepared *f*-SWCNTs is a promising biomaterial to use without any further treatment or calcination. It may be of great potential to widen the applications of *f*-SWCNTs in biomedical field, especially in bone tissue engineering. Further studies on mechanical properties and biocompatibility of *f*-SWCNTs are under way in our laboratory and will be reported in due course.

Research highlights

1. This method is facile and effective to graft HA on SWCNTs.
2. As *f*-SWCNTs is a promising biomaterial to use without any further processing.
3. This technique is economic, easy to repeat and can be optimized for mass production.
4. Ratio of Ca to P in *f*-SWCNTs was 1.65, which is near to ratio present in bone tissue.

Acknowledgments

The authors acknowledge the support from NIH-NIGMS grant #1SC3GM086245, NIH-NIGMS RISE grant #1R25GM078361 and the Welch foundation.

References

1. Iijima S. *Nature*. 1991; 354:56.
2. Ajayan PM. *Chem. Rev.* 1999; 99:1787. [PubMed: 11849010]
3. Cholpek J, Czajkowska B, Szaraniec B, Beguin F. *Carbon*. 2006; 44:1106.
4. Price RL, Waid MC, Haberstroh KM, Webster TJ. *Biomaterials*. 2003; 24:1877. [PubMed: 12615478]
5. Peigney A. *Nat. Mater.* 2003; 2:15. [PubMed: 12652665]
6. Zanello LP, Zhao B, Hu H, Haddon RC. *Nano Lett.* 2006; 6:562. [PubMed: 16522063]
7. Correa-Duarte MA, Wagner N, Rojas-Chapana J, Morsczeck C, Thie M, Giersig M. *Nano Lett.* 2004; 11:2233.
8. MacDonald RA, Laurenzi BF, Viswanathan G, Ajayan PM, Stegemann JP. *J. Biomed. Mater. Res.* 2005; 74:489.
9. Shi X, Hudson JL, Spicer PP, Tour JM, Krishnamoorti R, Mikos AG. *Biomacromolecules*. 2006; 7:2237. [PubMed: 16827593]
10. Sitharaman B, Shi X, Tran LA, Spicer PP, Rusakova I, Wilson LJ, Mikos AG. *J. Biomater. Sci., Polym. Ed.* 2007; 18:655. [PubMed: 17623549]
11. Gu YW, Loha NH, Khor KA, Tor SB, Cheang P. *Biomaterials*. 2002; 23:37–43. [PubMed: 11762852]
12. Yu LG, Khor KA, Li H, Cheang P. *Biomaterials*. 2003; 24:2695. [PubMed: 12711515]
13. White AA, Best SM, Kinloch IA. *Int. J. Appl. Ceram. Technol.* 2007; 4:1.
14. Zhao HY, Xu XX, Zhang JX, Zheng W, Zheng YF. *Bioelectrochemistry*. 2010; 78:124. [PubMed: 19762287]
15. Najafi H, Nemati ZA, Sadeghian Z. *Ceram. Int.* 2009; 35:2987.
16. Bai Y, Neupane MP, Park S, Lee MH, Bae TS, Watari F, Uo M. *Mater. Sci. Eng. C*. 2010; 30:1043.
17. Li A, Sun K, Dong W, Zhao D. *Mater. Lett.* 2007; 61:1839.
18. Liang C, Li H, Wang L, Chen X, Zhao W. *Mater. Chem. Phys.* 2010; 124:21.
19. Lahiri D, Singh V, Keshri AK, Seal S, Agarwal A. *Carbon*. 2010; 48:3103.
20. Singh MK, Shokuhfar T, Gracio JJA, Sousa ACM, Ferreira JMF, Garmestani H, Ahzi S. *Adv. Funct. Mater.* 2008; 18:694.
21. Xiao Y, Gong T, Zhou S. *Biomaterials*. 2010; 31:5182. [PubMed: 20392491]
22. Lobo AO, Corat MAF, Ramos SC, Matsushima JT, Granato AEC, Pacheco-Soares C, Corat EJ. *Langmuir*. 2010; 26:18308. [PubMed: 20961085]
23. White AA, Windle AH, Kinloch IA, Best SM. *Key Eng. Mater.* 2008; 361–363:419.
24. Neelgund GM, Oki A. *Appl. Catal. A: Gen.* (In Press).
25. Neelgund GM, Oki A. *J. Nanosci. Nanotechnol.* 2011; 11:3621. [PubMed: 21776746]
26. Venkatesan J, Qian ZJ, Ryu B, Kumar NA, Kim SK. *Carbohydr. Polym.* 2011; 83:569.
27. Xu JL, Khor KA, Sui JJ, Chen WN. *Mater. Sci. Eng. C*. 2009; 29:44.
28. Yuan W, Guohua J, Che J, Qi X, Xu R, Chang MW, Chen Y, Lim SY, Dai J, Chan-Park MB. *J. Phys. Chem. C*. 2008; 112:18754.
29. Kalita SJ, Verma S. *Mater. Sci. Eng. C*. 2010; 30:295.

Fig. 1.
FTIR spectra of (a) HA and (b) *f*-SWCNTs.

Fig. 2.
Powder XRD patterns of (a) *p*-SWCNTs and (b) *f*-SWCNTs.

Fig. 3.
Raman spectra of (a) *p*-SWCNTs and (b) *f*-SWCNTs.

Fig. 4.
SEM images of (a) SWCNTs-COOH and (b–d) *f*-SWCNTs.

Fig. 5.
TEM images of (a) SWCNTs-COOH and (b–d) *f*-SWCNTs.

Fig. 6.
EDS of *f*-SWCNTs.

Fig. 7.
TGA profiles of (a) HA and (b) *f*-SWCNTs.

Scheme 1.
Schematic representation of grafting of HA on SWCNTs.