Fabrication, Mechanical Properties, and Biocompatibility of Graphene-Reinforced Chitosan Composites

Hailong Fan,[†] Lili Wang,[‡] Keke Zhao,[†] Nan Li,[§] Zujin Shi,[§] Zigang Ge,[*,‡] and Zhaoxia Jin[*,†]

Department of Chemistry, Renmin University of China, Beijing, 100872, People's Republic of China, and College of Engineering and College of Chemical and Molecular Engineering, Peking University, Beijing 100871, People's Republic of China

Received May 2, 2010; Revised Manuscript Received July 13, 2010

Few-layered graphene sheets, synthesized by direct current arc-discharge method using NH_3 as one of the buffer gases, were dispersed in chitosan/acetic acid solutions. FTIR and X-ray photoelectron spectroscopy showed the presence of oxygen-containing functional groups on the surface of graphene sheets that may assist the good dispersion of graphene in chitosan solution. Graphene/chitosan films were produced by solution casting method. The mechanical properties of composite films were tested by nanoindentation method. With the addition of a small amount of graphene in chitosan (0.1−0.3 wt %), the elastic modulus of chitosan increased over ∼200%. The biocompatibility of graphene/chitosan composite films was checked by tetrazolium-based colorimetric assays in vitro. The cell adhesion result showed that the L929 cell can adhere to and develop on the graphene/chitosan composite films as well as on pure chitosan film, indicating that graphene/chitosan composites have good biocompatibility. Because there is no metallic impurity in graphene raw materials, the time-consuming purification process for removing metal nanoparticles entrapped in carbon nanotubes is thus avoided when graphene is used to prepare biomedical materials. Graphene/chitosan composites are potential candidates as scaffold materials in tissue engineering.

Introduction

Recently, carbon nanotube (CNT)−reinforced polymer composites have been extensively studied for their applications as biomedical materials.[1−7] The CNT-based scaffolds are mechanically strong and electro-active and are useful for stimulate-guided growth of cells.[5,6,8,9] However, because the metal catalyst used in the fabrication of carbon nanotubes can be trapped inside carbon nanotubes, metal-free carbon nanotubes are only obtained after extensive and time-consuming purification processes.[10−12] The metallic impurity inside carbon nanotubes has potentially negative influences on their cytotoxicity when used as biomaterials.[13] It is better to have a metal-free material that may enhance the mechanical properties and electric conductivities of polymer scaffolds.

After the success of micromechanical cleavage of highly ordered pyrolytic graphite (HOPG), graphene has become one of the most fascinating materials for its novel properties such as quantum Hall effect at room temperature,[14] tunable band gap,[15] and high elasticity.[16−19] Polymer composites containing graphene possess good electric conductivity, thermal conductivity, and mechanical stiffness.[20−22] Very recently, Rao's group reported a significant increase of mechanical properties of polyvinyl alcohol (PVA) with the addition of functionalized few-layer graphene.[23] They further observed an extraordinary synergistic effect in the mechanical properties of PVA reinforced with two nanocarbons.[24] Rafiee et al. compared the mechanical properties of graphene/epoxy and carbon nanotube/epoxy nanocomposites at a low filler content of 0.1 wt %. They found that graphene platelets significantly out-performed carbon nanotubes as a reinforced additive.[25] Such unique reinforcing behavior of graphene sheets attracted our interest to investigate graphene-reinforced biocompatible materials. The research of graphene/biomacromolecules composite materials is limited. Li et al. reported their pioneering work using graphene paper to culture cells.[26] The graphene paper showed ultrastrong and biocompatible properties which are highly desirable for scaffolds in bone tissue engineering. However, there is still no report on graphene-reinforced natural biomacromolecules, such as chitosan. In this study, we fabricated graphene/chitosan composite films, and then explored their mechanical properties and biocompatibility. The addition of graphene significantly increased the modulus of chitosan at a very low content (between 0.1 to 0.3 wt %). The composites showed good biocompatibility for L929 cells as confirmed by in vitro MTT assays.

Experimental Section

Materials. Graphene sheets used in our experiments were prepared by direct-current arc-discharge method as reported previously.[27] Chitosan (reagent grade) with deacetylation degree above 90% and acetic acid were purchased from Sinopharm Chemical Reagent Co., Ltd. All solutions were prepared using doubly distilled Millipore water and filtered by 0.45 μm membrane before use.

Preparation and Characterization of Graphene/Chitosan Composite Films. Graphene sheets (8.5 mg) were first dispersed in 250 mL of 3 M acetic acid aqueous solution by ultrasonication for 1 h. Then chitosan was dissolved in this graphene suspension by vigorous stirring. The graphene/chitosan films were fabricated by solution-casting method. Composite films with different graphene contents (0, 0.1, 0.2, 0.3, 0.6, and 2.3 wt %) were produced. The composite films were first dried at room temperature and then in vacuo at 50 °C overnight to completely remove acetic acid.

Graphene was characterized by X-ray photoelectron spectroscopy (XPS, Perkin-Elmer PHI-5300 ESCA) and FTIR (IR Prestige-21,

* To whom correspondence should be addressed. Phone: 86-10-62515757. Fax: 86-10-62516464. E-mail: jinzx@ruc.edu.cn (Z.X.J.); gez@pku.edu.cn (Z.G.G).

[†] Renmin University of China.

[‡] College of Engineering, Peking University.

[§] College of Chemical and Molecular Engineering, Peking University.

10.1021/bm100470q
Published on Web 08/05/2010

SHIMADZU) to identify their functional groups before being dispersed in chitosan/acetic acid solution. The suspension of graphene/chitosan in aqueous acetic acid solution was characterized by ZEN3600 zetasizer (Malvern Instruments) and the morphology of the graphene sheet dispersed in chitosan/acetic acid solution was observed using a transmission electron microscope (TEM, Hitachi H-9000NAR) with an accelerating voltage of 100 kV. The Raman spectra of graphene and graphene/chitosan composite films were recorded using a Jobin Yvon HR-800 spectrometer with an excitation wavelength of 633 nm.

In situ Nanomechanical Test System (Hysitron Triboindenter, Minneapolis, MN) was used to conduct nanoindentation experiments to determine the mechanical properties of graphene/chitosan composite films. A three-sided pyramidal diamond tip (50 nm) was used in the characterization, and the displacement-controlled mode was selected. The maximum excursion is 200 nm, with the approach rate and withdraw rate of 20 nm/s, and a hold time of tip of 0 s. A total of 5−8 indentations were made for each sample, and the average values were reported.

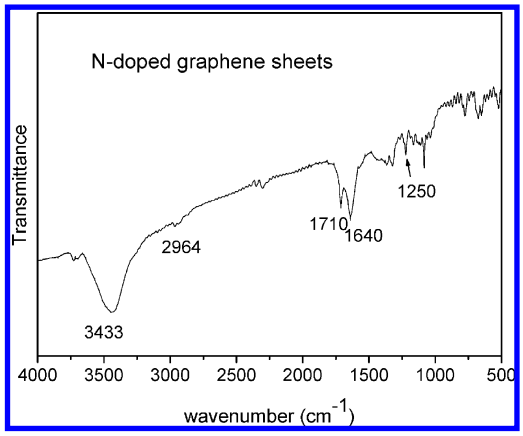

Figure 1. FTIR spectrum of graphene sheets.

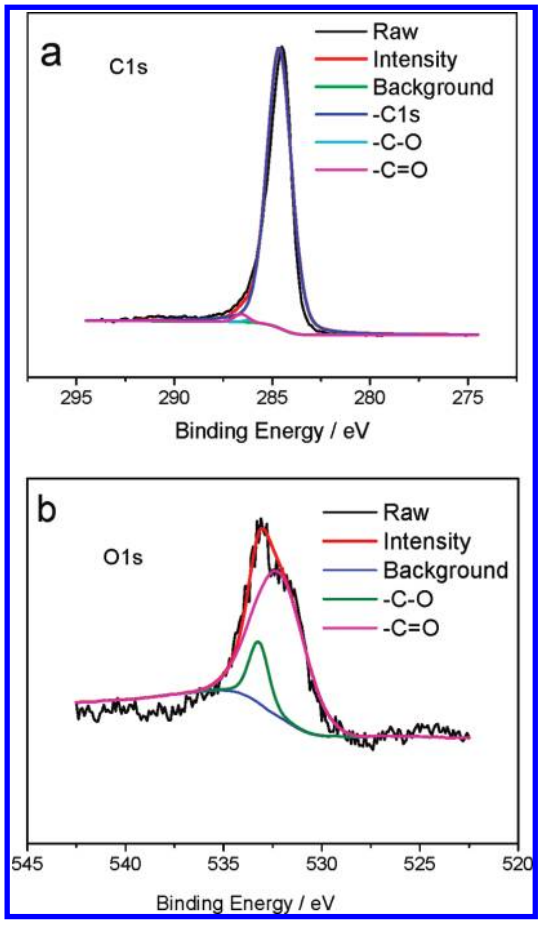

Figure 2. C1s (a) and O1s (b) spectra of graphene sheets.

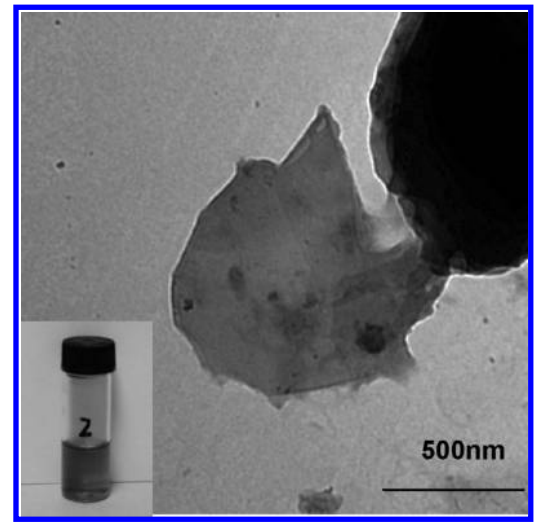

Figure 3. TEM picture of graphene sheets dispersed in chitosan/acetic acid solution. The picture inset was the graphene/chitosan solution.

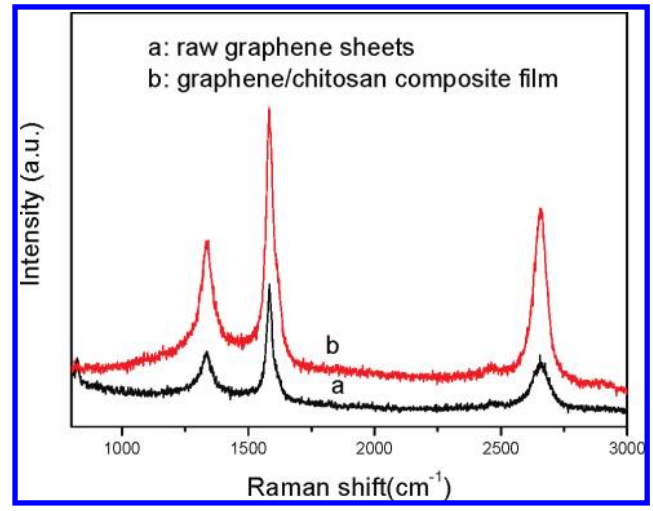

Figure 4. Raman spectra of raw graphene sheets (a) and graphene/chitosan composite film (b).

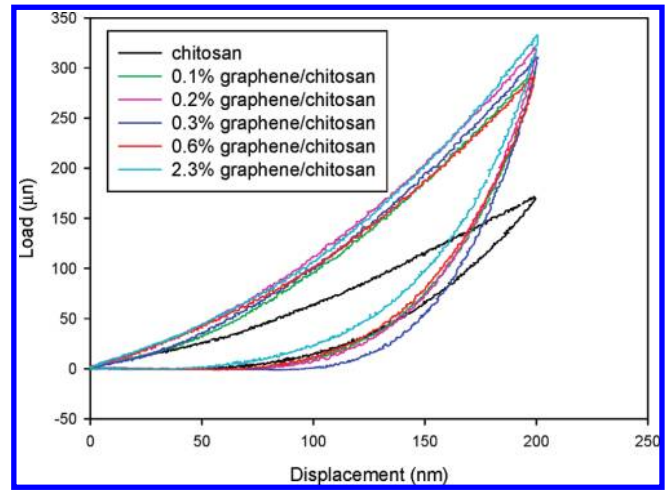

Figure 5. Load vs displacement curves of graphene/chitosan composites with different addition amount of graphene.

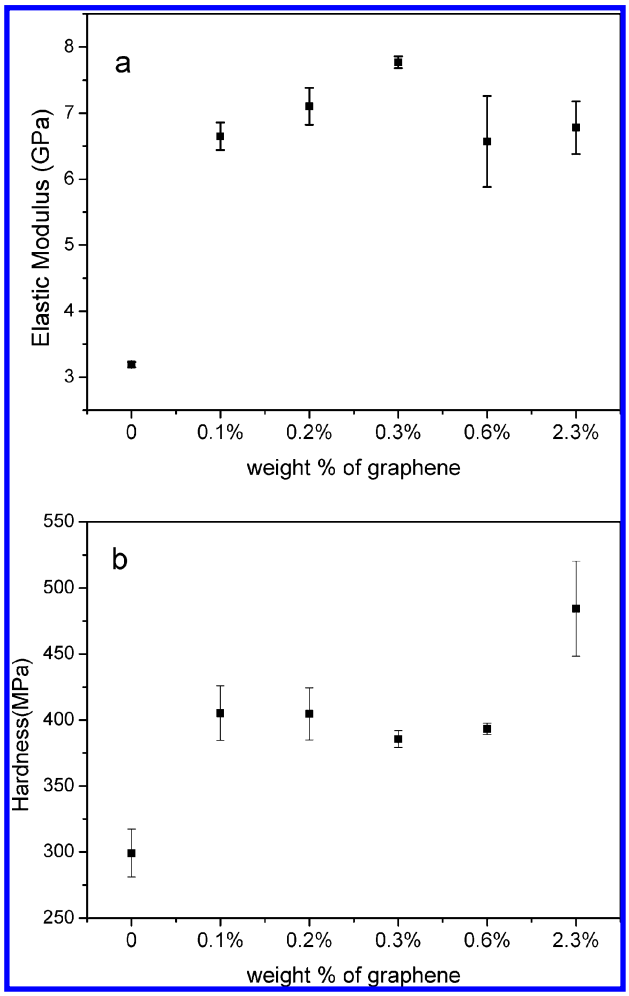

Figure 6. Mechanical properties of graphene/chitosan with different addition amount of graphene: (a) modulus; (b) hardness.

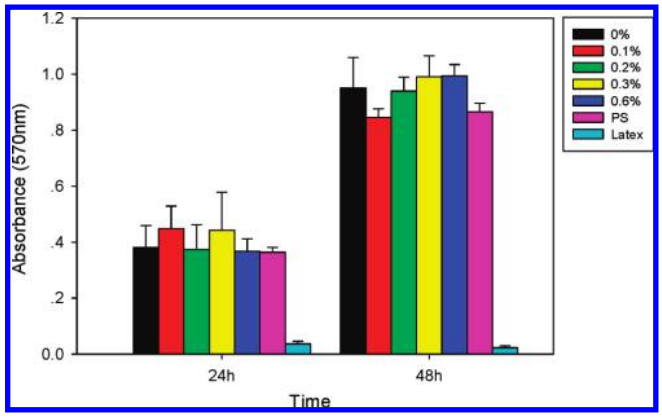

Figure 7. Results of MTT assay of L929 cells incubated with composite films at 24 and 48 h. The bar represented standard deviation of three replicates ($p < 0.05$).

Biocompatibility of Graphene/Chitosan Composite Films. *Cell Culture.* L929 cells were cultured in Dulbecco's modified Eagle's medium (12800-017, high glucose Gibco), supplemented with 10% fetal bovine serum (SV 30087.02, Hyclone) and 100 U/mL penicillin−100 μg/mL streptomycin, in a humidified 5% CO_2 balanced air incubator at 37 °C. Medium was changed every 3 days. The cells were passaged with 0.25% trypsin (Invitrogen) plus 0.02% EDTA (Sigma).

Biocompatibility. Cell viability was measured using [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] (MTT) assays. All material samples were cut into 0.5 × 0.5 cm blocks, sterilized in absolute ethanol overnight, and then rinsed with PBS solution three times. A total of 5000 cells in 100 μL of medium were seeded into each well of 96-well culture plates. Sterilized material samples were incubated with cells in individual wells for 24 or 48 h, respectively. A total of 20 μL of MTT solution (M5655, Sigma, 5 mg/mL in PBS solution) was added into each well and incubated for 3 h. After incubation, culture supernatants were aspirated, and purple insoluble MTT product formazan was dissolved in 150 μL of DMSO (Merck). Absorbance was measured at the wavelength of 570 nm with a microplate reader (Biorad 680). The polystyrene (PS) surface of the 96-well culture plate was adopted as a negative control, while latex rubber served as positive control. Three repeats were done for each group.

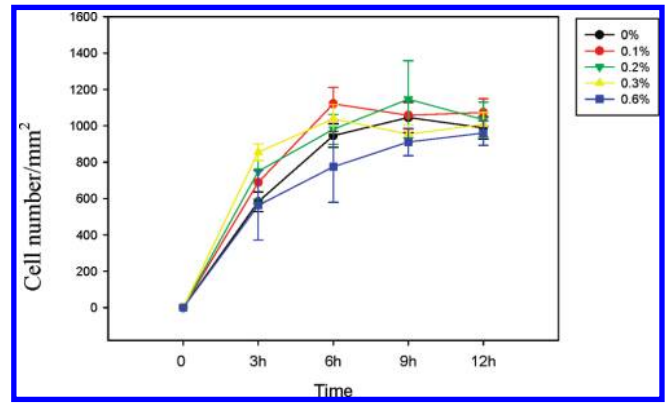

Figure 8. Cell attachment curves of L929 cells on different composite films. The bar represents standard deviation of four replicates.

Cell Attachment and Morphology Assessment. Material samples were fixed on coverslips (2 × 2 cm), sterilized in absolute ethanol overnight (water would destroy material samples), and rinsed in PBS solution three times. A total of 20000 L929 fibroblasts with 500 μL of medium were seeded on coverslips. Cell attachment and morphology were evaluated at 3, 6, 9, 12, and 24 h by phase contrast microscopy (Olympus CKX41) after unattached cells were washed out thoroughly. The extension of cells was compared by direct observation from micrographs. Four replicates were conducted at each group.

Statistics. The difference among individual groups was evaluated by the Student's *t*-test, with a p-value less than 0.05 being considered significantly different.

Results and Discussion

Following the fabrication of graphene sheets using CVD[28−30] or arc-discharge methods,[27] the direct dispersion of graphene sheets in aqueous solution is highly desirable for their further applications. However, those reported dispersions of graphene sheets in aqueous solution were based on lightly sulfonated graphene,[31] poly(sodium-4-styrene sulfonate),[32] or ssDNA[33] coated graphitic nanoplatelets, obtained from graphite oxide. The direct dispersion of graphene sheets in aqueous solution is still a challenge.[34] On the other hand, to enhance the compatibility of graphene to polymers, except chemically modified graphene,[20,35] graphene sheets should also be transformed from exfoliated graphite oxide in organic solvents.[36−39] In our experiments, the graphene sheets are N-doped graphene produced by the arc-discharge method.[27] First we used FTIR to characterize the functional groups on graphene sheets (Figure 1). The dominant peaks at ∼3430, ∼2960, and 1700−1600 cm^{-1} correspond to a stretching vibration from −OH or COOH, CH_3, and C═O, respectively,[40] demonstrating the presence of functional groups on the original graphene sheets. Peaks at ∼1710 and ∼1640 cm^{-1} may be assigned to the C═O stretching vibration in COOH and in quinone functional groups as found in functional carbon nanotubes.[40] The peak at ∼1250 cm^{-1} is

consistent with C−N stretching vibrations,[41] which was also observed previously in N-doped carbon nanotubes.[42] XPS analysis also indicated the presence of oxygen-containing functional groups, mainly C═O, on graphene sheets (Figure 2). Through FTIR and XPS studies, we have confirmed the presence of oxygen-containing functional groups on the graphene sheets, which may assist their dispersion in aqueous solution as in the case of functionalized carbon nanotubes.

After mixing with chitosan/acetic acid solution by vigorous stirring, the suspension showed good stability over a period of several months (Figure 3). The zeta potential value of graphene dispersed in chitosan/acetic acid solution is 28 mV, indicating a metastable dispersion of graphene in chitosan solution. Such a good dispersion of graphene sheets in chitosan/acetic acid solution may be the result of noncovalent modification of chitosan to carbon surface, as that in chitosan-assisted dispersion of carbon nanotubes.[43−45] Graphene sheet in chitosan solution was observed by TEM (Figure 3).

The good dispersion of graphene in chitosan solution was retained in their composite films after solution-casting. Raman spectroscopy of these composite films confirmed the existence of graphene sheets in chitosan matrix (Figure 4). There are three distinguished peaks at ∼1334, ∼1584, and 2655 cm^{-1}, representing the D band, G band, and 2D band in graphene, respectively. It is interesting to observe the change of the I_G/I_{2D} ratio of raw grapheme (1.99) and its chitosan composite (1.41). It is known that the ratio of I_G/I_{2D} is sensitive to the layers of graphene.[30,46] So the decrease of the I_G/I_{2D} ratio in

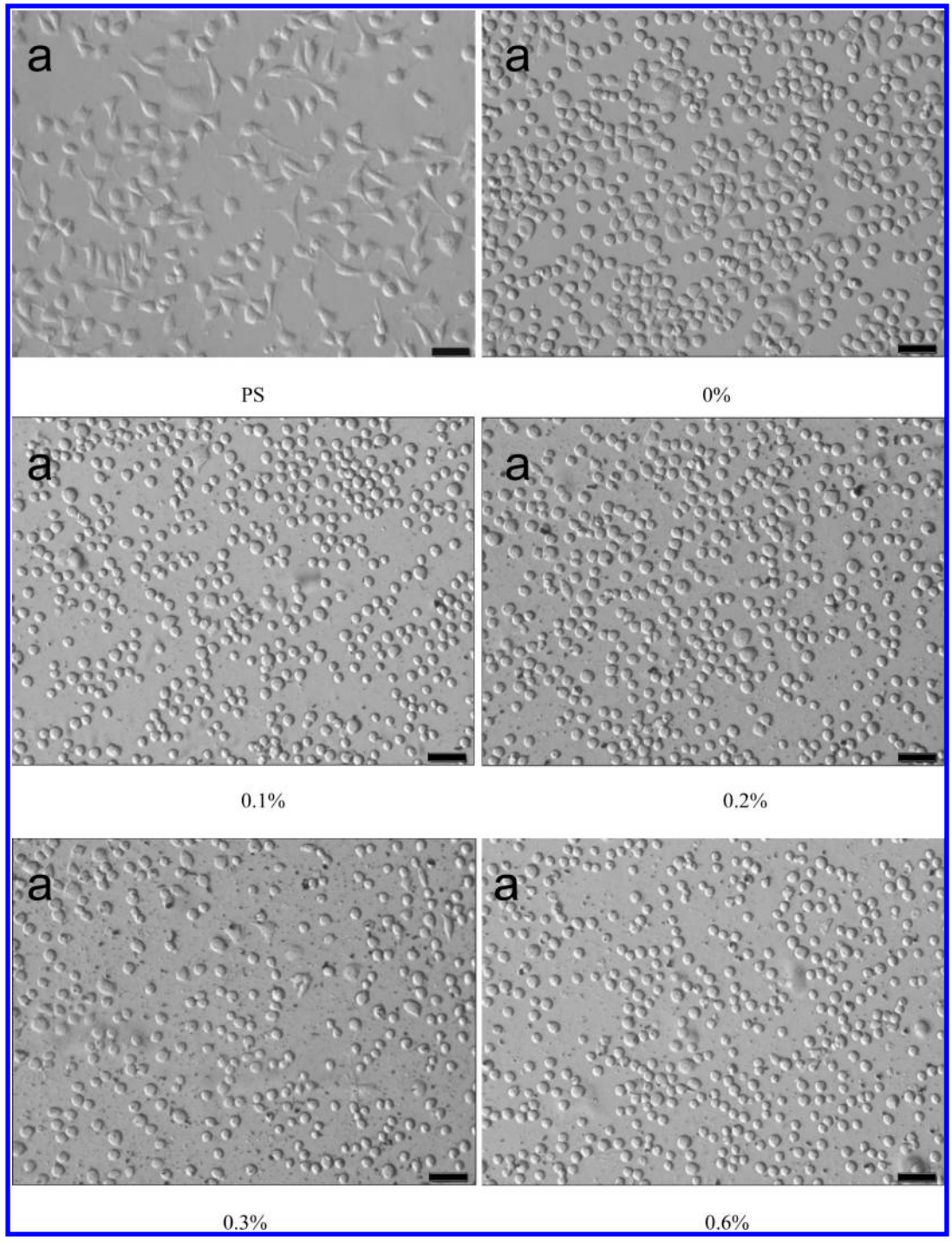

Figure 9

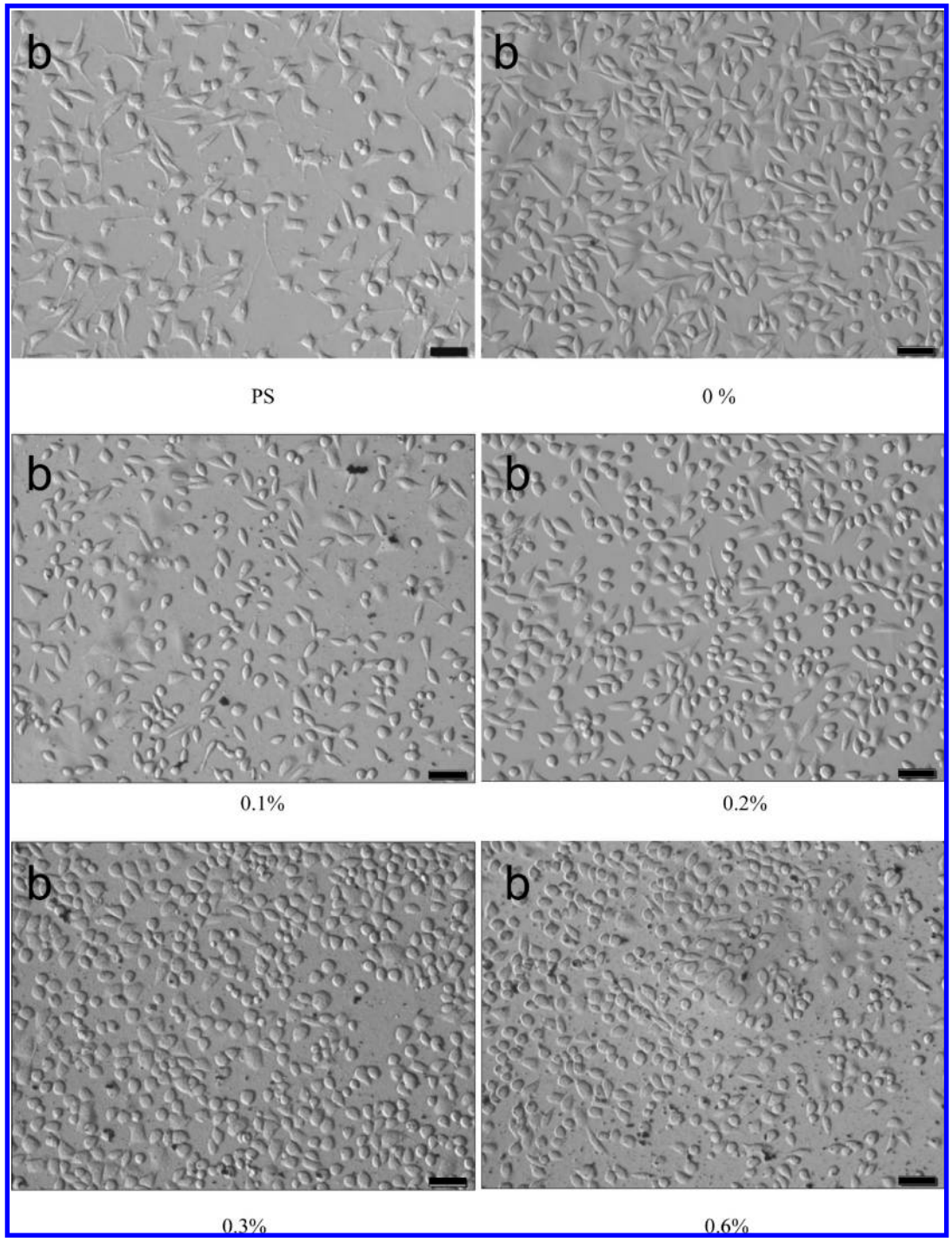

Figure 9. (a) Cell phase contrast micrographs at 12 h. The scale bar represents 50 μm. (b) Cell phase contrast micrographs at 24 h. As time increases, cells extend gradually. Cells on the lower concentration graphene/chitosan composite materials performed better. The scale bar represents 50 μm.

graphene/chitosan composite may be the result of intercalation of chitosan chains into the graphene sheets.

Although the amounts of graphene in all chitosan composite films are not very high, the enhancement of modulus by graphene addition is clearly observed, even at an amount as low as 0.1 wt %. The mechanical properties of graphene/chitosan composite films were measured by using a nanoindentation method, which is commonly used to evaluate the mechanical property of thin films. Figure 5 showed the typical indentation load−displacement curves of pure chitosan and graphene/chitosan composites. The modulus and hardness of all graphene/chitosan composite films are significantly higher than the pure chitosan film (Figure 6). This result is in agreement with other graphene-reinforced polymer composites.[23−25] Rafiee et al. observed that graphene as reinforced filler for the polymer matrix showed significant improvement in mechanical properties as compared with carbon nanotubes at a low content (~0.1 wt %).[25] Das et al. observed a significant increase in both the elastic modulus and hardness in polyvinyl alcohol (PVA) and poly(methyl methacrylate) (PMMA) composites with only 0.6 wt % of graphene added based on nanoindentation characterization.[23] Such a low graphene content offers many advantages in biomedical applications. Although graphene sheets are nonbiodegradable materials, the low graphene content in graphene/

chitosan composites may limit possible negative influence of graphene to cells after chitosan decomposed in body.

Because the graphene sheets we used are a new material with unknown biocompatibility, we next conducted MTT assays to test the cytotoxity of graphene/chitosan materials. L929 cell lines were selected in our MTT assays because they have been recommended by the International Standard Organization (ISO) as biocompatibility test model in vitro.[47] The viability of L929 cells that exposed to graphene/chitosan composites with an increasing concentration of graphene from 0 to 0.6 wt % was summarized in Figure 7. MTT results revealed no visible reduction in viability between the negative control and experimental groups at 24 and 48 h. In view of the insignificant variances ($P < 0.05$) compared with negative control groups, the graphene/chitosan composite materials showed good biological safety and displayed almost noncytotoxicity.

The cells seeded on graphene/chitosan composite films could be observed clearly by phase contrast microscope. We observed that the speed of cell attachment on tested materials was dependent on the concentration of graphene in chitosan composites (Figure 8). On the composite film with a higher concentration of graphene (0.6 wt %), the speed of cell attachment was slightly lower than that on other experimental groups. It took about 9 h to complete the attachment on 0.6 wt % graphene/chitosan composite samples, and only 6 h under other concentrations from 0 to 0.3 wt %. At 12 h, the percentages of attached cells on all tested material samples were similar and reached 80% (Figure 9). The morphology of these cells seeded on graphene/chitosan materials showed no obvious variances between different concentrations. Hence, graphene/chitosan materials had an acceptable biocompatibility in a limited graphene content range.

Compared with carbon nanotube-based composites, the graphene-based composites have one major advantage as biomaterials. The metallic impurities in CNTs limited their applications as drug carriers and biomedical sensors.[10,13,48] However, there was no such concern for graphene-based materials because of the absence of metal catalysts on graphene sheets.[35,49] Thus, graphene-reinforced biocompatible polymer scaffolds may be the next candidates as bioengineering materials. Our further studies will focus on the stimulate-guided growth of cells based on conductive graphene-based scaffolds.

Conclusions

Few-layered graphene sheets were produced by a direct arc-discharge method using NH_3 as a buffer gas. The presence of oxygen-containing functional groups on graphene sheets as identified by FTIR and XPS gave rise to their good dispersion in chitosan/acetic acid aqueous solution. The mechanical properties of graphene/chitosan composite films were measured by the nanoindentation method. Chitosan was significantly reinforced upon the addition of a small amount of graphene sheets. The graphene/chitosan composites were biocompatible to L929 cells as shown by MTT colorimetric assays. The absence of metallic impurities on graphene sheets makes them potential candidates as scaffolds for tissue engineering.

Acknowledgment. The authors gratefully acknowledge the National Nature Science Foundation of China (Grant 50503025), "Mingde Scholar Plan" from Renmin University of China, and Beijing National Laboratory for Molecular Sciences for financial support. We also thank Prof. Goh Suat Hong in National University of Singapore for his helpful discussion.

References and Notes

(1) Shi, X. F.; Hudson, J. L.; Spicer, P. P.; Tour, J. M.; Krishnamoorti, R.; Mikos, A. G. *Biomacromolecules* **2006**, *7*, 2237–2242.
(2) Veetil, J. V.; Ye, K. M. *Biotechnol. Prog.* **2009**, *25*, 709–721.
(3) Sitharaman, B.; Shi, X. F.; Walboomers, X. F.; Liao, H. B.; Cuijpers, V.; Wilson, L. J.; Mikos, A. G.; Jansen, J. A. *Bone* **2008**, *43*, 362–370.
(4) Bhattacharyya, S.; Guillott, S.; Dabboue, H.; Tranchant, J. F.; Salvetat, J. P. *Biomacromolecules* **2008**, *9*, 505–509.
(5) Lau, C.; Cooney, M. J.; Atanassov, P. *Langmuir* **2008**, *24*, 7004–7010.
(6) MacDonald, R. A.; Voge, C. M.; Kariolis, M.; Stegemann, J. P. *Acta Biomater.* **2008**, *4*, 1583–1592.
(7) Shi, X. F.; Sitharaman, B.; Pham, Q. P.; Liang, F.; Wu, K.; Billups, W. E.; Wilson, L. J.; Mikos, A. G. *Biomaterials* **2007**, *28*, 4078–4090.
(8) Zanello, L. P.; Zhao, B.; Hu, H.; Haddon, R. C. *Nano Lett.* **2006**, *6*, 562–567.
(9) Correa-Duarte, M. A.; Wagner, N.; Rojas-Chapana, J.; Morsczeck, C.; Thie, M.; Giersig, M. *Nano Lett.* **2004**, *4*, 2233–2236.
(10) Ge, C. C.; Lao, F.; Li, W.; Li, Y. F.; Chen, C. Y.; Qiu, Y.; Mao, X. Y.; Li, B.; Chai, Z. F.; Zhao, Y. L. *Anal. Chem.* **2008**, *80*, 9426–9434.
(11) Pumera, M. *Langmuir* **2007**, *23*, 6453–6458.
(12) Kolodiazhnyi, T.; Pumera, M. *Small* **2008**, *4*, 1476–1484.
(13) Isobe, H.; Tanaka, T.; Maeda, R.; Noiri, E.; Solin, N.; Yudasaka, M.; Iijima, S.; Nakamura, E. *Angew. Chem., Int. Ed.* **2006**, *45*, 6676–6680.
(14) Novoselov, K. S.; Jiang, Z.; Zhang, Y.; Morozov, S. V.; Stormer, H. L.; Zeitler, U.; Maan, J. C.; Boebinger, G. S.; Kim, P.; Geim, A. K. *Science* **2007**, *315*, 1379–1379.
(15) Castro, E. V.; Novoselov, K. S.; Morozov, S. V.; Peres, N. M. R.; Dos Santos, J. M. B. L.; Nilsson, J.; Guinea, F.; Geim, A. K.; Neto, A. H. C. *Phys. Rev. Lett.* **2007**, *99*, 216802.
(16) Novoselov, K. S.; Geim, A. K.; Morozov, S. V.; Jiang, D.; Zhang, Y.; Dubonos, S. V.; Grigorieva, I. V.; Firsov, A. A. *Science* **2004**, *306*, 666–669.
(17) Novoselov, K. S.; Jiang, D.; Schedin, F.; Booth, T. J.; Khotkevich, V. V.; Morozov, S. V.; Geim, A. K. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 10451–10453.
(18) Rao, C. N. R.; Sood, A. K.; Subrahmanyam, K. S.; Govindaraj, A. *Angew. Chem., Int. Ed.* **2009**, *48*, 7752–7777.
(19) Scarpa, F.; Adhikari, S.; Phani, A. S. *Nanotechnology* **2009**, *20*, 065709.
(20) Stankovich, S.; Dikin, D. A.; Dommett, G. H. B.; Kohlhaas, K. M.; Zimney, E. J.; Stach, E. A.; Piner, R. D.; Nguyen, S. T.; Ruoff, R. S. *Nature* **2006**, *442*, 282–286.
(21) Wang, D. W.; Li, F.; Zhao, J. P.; Ren, W. C.; Chen, Z. G.; Tan, J.; Wu, Z. S.; Gentle, I.; Lu, G. Q.; Cheng, H. M. *ACS Nano* **2009**, *3*, 1745–1752.
(22) Eda, G.; Chhowalla, M. *Nano Lett.* **2009**, *9*, 814–818.
(23) Das, B.; Prasad, K. E.; Ramamurty, U.; Rao, C. N. R. *Nanotechnology* **2009**, *20*, 125705.
(24) Prasad, K. E.; Das, B.; Maitra, U.; Ramamurty, U.; Rao, C. N. R. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 13186–13189.
(25) Rafiee, M. A.; Rafiee, J.; Wang, Z.; Song, H. H.; Yu, Z. Z.; Koratkar, N. *ACS Nano* **2009**, *3*, 3884–3890.
(26) Chen, H. Q.; Muller, M. B.; Gilmore, K. J.; Wallace, G. G.; Li, D. *Adv. Mater.* **2008**, *20*, 3557–3561.
(27) Li, N.; Wang, Z. Y.; Zhao, K. K.; Shi, Z. J.; Gu, Z. N.; Xu, S. K. *Carbon* **2009**, *48*, 255–259.
(28) Yuan, G. D.; Zhang, W. J.; Yang, Y.; Tang, Y. B.; Li, Y. Q.; Wang, J. X.; Meng, X. M.; He, Z. B.; Wu, C. M. L.; Bello, I.; Lee, C. S.; Lee, S. T. *Chem. Phys. Lett.* **2009**, *467*, 361–364.
(29) Malesevic, A.; Vitchev, R.; Schouteden, K.; Volodin, A.; Zhang, L.; Van Tendeloo, G.; Vanhulsel, A.; Van Haesendonck, C. *Nanotechnology* **2008**, *19*, 305604.
(30) Reina, A.; Jia, X. T.; Ho, J.; Nezich, D.; Son, H. B.; Bulovic, V.; Dresselhaus, M. S.; Kong, J. *Nano Lett.* **2009**, *9*, 30–35.
(31) Si, Y. C.; Samulski, E. T. *Nano Lett.* **2008**, *8*, 1679–1682.
(32) Stankovich, S.; Piner, R. D.; Chen, X. Q.; Wu, N. Q.; Nguyen, S. T.; Ruoff, R. S. *J. Mater. Chem.* **2006**, *16*, 155–158.
(33) Patil, A. J.; Vickery, J. L.; Scott, T. B.; Mann, S. *Adv. Mater.* **2009**, *21*, 3159–3164.
(34) Li, D.; Muller, M. B.; Gilje, S.; Kaner, R. B.; Wallace, G. G. *Nat. Nanotechnol.* **2008**, *3*, 101–105.

(35) McAllister, M. J.; Li, J. L.; Adamson, D. H.; Schniepp, H. C.; Abdala, A. A.; Liu, J.; Herrera-Alonso, M.; Milius, D. L.; Car, R.; Prud'homme, R. K.; Aksay, I. A. *Chem. Mater.* **2007**, *19*, 4396–4404.
(36) Cote, L. J.; Cruz-Silva, R.; Huang, J. X. *J. Am. Chem. Soc.* **2009**, *131*, 11027–11032.
(37) Cai, D. Y.; Yusoh, K.; Song, M. *Nanotechnology* **2009**, *20*, 085712.
(38) Paredes, J. I.; Villar-Rodil, S.; Martinez-Alonso, A.; Tascon, J. M. D. *Langmuir* **2008**, *24*, 10560–10564.
(39) Steurer, P.; Wissert, R.; Thomann, R.; Mulhaupt, R. *Macromol. Rapid Commun.* **2009**, *30*, 316–327.
(40) Zhang, J.; Zou, H. L.; Qing, Q.; Yang, Y. L.; Li, Q. W.; Liu, Z. F.; Guo, X. Y.; Du, Z. L. *J. Phys. Chem. B* **2003**, *107*, 3712–3718.
(41) Choi, H. C.; Bae, S. Y.; Park, J.; Seo, K.; Kim, C.; Kim, B.; Song, H. J.; Shin, H. J. *Appl. Phys. Lett.* **2004**, *85*, 5742–5744.
(42) Misra, A.; Tyagi, P. K.; Singh, M. K.; Misra, D. S. *Diamond Relat. Mater.* **2006**, *15*, 385–388.
(43) Yang, Q.; Li, S.; Pan, X. J. *Biomacromolecules* **2008**, *9*, 3422–3426.
(44) Zhang, J. P.; Wang, Q.; Wang, L.; Wang, A. Q. *Carbon* **2007**, *45*, 1917–1920.
(45) Yan, L. Y.; Poon, Y. F.; Chan-Park, M. B.; Chen, Y.; Zhang, Q. *J. Phys. Chem. C* **2008**, *112*, 7579–7587.
(46) Ferrari, A. C.; Meyer, J. C.; Scardaci, V.; Casiraghi, C.; Lazzeri, M.; Mauri, F.; Piscanec, S.; Jiang, D.; Novoselov, K. S.; Roth, S.; Geim, A. K. *Phys. Rev. Lett.* **2006**, *97*, 187401.
(47) Zange, R.; Kissel, T. *Eur. J. Pharm. Biopharm.* **1997**, *44*, 149–157.
(48) Smart, S. K.; Cassady, A. I.; Lu, G. Q.; Martin, D. J. *Carbon* **2006**, *44*, 1034–1047.
(49) Wu, Z. S.; Ren, W. C.; Gao, L. B.; Zhao, J. P.; Chen, Z. P.; Liu, B. L.; Tang, D. M.; Yu, B.; Jiang, C. B.; Cheng, H. M. *ACS Nano* **2009**, *3*, 411–417.

BM100470Q