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A novel three-dimensional graphene/bacterial cellulose nanocomposite prepared by *in situ* biosynthesis

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Abstract

Graphene has been widely used to reinforce various hydrogels while there is no report on the composite hydrogels of bacterial cellulose (BC) and graphene. In this work, a graphene/BC (GE/BC) nanocomposite hydrogel was prepared by in situ biosynthesis. The morphology and structure of the obtained GE/BC nanocomposite were characterized by SEM, TEM, XRD, and Raman. Results showed that the presence of graphene in the culture medium of BC changed the crystalline structure of BC while the *in situ* biosynthesis process had no influence on the structure of graphene. It was found that graphene nanoplates were uniformly dispersed in the three-dimensional (3D) BC matrix and tightly bound by BC nanofibers. This unique 3D structure will impart the GE/BC nanocomposite excellent mechanical, electrical, and biological

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properties.

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Polymer nanocomposites reinforced with nanosized carbonaceous fillers, in particular, carbon nanotube (CNT) and graphene have been extensively studied.[1-4] Compared with CNTs which may cause toxic reactions,[5] graphene is believed to raise fewer toxicity concerns[6] and a study carried out by Rafiee et al. confirmed that graphene significantly out-performed CNTs as a reinforced additive.[7] Therefore, polymer composites reinforced with graphene have attracted tremendous interest in recent years. Numerous polymers such as poly (vinyl alcohol),[8, 9] chitosan,[10] polyacrylamide,[11] epoxy,[12] poly (sodium acrylate),[13] and nanocellulose[14] have been used to form nanocomposites with graphene and its derivatives.

A recent study reported the preparation of a nanocomposite based on a graphene derivative (graphene oxide) and bacterial cellulose (BC) by a mechanical mixing method.[15] Though the method is simple, the obtained nanocomposite has broken the intrinsically three-dimensional (3D) structure of BC, which is believed to be the most striking features distinguishing BC from other natural polymers. BC, a nanofibrous cellulosic material produced by the bacteria, *Acetobacter xylinum* (*A. xylinum*), has been suggested to be suitable as a tissue engineering scaffold due to its 3D structure, high biocompatibility, sufficient mechanical strength, good stability, interconnected pores and tunable pore structure.[16-19] Therefore, exploring a new method that can ensure the uniform distribution of graphene in the BC matrix while retaining the advantageous 3D structure of BC is of vital importance for the development of a new tissue engineering scaffold. Herein we developed a one-pot *in situ* biosynthesis approach for the fabrication of graphene/BC (abbreviated as GE/BC hereinafter)

nanocomposite. It is assumed that the *in situ* biosynthesized GE/BC nanocomposite would show high mechanical properties, improved electrical conductivity and possible enhanced biological behavior as compared to the pristine BC.

The aim of the present study was to prepare the GE/BC nanocomposite and to evaluate its morphology and structure. The influence of graphene on the mechanical and electrical properties and cell compatibility of BC hydrogels will be addressed in another article.

Commercially available aqueous dispersion of graphene with a concentration of 0.2 mg/ml was purchased from Nanjing XFNANO Materials Technology Co. Ltd., China. According to the supplier, the graphene nanoplates had an average diameter of several microns. Reagents for BC production included yeast extract, tryptone, disodium phosphate (Na_2HPO_4), and acetic acid. All reagents were used without further purification.

In this work, the bacterial strain, *Acetobacter xylinum* X-2, was used to produce BC and GE/BC hydrogels. Prior to incubation, the culture medium was sterilized at 121 °C in autoclave for 30 min. The medium for the pristine BC was composed of 2.5% (w/v) glucose, 0.75% (w/v) yeast extract, 1% (w/v) tryptone, and 1% (w/v) Na_2HPO_4, and the pH was adjusted to 4. 5 by acetic acid. To prepare GE/BC nanocomposite hydrogel, a graphene-dispersed culture medium was first prepared. The schematic diagram of the synthesis of GE/BC nanocomposite hydrogel is illustrated in Fig. 1. In a typical process, 20 mL graphene suspension (0.2 mg/ml) was added to 200 mL culture medium followed by intense stirring for 60 min. The seed

broth (2 ml) was inoculated into a 500 ml Erlenmeyer flask containing 200 ml of the graphene-dispersed medium. The flasks were incubated under static condition at 30 °C for 10 days. For comparison purpose, the pristine BC was prepared under the same conditions for 10 days. The harvested GE/BC and BC pellicles were purified and cleansed following the procedures described previously.[20]

The morphology of BC and GE/BC samples freeze-dried for 24 h was observed using a field emission scanning electron microscope (FE-SEM, Nano 430, FEI, USA) and transmission electron microscope (TEM, Philips Tecnai G2 F20) operating at 200 kV. The Raman spectra of pristine graphene and freeze-dried GE/BC were recorded using a Jobin Yvon HR-800 spectrometer with an excitation wavelength of 633 nm. The crystalline structure of BC and GE/BC was studied using a Rigaku D/max 2,500 X-ray diffractometer with a thin film attachment. Cu-Kα radiation was utilized (λ = 0.154 nm) and the samples were scanned from 5 to 30 ° with a scan speed of 2 °/min. The crystallinity index (CI) was calculated by Segal's method.[21]

The FESEM images of BC and GE/BC samples are shown in Fig. 2. It was clearly seen from Fig. 2a that the pristine BC had a typical 3D network structure and interconnected pores. The images of GE/BC nanocomposite shown in Fig. 2b-d revealed that the 3D network structure remained unchanged and the spaces among BC nanofibers were still open after incorporating graphene nanoplates. Importantly, graphene nanoplates were evenly distributed within the BC matrix. As can be seen from Fig. 2c and d, graphene nanoplates appeared to be bound by BC nanofibers in a spider web-like manner. The formation of this strongly bonded structure is probably

due to the entrapment of graphene nanoplates by BC nanofibers during the BC growing process. The formation of this unique structure is also illustrated in Fig. 1. This special structure is obviously beneficial to obtaining improved mechanical properties. SEM observation demonstrated that *in situ* biosynthesis was a facile and effective method to prepare GE/BC nanocomposite.

In order to further characterize the structure of graphene nanoplates in the GE/BC nanocomposite, TEM was used to investigate the morphology (Fig. 3). Fig. 3a showed that BC and graphene nanoplates were closely entangled, consistent with SEM findings. As shown in Fig. 3b, the graphene crystal lattices could be seen clearly in the HRTEM image, indicating that graphene could maintain its perfect crystal structure after the biosynthesis process.

XRD results (Fig. 4) show that three peaks in the BC spectrum, corresponding to $(1\bar{1}0)$, (110), and (200) planes, respectively were identified to cellulose I.[22] The peak sharpness indicated that the BC was semi-crystalline.[23] The spectrum of graphene showed a straight line without apparent diffraction peak at $2\theta = 13.7$ °, consistent with previous report by Zhang et al,[24] which was an indication of single-layer graphene.[25] As expected, no obvious diffraction peaks for graphene could be observed in the GE/BC nanocomposite spectrum, which may be due to the single-layered graphene in the composite and the low amount of graphene nanoplates in the GE/BC nanocomposite. Furthermore, it was shown that adding graphene in the culture medium significantly reduced the crystallinity of BC from 89 to 81%. The change of crystillinity caused by addition of foreign substances was previously reported in the

literature.[15, 26] It was believed that adding foreign supplement changed the viscosity of culture medium and thus disturbed the movement of bacteria.[26, 27] This disturbance might impede the crystallization process of nascent nanofibril, resulting in a lower crystallinity. Even though further studies are needed to clarify the exact mechanisms on the influence of GE on the crystallinity, the XRD results clearly suggested that the addition of graphene influenced the crystal structure of BC.

Raman spectroscopy has been one of the most widely used techniques to characterize the structural and electronic conjugation of graphene materials.[28, 29] Fig. 5 shows the Raman spectra of GE/BC and the pristine graphene. The spectra showed two distinguished D band and G band, which corresponded to the A_{1g} breathing mode and in-plane E_{2g} vibrational mode, respectively. Moreover, other two weak peaks were also observed, which were due to the 2D and S3 bands [28, 29]. Notably, the peak intensity ratio, I_D/I_G, which is an indication of the defect population and is proportional to size of crystalline domains,[30, 31] did not show significant difference (I_D/I_G = 1.1) between the pristine graphene and GE/BC, indicating that the structure of graphene did not change after *in situ* biosynthesis.

In conclusion, a novel GE/BC nanocomposite hydrogel has been prepared by simply adding graphene suspension into the culture medium of BC. The addition of graphene reduced the crystallinity of BC while the structure of graphene remained unchanged after *in situ* biosynthesis. The graphene nanoplates were well-dispersed throughout the BC matrix and a well-bound and entangled network structure of GE/BC was formed. This structure was presumed to endow BC with excellent

mechanical properties and electrical performance. Although characterizations of mechanical, electrical, and biological properties are still in progress, we believe that the GE/BC nanocomposite described herein shows promise as a tissue engineering scaffold.

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Notes and references

1. J. N. Coleman, U. Khan and Y. K. Gun'ko, *Adv Mater* 2006, **18**, 689-706.
2. D. Y. Lewitus, J. Landers, J. R. Branch, K. L. Smith, G. Callegari, J. Kohn and A. V. Neimark, *Adv Funct Mater* 2011, **21**, 2624-2632.
3. K. S. Novoselov, V. I. Fal'ko, L. Colombo, P. R. Gellert, M. G. Schwab and K. Kim, *Nature* 2012, **490**, 192-200.
4. V. C. Sanchez, A. Jachak, R. H. Hurt and A. B. Kane, *Chem Res Toxicol* 2012, **25**, 15-34.
5. C. A. Poland, R. Duffin, I. Kinloch, A. Maynard, W. A. H. Wallace, A. Seaton, V. Stone, S. Brown, W. MacNee and K. Donaldson, *Nat Nanotechnol* 2008, **3**, 423-428.
6. C. Chung, Y.-K. Kim, D. Shin, S.-R. Ryoo, B. H. Hong and D.-H. Min, *Acc Chem Res* 2013, **46**, 2211-2224.
7. M. A. Rafiee, J. Rafiee, Z. Wang, H. Song, Z.-Z. Yu and N. Koratkar, *ACS Nano* 2009, **3**, 3884-3890.

8. H. Feng, Y. Li and J. Li, *RSC Adv* 2012, **2**, 6988-6993.

9. H.-W. Liu, S.-H. Hu, Y.-W. Chen and S.-Y. Chen, *J Mater Chem* 2012, **22**, 17311-17320.

10. Y. Chen, L. Chen, H. Bai and L. Li, *J Mater Chem A* 2013, **1**, 1992-2001.

11. R. Liu, S. Liang, X.-Z. Tang, D. Yan, X. Li and Z.-Z. Yu, *J Mater Chem* 2012, **22**, 14160-14167.

12. S. Ye, J. Feng and P. Wu, *J Mater Chem A* 2013, **1**, 3495-3502.

13. Y. Zeng, L. Qiu, K. Wang, J. Yao, D. Li, G. P. Simon, R. Wang and H. Wang, *RSC Adv* 2013, **3**, 887-894.

14. J.-M. Malho, P. Laaksonen, A. Walther, O. Ikkala and M. B. Linder, *Biomacromolecules* 2012, **13**, 1093-1099.

15. Y. Feng, X. Zhang, Y. Shen, K. Yoshino and W. Feng, *Carbohyd Polym* 2012, **87**, 644-649.

16. J. M. Dugan, J. E. Gough and S. J. Eichhorn, *Nanomedicine* 2013, **8**, 287-298.

17. P. M. Favi, R. S. Benson, N. R. Neilsen, R. L. Hammonds, C. C. Bates, C. P. Stephens and M. S. Dhar, *Mater Sci Eng C* 2013, **33**, 1935-1944.

18. K. Hirayama, T. Okitsu, H. Teramae, D. Kiriya, H. Onoe and S. Takeuchi, *Biomaterials* 2013, **34**, 2421-2427.

19. J. Wang, C. Yang, Y. Wan, H. Luo, F. He, K. Dai and Y. Huang, *Soft Mater* 2013, **11**, 173-180.

20. H. Luo, G. Xiong, Y. Huang, F. He, W. Wang and Y. Wan, *Mater Chem Phys* 2008, **110**, 193-196.

21. L. Segal, J. J. Creely, A. E. Martin and C. M. Conrad, *J Text Res* 1959, **29**, 786-794.

22. C. Tokoh, K. Takabe, M. Fujita and H. Saiki, *Cellulose* 1998, **5**, 249-261.

23. D. Klemm, B. Heublein, H. P. Fink and A. Bohn, *Angew Chem Int* 2005, **44**, 3358-3393.

24. H.-B. Zhang, W.-G. Zheng, Q. Yan, Y. Yang, J.-W. Wang, Z.-H. Lu, G.-Y. Ji and Z.-Z. Yu, *Polymer* 2010, **51**, 1191-1196.

25. T. Kuila, S. Bose, A. K. Mishra, P. Khanra, N. H. Kim and J. H. Lee, *Prog Mater Sci* 2012, **57**, 1061-1105.

26. S. Taokaew, S. Seetabhawang, P. Siripong and M. Phisalaphong, *Materials* 2013, **6**, 782-794.

27. C. H. Haigler, A. R. White, R. M. Brown, Jr. and K. M. Cooper, *J Cell Biol* 1982, **94**, 64-69.

28. I. K. Moon, J. Lee, R. S. Ruoff and H. Lee, *Nat Commun* 2010, **1**.

29. L. Zhang, G. Chen, M. N. Hedhili, H. Zhang and P. Wang, *Nanoscale* 2012, **4**, 7038-7045.

30. L. G. Cancado, K. Takai, T. Enoki, M. Endo, Y. A. Kim, H. Mizusaki, N. L. Speziali, A. Jorio and M. A. Pimenta, *Carbon* 2008, **46**, 272-275.

31. M. A. Pimenta, G. Dresselhaus, M. S. Dresselhaus, L. G. Cancado, A. Jorio and R. Saito, *Phys Chem Chem Phys* 2007, **9**, 1276-1291.

Figure captions

Fig. 1 Biosynthesis scheme depicting the synthesis of in situ formation of GE/BC nanocomposite hydrogel.

Fig. 2 SEM micrographs (a-d) TEM image (e), and HRTEM image (f) of GE/BC nanocomposite.

Fig. 3 Mechanism showing the formation of GE/BC nanocomposite with spider web-like microstructure.

Fig. 4 XRD patterns of pristine BC, graphene, and GE/BC nanocomposite.

Fig. 5 Raman spectra of GE/BC nanocomposite and pristine graphene.

Fig. 1.

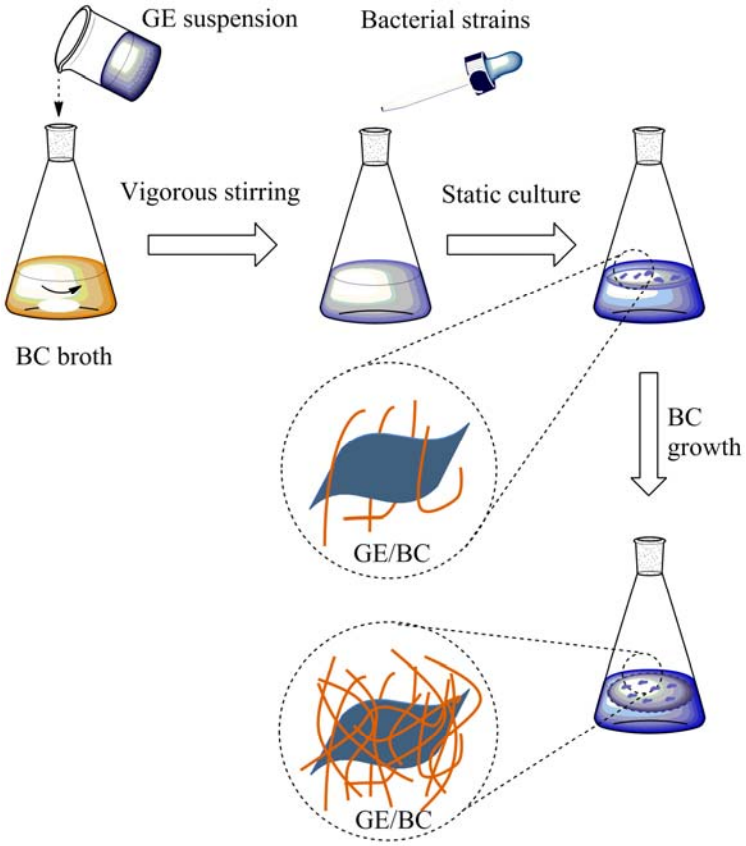

Fig. 2.

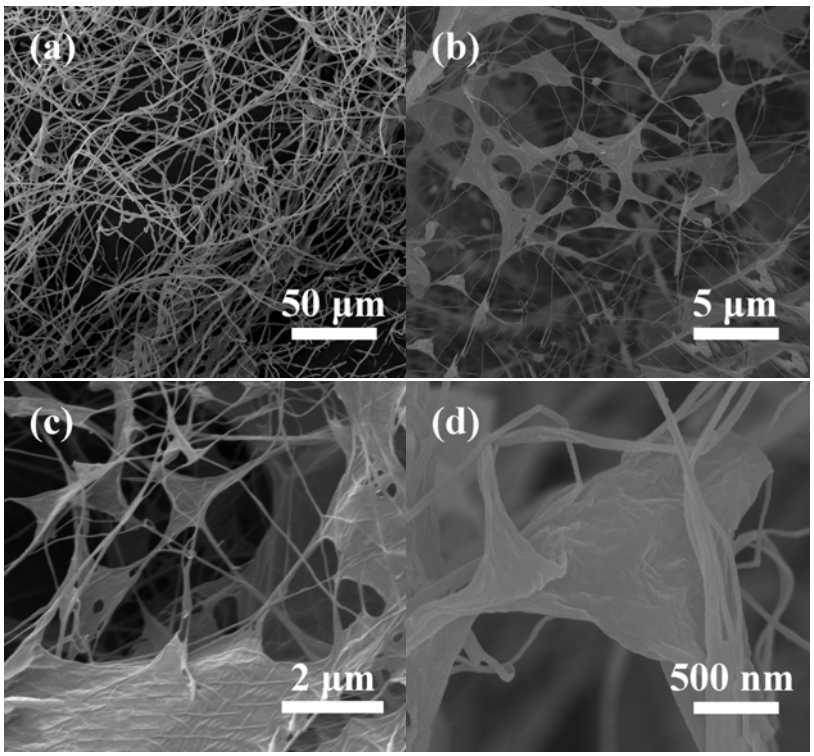

Fig. 3.

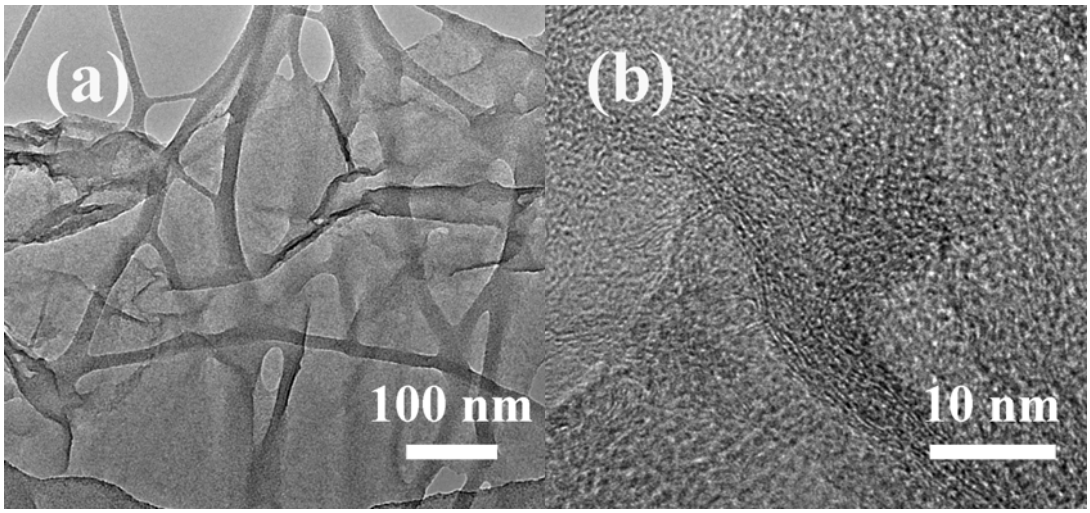

Fig. 4.

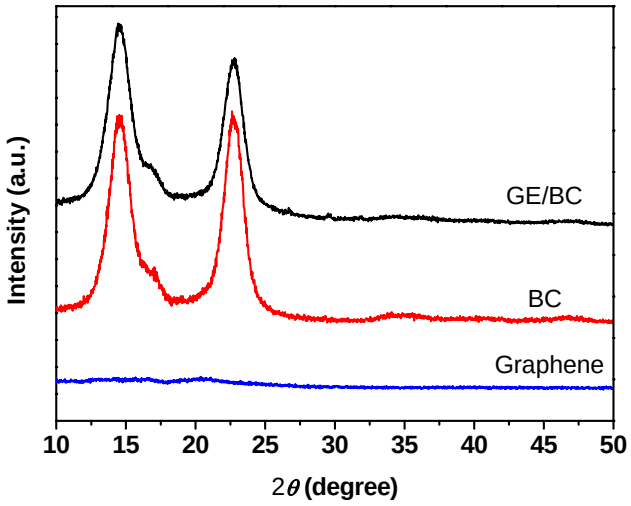

Fig. 5.

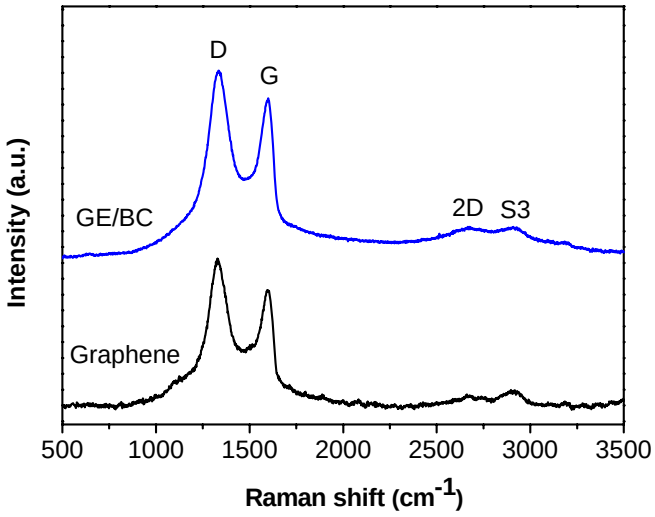

Table of content entry

A novel graphene/bacterial cellulose nanocomposite hydrogel was prepared by adding graphene suspension into the culture medium of bacterial cellulose.