three-dimensional (3D) diamond and graphite. Graphene has been discussed in theory for about 60 years. In 2004, graphene was first isolated by Novoselov and Geim.[13] Since then, graphene has attracted much interest from various scientific fields, and its discovery won the 2010 Nobel Prize in physics.

Graphene is a one-atom-thick sheet of carbon atoms arranged in a 2D honeycomb structure. The strong carbon-carbon bonding in the plane, the aromatic structure, the presence of free p electrons, and reactive sites for surface reactions make graphene a unique material with excellent properties: the thinnest material ever measured; a large specific surface area ($2600\ m^2\ g^{-1}$)[15]; a Young's modulus of 1 TPa and intrinsic strength of 130 GPa, which is close to that predicted by theory[16,17]; a high room temperature electron mobility of 2.5

Table 1. The Cell Behaviors on Graphene and Its Derivatives

Graphene types	Preparation methods	Application modes	Cell types	Outcomes	Possible mechanisms	Reference
G	CVD	Sheet	Human OB, hMSCs	The cells on G adhered and proliferated better than on an SiO_2 substrate	The electrical conductivity of G is of particular importance, because electricity as well as cocktails of growth factors and substrate properties is able to stimulate cell growth and differentiation.	Kalbacova et al.[51]
G	CVD	Sheet	hMSCs	G provides a promising biocompatible scaffold that does not curb the proliferation of hMSCs. The osteogenic differentiation rate induced by G is comparable to BMP-2.	Surface morphology; lateral stress	Nayak et al.[4]
G, GO	CVD	Sheet	hMSCs	The strong noncovalent binding abilities of G enable it to act as a preconcentration platform for osteogenic inducers, which accelerate the osteogenesis of MSCs. However, G suppresses adipogenesis of MSCs. GO does not interfere with adipogenesis of MSCs.	G has the ability to bind dexamethasone and b-glycerolphosphate, which is good for osteogenesis. Insulin is denatured on adsorption of G through a strong p-p interaction. GO does not interfere with adipogenesis due to electrostatic binding with insulin.	Lee et al.[5]

(continued)

Table 1. (Continued)

Graphene types	Preparation methods	Application modes	Cell types	Outcomes	Possible mechanisms	Reference
GO	Modified Hummers method	Nanosheets	hASCs	The GO was proved to be a suitable environment for the time-dependent viability of hASCs. The enhanced differentiation of hASCs included osteogenesis, adipogenesis, and epithelial genesis; while the chondrogenic differentiation of hASCs was decreased, compared tissue culture polystyrene as a control substrate.	It is hypothesized that a combination of factors, including nanoscale structure, strong stiffness, roughness, reactive oxygen functional groups, and adsorption of biomolecules on GO sheets, affects the hASCs behaviors.	Kim et al.[6]
rGO	Modified Hummers method; Spin coating	Film	hFOB	rGO is biocompatible with hFOB, whereas single-walled carbon nanotube network is inhibitory to the proliferation of hFOB.	The distinct nanotopographic features of these two kinds of nanocarbon substrates cause the difference.	Agarwal et al.[54]
GO-$CaCO_3$ G-$CaCO_3$ GO-HA G-HA	Modified Hummers method	Film	Mouse OB	Compared with bare GO and G sheets, GO/G-$CaCO_3$ composites exhibited remarkably enhanced HA formation when incubated in a simulated body fluid solution. The GO/G-HA composites supported high viability of OB with an elongated morphology.	GO/G-HA hybrid materials induce a 3D matrix adhesion of OB with high cell viability and provide a similar microenvironment to that found in vivo	Kim et al.[52]

(continued)

Table 1. (Continued)

Graphene types	Preparation methods	Application modes	Cell types	Outcomes	Possible mechanisms	Reference
FRGO	Hummer's method; low-temperature thermal reduction method	Film	OB	The surface oxygen content of FRGO significantly influences the cellular behaviors, with the best performance for cell attachment, proliferation, calcium deposition, and collagen secretion being obtained in moderately reduced FRGO. Cell performance significantly decreased as the FRGO was highly reduced. Moderate performance was found in nonreduced pure GO and control glass slides.	Enhancement of cell adhesion and proliferation may be induced by enhanced extracellular matrix protein adsorption in moderately reduced FRGO by noncovalent interactions.	Shi et al.[7]
Nylon 6, 6-GO hybrid	Hybrid Nylon 6,6 with 0.25 wt% graphene oxide.	Film	Mouse pre-OB	The cell attachment and proliferation on Nylon 6, 6-GO hybrid polymer greatly exceed those of Nylon 6,6.	The incorporation of GO in Nylon 6,6 matrix modifies the physiochemical properties of the surface, including chemistry and wettability, favoring attachment, cell-substrate interactions, and biological response required for cell attachment and proliferation. Furthermore, the negative polarity of G is instrumental in favorably influencing biological function.	Misra and Chaudhari[53]

(continued)

Table 1. (Continued)

Graphene types	Preparation methods	Application modes	Cell types	Outcomes	Possible mechanisms	Reference
Chitosan-GO scaffolds	Covalent linking of the carboxyl groups of GO with the amino groups of CS	Nanosheets	Mouse pre-OB	The covalent incorporation of GO into a chitosan network favorably modulated the biological response of osteoblasts, such that cell attachment, proliferation, and formation of extracellular matrix are signi cantly enhanced.	The signi cant enhancement of biological function is related to a combination of a number of physico-chemical factors, including a large surface area, nanoscale roughness, the presence of pendant groups, a hydrophilic nature, and a high water retention ability.	Depan et al.[8]
GO-modi ed CS scaffold	The carboxyl groups of GO are covalently attached with the amine group of CS.	Nanosheets	Mouse pre-OB	Biological functions of the pre-OB including cell attachment, proliferation, growth and mineralization, are signi cantly enhanced in the presence of GO, compared with unmodi ed chitosan.	The difference of cell behavior is related to the degree and topography of protein adsorption on the scaffolds. BSA is widely spread on CS-GO as small globules, whereas the globules are large and randomly distributed on pure CS. This subtle but important difference in the adsorption of protein is responsible for the observed differences in cellular interactions.	Depan and Misra[9]
rGO-CS substrata	Hummers method; hydrazine treatment; spin-coating	Nanosheets; thin	hMSCs	G-incorporated CS substrate promotes adhesion and differentiation of hMSCs.	rGO-CS substrata with asymmetrical nanotopology and its secondary effects such as stiffness and roughness provided a suitable environment for adhesion and proliferation of hMSCs as well as enhanced cell-substrata interactions and cell-cell contacts.	Kim et al.[10]

(continued)

Table 1. (Continued)

Graphene types	Preparation methods	Application modes	Cell types	Outcomes	Possible mechanisms	Reference
3D GFs	Growing graphene on 3D Nickel scaffolds	Film	hMSCs	3D GFs can maintain hMSCs viability and promote osteogenic differentiation without the need for extrinsic biochemical manipulation.	The mechanism driving spontaneous differentiation remains unclear, but is suspected to be an intrinsic cellular response to the stress derived from the interaction with a high stiffness material.	Crowder et al.[11]
GO-ac	Hummer's method; self-assemblies on water	Film	hMSCs	hMSCs show spontaneous osteogenic differentiation on rough GO-ac films without the use of any chemical inducers. The extent of mineralization increases with increasing roughness of the GO-ac films.	The rough topology of the cross-linked films creates increased cytoskeletal tension to grow and differentiate. The rough surface topology provides more anchoring points for the adhesion and proliferation of hMSCs.	Tang et al.[12]

G, graphene; GO, graphene oxide; rGO, reduced graphene oxide; OB,osteoblasts; BSA, bovine serum albumin; HA, hydroxyapatite; CS, chitosan; BMP-2, bone morphogenetic protein-2; CVD, chemical vapor deposition; FRGO, few-layer rGO films; GO-ac, acrylated GO; hASCs, human adipose-derived stem cells; hMSCs, human mesenchymal stem cells; hFOB, human fetal OB; MSC, mesenchymal stem cells; 3D, three-dimensional; 3D GFs, 3D graphene foams.

the surface charge of graphene have a large impact on the biological and toxicological response to red blood cells. At the smallest size, GO showed the greatest hemolytic activity, whereas aggregated graphene sheets exhibited the lowest hemolytic activity. Their study also demonstrated that compacted graphene sheets are more damaging to

FIG. 1. Schematic diagram depicting the possible mechanisms of the biological effects of graphenes on mesenchymal stem cells or osteoblastic cells in vitro. ? Means inconclusive results. Arrow, promotion; bar, inhibition. G, graphene; Dex, dexamethasone; b-GP, b-glycerophosphate. Color images available online at www.liebertpub.com/teb

concentration above 50 mg/L.[88] In another study by Kim et al., the proliferation rate of hMSCs decreased with the incorporation of higher amounts of rGO into the rGO-chitosan substrata.[10] On the other hand, G and GO exhibited negligible in vitro toxicity to BMMSCs, ASCs, or osteoblasts when they were used as coating materials, as mentioned earlier. Thus, further systemic studies are required to wholly understand the potential biological effects and to address concerns over health hazards before any practical application.

The differentiation of MSCs into osteogenic lineages on graphene

A study by Kim et al. showed that GO/graphene-$CaCO_3$, hybrid materials exhibited remarkably enhanced hydroxyapatite formation when incubated in a simulated body fluid solution compared with bare GO and graphene films.[52] Later, other studies indicated that the formation of extracellular matrix of osteoblasts or preosteoblasts on CS-GO and FRGO was significantly enhanced.[7?]

In addition, positive results for the osteogenic differentiation of MSCs on graphene have been obtained.[4?,12] Nayak et al. cultured BMMSCs in osteogenic medium without bone morphogenetic protein-2 (BMP-2), which did not lead to osteogenic differentiation over the whole duration of the experiment (15 days). However, once the substrates (PDMS, PET, glass slide, and silicon wafer) were coated with CVD-grown graphene, BMMSCs successfully differentiated into osteoblasts, which was confirmed by quantitative alizarin red staining. Interestingly, both BMP-2-treated and CVD-grown graphene-coated substrates induced cell differentiation at the same rate, suggesting that graphene might be a driving force of bone cell formation.[4] In another study, after 12 days of osteogenic induction, there was a sevenfold increase in the extent of mineralization in BMMSCs cultured on CVD-grown graphene compared with those on PDMS.[5] Tang et al. reported spontaneous osteogenic differentiation of MSCs on a cross-linked GO film, without the use of any chemical inducers. Furthermore, the extent of mineralization increased with an increase in the roughness of the GO film. Thus, the GO film may represent a chemical-free method of inducing osteoblastic differentiation.[12] The enhanced differentiation of ASCs, including osteogenesis and adipogenesis, on GO film has also been reported.[6] In addition, Alizarin Red S staining and western blot analysis by Kim et al. showed up-regulation of the osteogenic differentiation of hMSCs in the rGO-chitosan substrata compared with the chitosan substrata and tissue culture polystyrene, in both the proliferative and osteogenic induction media.[10] These recent investigations indicate an active or potential pro-osteodifferentiation capability of G or GO.

Cell behavior of MSCs on 3D graphene

All the reports mentioned earlier investigated stem cell behavior on 2D graphene sheets. Crowder et al. employed 3D graphene foams (3D GFs) as culture substrates for BMMSCs for the first time. GFs were fabricated by growing graphene on 3D nickel scaffolds, and the nickel was subsequently removed by $FeCl_3$ etching. GFs were shown to maintain BMMSCs viability and to stimulate changes in morphology. When cultured on GFs for 7 days, BMMSCs exhibited spindle-shaped, elongated morphology with thin, aligned nuclei and strongly expressed both osteocalcin and osteopontin, indicating spontaneous osteogenic differentiation of BMMSCs without the need for extrinsic biochemical manipulation.[11]

Mechanism of Graphene's Positive Effects on the Proliferation and Osteogenic Differentiation of MSCs

The ability of graphene to promote the proliferation and osteogenic differentiation of BMMSCs and ASCs has been demonstrated (Fig. 1). It has been reported that topography, chemistry, and physical properties of biomaterials are critical parameters for directing cell fates.[81] In recent years, the mechanism has been explored from different aspects (Table 1).

Adhesion mechanism

FAs are large protein complexes that indicate the connections between cells and the extracellular matrix. FAs play a key role in the mediation of adhesion and migration of cells.[89] Several studies have suggested that nanoscale structures of substrates may regulate FAs. Kim et al. hypothesized that the unique nanotopography of the GO film would influence the formation of FAs.[6]

Cell morphology mechanism

Micromorphology regulates multiple biological processes, including adhesion,[90] proliferation,[91] and differentiation.[92] Moreover, studies suggest that cell shape is a key

with regard to graphene's effects on the adhesion, proliferation, and differentiation of MSCs, and its potential to promote osteodifferentiation, which point to its future use in surface modification of implants or scaffold materials. To date, studies on graphene's contribution to bone tissue engineering are still quite new, and knowledge on the effects of graphene is limited. Within the limitation of the present investigation, we believe that graphene may have a promising future, and further research will realize its potential. In spite of the significant progress mentioned earlier, there are still some important challenges. First, the potential long-term toxicity and the nonbiodegradable nature of graphene should be further investigated. Second, our understanding of graphene-cell interactions and its internal mechanisms are incomplete, and many hypotheses remain to be tested. Third, a comparison of the osteogenic effects of graphene with those of current successful implants or scaffold materials should be performed before we can conclude whether graphene is a promising nano-material for promoting surface modification of implants or scaffold materials. Lastly, the exact effects of graphene on cells, tissues, or organs, and their metabolic pathway in vivo remain unclear and require further studies.

Acknowledgments

This study was supported by grants from the Program for New Century Excellent Talents in University from Ministry of Education of China (NCET-11-0026) and Peking University 985 Program.

Disclosure Statement

The authors indicated no potential conflicts of interest.

References

1. Rose, F.R., and Oreffo, R.O. Bone tissue engineering: hope vs hype. Biochem Biophys Res Commun 292, 1, 2002.
2. Dominici, M., Le Blanc, K., Mueller, I., Slaper-Cortenbach, I., Marini, F., Krause, D., Deans, R., Keating, A., Prockop, D., and Horwitz, E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8, 315, 2006.
3. Treiser, M.D., Yang, E.H., Gordonov, S., Cohen, D.M., Androulakis, I.P., Kohn, J., Chen, C.S., and Moghe, P.V. Cytoskeleton-based forecasting of stem cell lineage fates. Proc Natl Acad Sci U S A 107, 610, 2010.
4. Nayak, T.R., Andersen, H., Makam, V.S., Khaw, C., Bae, S., Xu, X., Ee, P.L., Ahn, J.H., Hong, B.H., Pastorin, G., and Ozyilmaz, B. Graphene for controlled and accelerated osteogenic differentiation of human mesenchymal stem cells. ACS Nano 5, 4670, 2011.
5. Lee, W.C., Lim, C.H., Shi, H., Tang, L.A., Wang, Y., Lim, C.T., and Loh, K.P. Origin of enhanced stem cell growth and differentiation on graphene and graphene oxide. ACS Nano 5, 7334, 2011.
6. Kim, J., Choi, K.S., Kim, Y., Lim, K.T., Seonwoo, H., Park, Y., Kim, D.H., Choung, P.H., Cho, C.S., Kim, S.Y., Choung, Y.H., and Chung, J.H. Bioactive effects of graphene oxide cell culture substratum on structure and function of human adipose-derived stem cells. J Biomed Mater Res A 101, 3520, 2013.
7. Shi, X.T., Chang, H.X., Chen, S., Lai, C., Khademhosseini, A., and Wu, H.K. Regulating cellular behavior on few-layer reduced graphene oxide films with well-controlled reduction states. Adv Funct Mater 22, 751, 2012.
8. Depan, D., Girase, B., Shah, J.S., and Misra, R.D. Structure-process-property relationship of the polar graphene oxide-mediated cellular response and stimulated growth of osteoblasts on hybrid chitosan network structure nanocomposite scaffolds. Acta Biomater 7, 3432, 2011.
9. Depan, D., and Misra, R.D. The interplay between nanostructured carbon-grafted chitosan scaffolds and protein adsorption on the cellular response of osteoblasts: structure-function property relationship. Acta Biomater 9, 6084, 2013.
10. Kim, J., Kim, Y.R., Kim, Y., Lim, K.T., Seonwoo, H., Park, S., et al. Graphene-incorporated chitosan substrata for adhesion and differentiation of human mesenchymal stem cells. J Mater Chem B 1, 933, 2013.
11. Crowder, S.W., Prasai, D., Rath, R., Balikov, D.A., Bae, H., Bolotin, K.I., and Sung, H.J. Three-dimensional graphene foams promote osteogenic differentiation of human mesenchymal stem cells. Nanoscale 5, 4171, 2013.
12. Tang, L.A., Lee, W.C., Shi, H., Wong, E.Y., Sadovoy, A., Gorelik, S., Hobley, J., Lim, C.T., and Loh, K.P. Highly wrinkled cross-linked graphene oxide membranes for biological and charge-storage applications. Small 8, 423, 2012.
13. Novoselov, K.S., Geim, A.K., Morozov, S.V., Jiang, D., Zhang, Y., Dubonos, S.V., Grigorieva, I.V., and Firsov, A.A. Electric field effect in atomically thin carbon films. Science 306, 666, 2004.
14. Wei, W., and Qu, X. Extraordinary physical properties of functionalized graphene. Small 8, 2138, 2012.
15. Yang, M., Yao, J., and Duan, Y. Graphene and its derivatives for cell biotechnology. Analyst 138, 72, 2013.
16. Lee, C., Wei, X., Kysar, J.W., and Hone, J. Measurement of the elastic properties and intrinsic strength of monolayer graphene. Science 321, 385, 2008.
17. Novoselov, K.S., Fal'ko, V.I., Colombo, L., Gellert, P.R., Schwab, M.G., and Kim, K. A roadmap for graphene. Nature 490, 192, 2012.
18. Mayorov, A.S., Gorbachev, R.V., Morozov, S.V., Britnell, L., Jalil, R., and Ponomarenko, L.A. Micrometer-scale ballistic transport in encapsulated graphene at room temperature. Nano Lett 11, 2396, 2011.
19. Morozov, S.V., Novoselov, K.S., Katsnelson, M.I., Schedin, F., Elias, D.C., and Jaszczak, J.A. Giant intrinsic carrier mobilities in graphene and its bilayer. Phys Rev Lett 100, 016602, 2008.
20. Balandin, A.A. Thermal properties of graphene and nanostructured carbon materials. Nat Mater 10, 569, 2011.
21. Nair, R.R., Blake, P., Grigorenko, A.N., Novoselov, K.S., Booth, T.J., and Stauber, T. Fine structure constant defines visual transparency of graphene. Science 320, 1308, 2008.
22. Bunch, J.S., Verbridge, S.S., Alden, J.S., van der Zande, A.M., Parpia, J.M., and Craighead, H.G. Impermeable atomic membranes from graphene sheets. Nano Lett 8, 2458, 2008.
23. Novoselov, K.S., Jiang, Z., Zhang, Y., Morozov, S.V., Stormer, H.L., Zeitler, U., Maan, J.C., Boebinger, G.S., Kim, P., and Geim, A.K. Room-temperature quantum Hall effect in graphene. Science 315, 1379, 2007.
24. Wang, Y., Huang, Y., Song, Y., Zhang, X., Ma, Y., Liang, J., and Chen, Y. Room-temperature ferromagnetism of graphene. Nano Lett 9, 220, 2009.

25. Yan, L., Zheng, Y.B., Zhao, F., Li, S., Gao, X., Xu, B., Weiss, P.S., and Zhao, Y. Chemistry and physics of a single atomic layer: strategies and challenges for functionalization of graphene and graphene-based materials. Chem Soc Rev 41, 97, 2012.
26. Salas, E.C., Sun, Z., Luttge, A., and Tour, J.M. Reduction of graphene oxide via bacterial respiration. ACS Nano 4, 4852, 2010.
27. Park, S., Mohanty, N., Suk, J.W., Nagaraja, A., An, J., Piner, R.D., Cai, W., Dreyer, D.R., Berry, V., and Ruoff, R.S. Biocompatible, robust free-standing paper composed of a TWEEN/graphene composite. Adv Mater 22, 1736, 2010.
28. Liu, S., Zeng, T.H., Hofmann, M., Burcombe, E., Wei, J., Jiang, R., Kong, J., and Chen, Y. Antibacterial activity of graphite, graphite oxide, graphene oxide, and reduced graphene oxide: membrane and oxidative stress. ACS Nano 5, 6971, 2011.
29. Chang, Y., Yang, S.T., Liu, J.H., Dong, E., Wang, Y., Cao, A., Liu, Y., and Wang, H. In vitro toxicity evaluation of graphene oxide on A549 cells. Toxicol Lett 200, 201, 2011.
30. Dean, C.R., Young, A.F., Meric, I., Lee, C., Wang, L., and Sorgenfrei, S. Boron nitride substrates for high-quality graphene electronics. Nat Nanotechnol 5, 722, 2010.
31. Zhang, Y., Zhang, L., and Zhou, C. Review of chemical vapor deposition of graphene and related applications. Acc Chem Res 46, 2329, 2013.
32. Berger, C., Song, Z., Li, X., Wu, X., Brown, N., Naud, C., Mayou, D., Li, T., Hass, J., Marchenkov, A.N., Conrad, E.H., First, P.N., and de Heer, W.A. Electronic confinement and coherence in patterned epitaxial graphene. Science 312, 1191, 2006.
33. Stankovich, S., Dikin, D.A., Dommett, G.H., Kohlhaas, K.M., Zimney, E.J., Stach, E.A., Piner, R.D., Nguyen, S.T., and Ruoff, R.S. Graphene-based composite materials. Nature 442, 282, 2006.
34. Li, X., Cai, W., An, J., Kim, S., Nah, J., Yang, D., Piner, R., Velamakanni, A., Jung, I., Tutuc, E., Banerjee, S.K., Colombo, L., and Ruoff, R.S. Large-area synthesis of high-quality and uniform graphene films on copper foils. Science 324, 1312, 2009.
35. Bae, S., Kim, H., Lee, Y., Xu, X., Park, J.S., Zheng, Y., Balakrishnan, J., Lei, T., Kim, H.R., Song, Y.I., Kim, Y.J., Kim, K.S., Ozyilmaz, B., Ahn, J.H., Hong, B.H., and Iijima, S. Roll-to-roll production of 30-inch graphene films for transparent electrodes. Nat Nanotechnol 5, 574, 2010.
36. Reina, A., Jia, X., Ho, J., Nezich, D., Son, H., Bulovic, V., Dresselhaus, M.S., and Kong, J. Large area, few-layer graphene films on arbitrary substrates by chemical vapor deposition. Nano Lett 9, 30, 2009.
37. Singh, V., Joung, D., Zhai, L., Das, S., Khondaker, S.I., and Seal, S. Graphene based materials: past, present and future. Prog Mater Sci 56, 1178, 2011.
38. Hummers, W.S., Jr., and Offeman, R.E. Preparation of graphitic oxide. J Am Chem Soc 80, 1339, 1958.
39. Yamaguchi, H., Eda, G., Mattevi, C., Kim, H., and Chhowalla, M. Highly uniform 300 mm wafer-scale deposition of single and multilayered chemically derived graphene thin films. ACS Nano 4, 524, 2010.
40. Becerril, H.A., Mao, J., Liu, Z., Stoltenberg, R.M., Bao, Z., and Chen, Y. Evaluation of solution-processed reduced graphene oxide films as transparent conductors. ACS Nano 2, 463, 2008.
41. Kim, J., Cote, L.J., Kim, F., Yuan, W., Shull, K.R., and Huang, J. Graphene oxide sheets at interfaces. J Am Chem Soc 132, 8180, 2010.
42. Cote, L.J., Kim, F., and Huang, J. Langmuir-Blodgett assembly of graphite oxide single layers. J Am Chem Soc 131, 1043, 2009.
43. Dikin, D.A., Stankovich, S., Zimney, E.J., Piner, R.D., Dommett, G.H., Evmenenko, G., Nguyen, S.T., and Ruoff, R.S. Preparation and characterization of graphene oxide paper. Nature 448, 457, 2007.
44. Chen, C.M., Yang, Q.H., Yang, Y.G., Lv, W., Wen, Y.F., Hou, P.X., Wang, M.Z., and Cheng, H.M. Self-assembled free-standing graphite oxide membrane. Adv Mater 21, 3007, 2009.
45. Azevedo, J., Costa-Coquelard, C., Jegou, P., Yu, T., and Benattar, J.J. Highly ordered monolayer, multilayer, and hybrid films of graphene oxide obtained by the bubble deposition method. J Phys Chem C 115, 14678, 2011.
46. Shih, C.J., Lin, S., Sharma, R., Strano, M.S., and Blankschtein, D. Understanding the pH-dependent behavior of graphene oxide aqueous solutions: a comparative experimental and molecular dynamics simulation study. Langmuir 28, 235, 2012.
47. Park, S., An, J., Jung, I., Piner, R.D., An, S.J., and Li, X. Colloidal suspensions of highly reduced graphene oxide in a wide variety of organic solvents. Nano Lett 9, 1593, 2009.
48. Bagri, A., Mattevi, C., Acik, M., Chabal, Y.J., Chhowalla, M., and Shenoy, V.B. Structural evolution during the reduction of chemically derived graphene oxide. Nat Chem 2, 581, 2010.
49. Salavagione, H.J., Martinez, G., and Ellis, G. Recent advances in the covalent modification of graphene with polymers. Macromol Rapid Commun 32, 1771, 2011.
50. Menard-Moyon, C., Kostarelos, K., Prato, M., and Bianco, A. Functionalized carbon nanotubes for probing and modulating molecular functions. Chem Biol 17, 107, 2010.
51. Kalbacova, M., Broz, A., Kong, J., and Kalbac, M. Graphene substrates promote adherence of human osteoblasts and mesenchymal stromal cells. Carbon 48, 4323, 2010.
52. Kim, S., Ku, S.H., Lim, S.Y., Kim, J.H., and Park, C.B. Graphene-biomineral hybrid materials. Adv Mater 23, 2009, 2011.
53. Misra, R.D., and Chaudhari, P.M. Cellular interactions and stimulated biological functions mediated by nanostructured carbon for tissue reconstruction and tracheal tubes and sutures. J Biomed Mater Res A 101, 528, 2013.
54. Agarwal, S., Zhou, X., Ye, F., He, Q., Chen, G.C., Soo, J., Boey, F., Zhang, H., and Chen, P. Interfacing live cells with nanocarbon substrates. Langmuir 26, 2244, 2010.
55. Feng, L., and Liu, Z. Graphene in biomedicine: opportunities and challenges. Nanomedicine (Lond) 6, 317, 2011.
56. Chung, C., Kim, Y.K., Shin, D., Ryoo, S.R., Hong, B.H., and Min, D.H. Biomedical applications of graphene and graphene oxide. Acc Chem Res 46, 2211, 2013.
57. Pinto, A.M., Goncalves, I.C., and Magalhaes, F.D. Graphene-based materials biocompatibility: a review. Colloids surf B Biointerfaces 111C, 188, 2013.
58. Jastrzebska, A.M., Kurtycz, P., and Olszyna, A.R. Recent advances in graphene family materials toxicity investigations. J Nanopart Res 14, 1320, 2012.
59. Cai, X., Tan, S., Yu, A., Zhang, J., Liu, J., and Mai, W. Sodium 1-naphthalen esulfonate-functionalized reduced graphene oxide stabilizes silver nanoparticles with lower cytotoxicity and long-term antibacterial activity. Chem Asian J 7, 1664, 2012.

60. Liao, K.H., Lin, Y.S., Macosko, C.W., and Haynes, C.L. Cytotoxicity of graphene oxide and graphene in human erythrocytes and skin fibroblasts. ACS Appl Mater Interfaces 3, 2607, 2011.
61. Sasidharan, A., Panchakarla, L.S., Chandran, P., Menon, D., Nair, S., and Rao, C.N. Differential nano-bio interactions and toxicity effects of pristine versus functionalized graphene. Nanoscale 3, 2461, 2011.
62. Hong, B.J., Compton, O.C., An, Z., Eryazici, I., and Nguyen, S.T. Successful stabilization of graphene oxide in electrolyte solutions: enhancement of biofunctionalization and cellular uptake. ACS Nano 6, 63, 2012.
63. Chng, E.L., and Pumera, M. The toxicity of graphene oxides: dependence on the oxidative methods used. Chemistry 19, 8227, 2013.
64. Zhang, Y., Ali, S.F., Dervishi, E., Xu, Y., Li, Z., Casciano, D., and Biris, A.S. Cytotoxicity effects of graphene and single-wall carbon nanotubes in neural phaeochromocytoma-derived PC12 cells. ACS Nano 4, 3181, 2010.
65. Peng, C., Hu, W., Zhou, Y., Fan, C., and Huang, Q. Intracellular imaging with a graphene-based fluorescent probe. Small 6, 1686, 2010.
66. Wen, H., Dong, C., Dong, H., Shen, A., Xia, W., and Cai, X. Engineered redox-responsive PEG detachment mechanism in PEGylated nano-graphene oxide for intracellular drug delivery. Small 8, 760, 2012.
67. Sahu, A., Choi, W.I., and Tae, G. A stimuli-sensitive injectable graphene oxide composite hydrogel. Chem Commun 48, 5820, 2012.
68. Duch, M.C., Budinger, G.R., Liang, Y.T., Soberanes, S., Urich, D., and Chiarella, S.E. Minimizing oxidation and stable nanoscale dispersion improves the biocompatibility of graphene in the lung. Nano Lett 11, 5201, 2011.
69. Gollavelli, G., and Ling, Y.C. Multi-functional graphene as an in vitro and in vivo imaging probe. Biomaterials 33, 2532, 2012.
70. Yan, L., Wang, Y., Xu, X., Zeng, C., Hou, J., and Lin, M. Can graphene oxide cause damage to eyesight? Chem Res Toxicol 25, 1265, 2012.
71. Yang, K., Wan, J., Zhang, S., Zhang, Y., Lee, S.T., and Liu, Z. In vivo pharmacokinetics, long-term biodistribution, and toxicology of PEGylated graphene in mice. ACS Nano 5, 516, 2011.
72. Sasidharan, A., Panchakarla, L.S., Sadanandan, A.R., Ashokan, A., Chandran, P., and Girish, C.M. Hemocompatibility and macrophage response of pristine and functionalized graphene. Small 8, 1251, 2012.
73. Nahain, A.A., Lee, J.E., Jeong, J.H., and Park, S.Y. Photoresponsive fluorescent reduced graphene oxide by spiropyran conjugated hyaluronic Acid for in vivo imaging and target delivery. Biomacromolecules 14, 4082, 2013.
74. Shah, A.K., Sinha, R.K., Hickok, N.J., and Tuan, R.S. High-resolution morphometric analysis of human osteoblastic cell adhesion on clinically relevant orthopedic alloys. Bone 24, 499, 1999.
75. Wu, S., Liu, X., Wu, G., Yeung, K.W., Zheng, D., Chung, C.Y., Xu, Z.S., and Chu, P.K. Wear mechanism and tribological characteristics of porous NiTi shape memory alloy for bone scaffold. J Biomed Mater Res A 101, 2586, 2013.
76. Roy, R.K., and Lee, K.R. Biomedical applications of diamond-like carbon coatings: a review. J Biomed Mater Res B Appl Biomater 83, 72, 2007.
77. Huang, N., Yang, P., Leng, Y.X., Chen, J.Y., Sun, H., Wang, J., Wang, G.J., Ding, P.D., Xi, T.F., and Leng, Y. Hemocompatibility of titanium oxide films. Biomaterials 24, 2177, 2003.
78. Gutensohn, K., Beythien, C., Bau, J., Fenner, T., Grewe, P., Koester, R., Padmanaban, K., and Kuehnl, P. In vitro analyses of diamond-like carbon coated stents. Reduction of metal ion release, platelet activation, and thrombogenicity. Thromb Res 99, 577, 2000.
79. Podila, R., Moore, T., Alexis, F., and Rao, A. Graphene coatings for biomedical implants. J Vis Exp e50276, 2013.
80. Edwards, S.L., Werkmeister, J.A., and Ramshaw, J.A. Carbon nanotubes in scaffolds for tissue engineering. Expert Rev Med Devices 6, 499, 2009.
81. Zanello, L.P., Zhao, B., Hu, H., and Haddon, R.C. Bone cell proliferation on carbon nanotubes. Nano Lett 6, 562, 2006.
82. Abarrategi, A., Gutierrez, M.C., Moreno-Vicente, C., Hortiguela, M.J., Ramos, V., Lopez-Lacomba, J.L., Ferrer, M.L., and Del, M.F. Multiwall carbon nanotube scaffolds for tissue engineering purposes. Biomaterials 29, 94, 2008.
83. Firkowska, I., Olek, M., Pazos-Perez, N., Rojas-Chapana, J., and Giersig, M. Highly ordered MWNT-based matrixes: topography at the nanoscale conceived for tissue engineering. Langmuir 22, 5427, 2006.
84. Kim, K.H., Oh, Y., and Islam, M.F. Graphene coating makes carbon nanotube aerogels superelastic and resistant to fatigue. Nat Nanotechnol 7, 562, 2012.
85. Yaszemski, M.J., Payne, R.G., Hayes, W.C., Langer, R.S., Aufdemorte, T.B., and Mikos, A.G. The ingrowth of new bone tissue and initial mechanical properties of a degrading polymeric composite scaffold. Tissue Eng 1, 41, 1995.
86. Lalwani, G., Henslee, A.M., Farshid, B., Lin, L., Kasper, F.K., Qin, Y.X., Mikos, A.G., and Sitharaman, B. Two-dimensional nanostructure-reinforced biodegradable polymeric nanocomposites for bone tissue engineering. Biomacromolecules 14, 900, 2013.
87. Some, S., Ho, S.M., Dua, P., Hwang, E., Shin, Y.H., Yoo, H., Kang, J.S., Lee, D.K., and Lee, H. Dual functions of highly potent graphene derivative-poly-L-lysine composites to inhibit bacteria and support human cells. ACS Nano 6, 7151, 2012.
88. Wang, K., Ruan, J., Song, H., Zhang, J., Wo, Y., Guo, S., and Cui, D. Bioccompatibility of graphene oxide. Nanoscale Res Lett 6, 8, 2011.
89. Humphries, J.D., Wang, P., Streuli, C., Geiger, B., Humphries, M.J., and Ballestrem, C. Vinculin controls focal adhesion formation by direct interactions with talin and actin. J Cell Biol 179, 1043, 2007.
90. Hamilton, D.W., and Brunette, D.M. The effect of substratum topography on osteoblast adhesion mediated signal transduction and phosphorylation. Biomaterials 28, 1806, 2007.
91. Chen, C.S., Mrksich, M., Huang, S., Whitesides, G.M., and Ingber, D.E. Geometric control of cell life and death. Science 276, 1425, 1997.
92. Roskelley, C.D., Desprez, P.Y., and Bissell, M.J. Extracellular matrix-dependent tissue-specific gene expression in mammary epithelial cells requires both physical and biochemical signal transduction. Proc Natl Acad Sci U S A 91, 12378, 1994.
93. McBeath, R., Pirone, D.M., Nelson, C.M., Bhadriraju, K., and Chen, C.S. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. Dev Cell 6, 483, 2004.

94. Dalby, M.J., Gadegaard, N., Tare, R., Andar, A., Riehle, M.O., Herzyk, P., Wilkinson, C.D., and Oreffo, R.O. The control of human mesenchymal cell differentiation using nanoscale symmetry and disorder. Nat Mater 6, 997, 2007.
95. Oh, S., Brammer, K.S., Li, Y.S., Teng, D., Engler, A.J., Chien, S., and Jin, S. Stem cell fate dictated solely by altered nanotube dimension. Proc Natl Acad Sci U S A 106, 2130, 2009.
96. Engler, A.J., Sen, S., Sweeney, H.L., and Discher, D.E. Matrix elasticity directs stem cell lineage specification. Cell 126, 677, 2006.
97. Kawai, M., and Rosen, C.J. PPARgamma: a circadian transcription factor in adipogenesis and osteogenesis. Nat Rev Endocrinol 6, 629, 2010.

Address correspondence to:
Yongsheng Zhou, DDS, PhD
Department of Prosthodontics
School and Hospital of Stomatology
Peking University
22 Zhongguancun South Avenue
Haidian District
Beijing 100081
P.R. China

E-mail: kqzhouysh@hsc.pku.edu.cn

Received: October 13, 2013
Accepted: January 9, 2014
Online Publication Date: February 26, 2014