

Preparation and functionalization of graphene nanocomposites for biomedical applications

[Kai Yang](#), [Liangzhu Feng](#), [Hao Hong](#), [Weibo Cai](#) & [Zhuang Liu](#) 

Nature Protocols **8**, 2392–2403 (2013)

7448 Accesses | **258** Citations | **13** Altmetric | [Metrics](#)

Abstract

Functionalized nano-graphene- and graphene-based nanocomposites have gained tremendous attention in the area of biomedicine in recent years owing to their biocompatibility, the ease with which they can be functionalized and their properties such as thermal and electrical conductivity. Potential applications for functionalized nanoparticles range from drug delivery and multimodal imaging to exploitation of the electrical properties of graphene toward the preparation of biosensing devices. This protocol covers the preparation, functionalization and bioconjugation of various graphene derivatives and nanocomposites. Starting from graphite, the preparations of graphene oxide (GO), reduced GO (RGO) and magnetic GO-based nanocomposite, as well as how to functionalize them with biocompatible polymers such as polyethylene glycol (PEG), are described in detail. We also provide procedures for ^{125}I radiolabeling of PEGylated GO and the preparation of GO-based gene carriers; other bioconjugation approaches including drug loading, antibody conjugation and fluorescent labeling are similar to those described previously and used for bioconjugation of PEGylated carbon nanotubes. We hope this article will help researchers in this field to fabricate graphene-based bioconjugates with high reproducibility for various applications in biomedicine. The sample preparation procedures take various times ranging from 1 to 2 d.

Access options

Buy this article

- Purchase on SpringerLink
- Instant access to full article PDF

Buy now

Subscribe to this journal

Receive 12 print issues and online access

265,23 € per year

only 22,10 € per issue

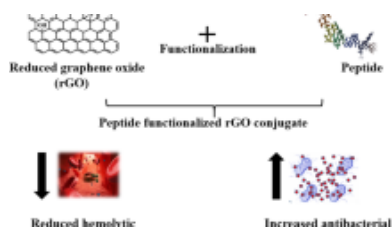
Learn more

Prices may be subject to local taxes which are calculated during checkout

Additional access options:

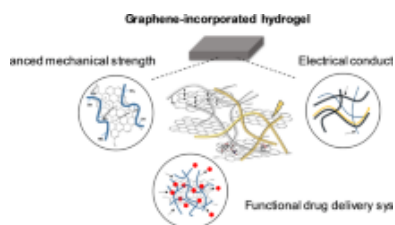
- [Log in](#)
- [Learn about institutional subscriptions](#)
- [Read our FAQs](#)
- [Contact customer support](#)

Similar content being viewed by others



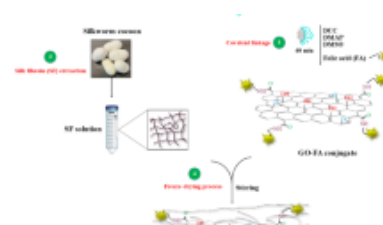
Green synthesis of peptide functionalized reduced graphene oxide (rGO) nano bioconjugat...

Article | Open access
10 June 2020



Graphene oxide-incorporated hydrogels for biomedical applications

Article | 08 May 2020



Functionalized graphene oxide nanosheets with folic acid and silk fibroin as a novel...

Article | Open access
13 April 2022

References

- 1 Yang, K., Feng, L., Shi, X. & Liu, Z. Nano-graphene in biomedicine: theranostic applications. *Chem. Soc. Rev.* **42**, 530–547 (2013).
 - 2 Yang, K., Li, Y., Tan, X., Peng, R. & Liu, Z. Behavior and toxicity of graphene and its functionalized derivatives in biological systems. *Small* **9**, 1492–1503 (2013).
 - 3 Feng, L.Z. & Liu, Z.A. Graphene in biomedicine: opportunities and challenges. *Nanomedicine* **6**, 317–324 (2011).
 - 4 Bitounis, D., Ali-Boucetta, H., Hong, B.H., Min, D.-H. & Kostarelos, K. Prospects and challenges of graphene in biomedical applications. *Adv. Mater.* **25**, 2258–2268 (2013).
 - 5 Shen, H., Zhang, L., Liu, M. & Zhang, Z. Biomedical applications of graphene. *Theranostics* **2**, 283–294 (2012).
 - 6 Zhang, Y., Nayak, T.R., Hong, H. & Cai, W. Graphene: a versatile nanoplatform for biomedical applications. *Nanoscale* **4**, 3833–3842 (2012).
 - 7 Feng, L., Wu, L. & Qu, X. New horizons for diagnosis and therapeutic applications of graphene and graphene oxide. *Adv. Mater.* 168–186 (2013).
 - 8 Yang, K. et al. Graphene in mice: ultra-high *in vivo* tumor uptake and photothermal therapy. *Nano Lett.* **10**, 3318–3323 (2010).
-

- 9 Zhang, S.A., Yang, K., Feng, L.Z. & Liu, Z. *In vitro* and *in vivo* behaviors of dextran functionalized graphene. *Carbon* **49**, 4040–4049 (2011).
-
- 10 Lu, C.-H., Yang, H.-H., Zhu, C.-L., Chen, X. & Chen, G.-N. A graphene platform for sensing biomolecules. *Angew. Chem. Int. Ed.* **48**, 4785–4787 (2009).
-
- 11 He, S. et al. A Graphene nanoprobe for rapid, sensitive, and multicolor fluorescent DNA analysis. *Adv. Funct. Mater.* **20**, 453–459 (2010).
-
- 12 Tang, L.A.L., Wang, J. & Loh, K.P. Graphene-based SELDI probe with ultrahigh extraction and sensitivity for DNA oligomer. *J. Am. Chem. Soc.* **132**, 10976–10977 (2010).
-
- 13 Lee, W.C. et al. Origin of enhanced stem cell growth and differentiation on graphene and graphene oxide. *ACS Nano*. **5**, 7334–7341 (2011).
-
- 14 Nayak, T.R. et al. Graphene for controlled and accelerated osteogenic differentiation of human mesenchymal stem cells. *ACS Nano*. **5**, 4670–4678 (2011).
-
- 15 Park, S.Y. et al. Enhanced differentiation of human neural stem cells into neurons on graphene. *Adv. Mater.* **23**, H263–H267 (2011).
-
- 16 Cohen-Karni, T., Qing, Q., Li, Q., Fang, Y. & Lieber, C.M. Graphene and nanowire transistors for cellular interfaces and electrical recording. *Nano Lett.* **10**, 1098–1102 (2010).
-

- 17 Nguyen, P. & Berry, V. Graphene interfaced with biological cells: opportunities and challenges. *J. Phys. Chem. Lett.* **3**, 1024–1029 (2012).
-
- 18 Liu, Z., Robinson, J.T., Sun, X.M. & Dai, H.J. PEGylated nanographene oxide for delivery of water-insoluble cancer drugs. *J. Am. Chem. Soc.* **130**, 10876–10877 (2008).
-
- 19 Sun, X. et al. Nano-graphene oxide for cellular imaging and drug delivery. *Nano Res.* **1**, 203–212 (2008).
-
- 20 Feng, L., Zhang, S. & Liu, Z. Graphene based gene transfection. *Nanoscale* **3**, 1252–1257 (2011).
-
- 21 Feng, L. et al. Polyethylene glycol and polyethylenimine dual-functionalized nanographene oxide for photothermally enhanced gene delivery. *Small* **9**, 1989–1997 (2013).
-
- 22 Zhang, W. et al. Synergistic effect of chemo-photothermal therapy using PEGylated graphene oxide. *Biomaterials* **32**, 8555–8561 (2011).
-
- 23 Zhang, L., Xia, J., Zhao, Q., Liu, L. & Zhang, Z. Functional graphene oxide as a nanocarrier for controlled loading and targeted delivery of mixed anticancer drugs. *Small* **6**, 537–544 (2010).
-
- 24 Yang, K. et al. Multimodal imaging guided photothermal therapy using functionalized graphene nanosheets anchored with magnetic nanoparticles. *Adv. Mater.* **24**, 1868–1872 (2012).
-

- 25 Yang, K. et al. The influence of surface chemistry and particle size of nanoscale graphene oxide on photothermal therapy of cancer using ultra-low laser power. *Biomaterials* **33**, 2206–2214 (2012).
-
- 26 Huang, P. et al. Folic acid-conjugated graphene oxide loaded with photosensitizers for targeting photodynamic therapy. *Theranostics* **1**, 240–250 (2011).
-
- 27 Wang, Y. et al. Multifunctional mesoporous silica-coated graphene nanosheet used for chemo-photothermal synergistic targeted therapy of glioma. *J. Am. Chem. Soc.* **135**, 4799–4804 (2013).
-
- 28 Shi, S. et al. Tumor vasculature targeting and imaging in living mice with reduced graphene oxide. *Biomaterials* 3002–3009 (2013).
-
- 29 Hong, H. et al. *In vivo* targeting and imaging of tumor vasculature with radiolabeled, antibody-conjugated nanographene. *ACS Nano*. **6**, 2361–2370 (2012).
-
- 30 Hong, H. et al. *In vivo* targeting and positron emission tomography imaging of tumor vasculature with Ga-66-labeled nano-graphene. *Biomaterials* **33**, 4147–4156 (2012).
-
- 31 Ma, X. et al. A functionalized graphene oxide-iron oxide nanocomposite for magnetically targeted drug delivery, photothermal therapy, and magnetic resonance imaging. *Nano Res.* **5**, 199–212 (2012).
-
- 32 Li, Y. et al. The triggering of apoptosis in macrophages by pristine graphene through the MAPK and TGF-beta signaling pathways. *Biomaterials* **33**, 402–411

(2012).

- 33** Yang, K. et al. *In vivo* pharmacokinetics, long-term biodistribution, and toxicology of PEGylated graphene in mice. *ACS Nano*. **5**, 516–522 (2011).
-
- 34** Duch, M.C. et al. Minimizing oxidation and stable nanoscale dispersion improves the biocompatibility of graphene in the lung. *Nano Lett.* **11**, 5201–5207 (2011).
-
- 35** Yang, K. et al. *In vivo* biodistribution and toxicology of functionalized nano-graphene oxide in mice after oral and intraperitoneal administration. *Biomaterials* **34**, 2787–2795 (2013).
-
- 36** Chen, W., Yan, L. & Bangal, P.R. Preparation of graphene by the rapid and mild thermal reduction of graphene oxide induced by microwaves. *Carbon* **48**, 1146–1152 (2010).
-
- 37** Hernandez, Y. et al. High-yield production of graphene by liquid-phase exfoliation of graphite. *Nat. Nanotech.* **3**, 563–568 (2008).
-
- 38** Mao, S. et al. A new reducing agent to prepare single-layer, high-quality reduced graphene oxide for device applications. *Nanoscale* **3**, 2849–2853 (2011).
-
- 39** Tang, X.-Z., Cao, Z., Zhang, H.-B., Liu, J. & Yu, Z.-Z. Growth of silver nanocrystals on graphene by simultaneous reduction of graphene oxide and silver ions with a rapid and efficient one-step approach. *Chem. Commun.* **47**, 3084–3086 (2011).
-

- 40 Ding, Y.H. et al. A green approach to the synthesis of reduced graphene oxide nanosheets under UV irradiation. *Nanotechnology* **22**, 215601 (2011).
-
- 41 Gurunathan, S., Han, J.W., Eppakayala, V., Jeyaraj, M. & Kim, J.-H. An environmentally friendly approach to the reduction of graphene oxide by *Escherichia fergusonii*. *J. Nanosci. Nanotechnol.* **13**, 2091–2098 (2013).
-
- 42 Zhou, T. et al. A simple and efficient method to prepare graphene by reduction of graphite oxide with sodium hydrosulfite. *Nanotechnology* **22** (2011).
-
- 43 Liu, Z., Tabakman, S.M., Chen, Z. & Dai, H. Preparation of carbon nanotube bioconjugates for biomedical applications. *Nat. Protoc.* **4**, 1372–1382 (2009).
-
- 44 Hummers, W.S. & Offeman, R.E. Preparation of graphitic oxide. *J. Am. Chem. Soc.* **80**, 1339–1339 (1958).
-
- 45 Robinson, J.T. et al. Ultrasmall reduced graphene oxide with high near-infrared absorbance for photothermal therapy. *J. Am. Chem. Soc.* **133**, 6825–6831 (2011).
-
- 46 Bao, H.Q. et al. Chitosan-functionalized graphene oxide as a nanocarrier for drug and gene delivery. *Small* **7**, 1569–1578 (2011).
-
- 47 Zhang, L. et al. Enhanced chemotherapy efficacy by sequential delivery of siRNA and anticancer drugs using PEI-grafted graphene oxide. *Small* **7**, 460–464 (2011).
-

48 Liu, X. et al. Optimization of surface chemistry on single-walled carbon nanotubes for *in vivo* photothermal ablation of tumors. *Biomaterials* **32**, 144–151 (2011).

Acknowledgements

This work was supported by the National Basic Research Program of China (973 Program, 2012CB932600 and 2011CB911002), the National Science Foundation of China (NSFC, 51002100, 51222203, 51132006) and a project funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD), the University of Wisconsin at Madison, the US National Institute of Biomedical Imaging and Bioengineering/National Cancer Institute (1R01CA169365), and the US Department of Defense (W81XWH-11-1-0644).

Author information

Authors and Affiliations

Institute of Functional Nano and Soft Materials (FUNSOM), Collaborative Innovation Center of Suzhou Nano Science and Technology, Soochow University, Suzhou, Jiangsu, China

Kai Yang, Liangzhu Feng & Zhuang Liu

Department of Radiology, University of Wisconsin, Madison, Wisconsin, USA

Hao Hong & Weibo Cai

Department of Medical Physics, University of Wisconsin, Madison, Wisconsin, USA

Hao Hong & Weibo Cai

University of Wisconsin Carbone Cancer Center, Madison, Wisconsin, USA

Weibo Cai

Contributions

Z.L. and W.C. designed the experiments and wrote the manuscript; K.Y., L.F. and H.H. performed the experiments, analyzed the results and wrote the manuscript.

Corresponding author

Correspondence to [Zhuang Liu](#).

Ethics declarations

Competing interests

The authors declare no competing financial interests.

Integrated supplementary information

[Supplementary Figure 1 Biodistribution of free \$^{125}\text{I}\$ and \$^{125}\text{I}\$ -nGO-PEG at 6 h post i.v. injection.](#)

Minimal uptake of free ^{125}I was observed in the liver, spleen, as well as most other organs except thyroid and stomach due to the fast renal excretion of small iodine ions³³.

Supplementary information

[Supplementary Figure 1](#)

(PDF 147 kb)

[Supplementary Table 1](#)

Summary of *in vivo* toxicity of different polymer-functionalized GO. (PDF 169 kb)

Rights and permissions

[Reprints and permissions](#)

About this article

Cite this article

Yang, K., Feng, L., Hong, H. *et al.* Preparation and functionalization of graphene nanocomposites for biomedical applications. *Nat Protoc* **8**, 2392–2403 (2013).

<https://doi.org/10.1038/nprot.2013.146>

Published

Issue Date

DOI

<https://doi.org/10.1038/nprot.2013.146>

Subjects [Graphene](#) • [Nanoparticle synthesis](#) • [Sensors and probes](#)

This article is cited by

[Radiolabeled florescent-magnetic graphene oxide nanosheets: probing the biodistribution of a potential PET-MRI hybrid imaging agent for detection of fibrosarcoma tumor](#)

Ahad Amiri, Yousef Fazaeli ... Mohammadreza Afrasyabi

Annals of Nuclear Medicine (2024)

[Pristine/folate-functionalized graphene oxide as two intrinsically radioiodinated nano-theranostics: self/dual in vivo targeting comparative study](#)

Mohamed M. Swidan, Basma M. Essa & Tamer M. Sakr

Cancer Nanotechnology (2023)

[Synthesis and evaluation of biological effects of modified graphene oxide nanoparticles containing Lawson \(Henna extract\) on gastric cancer cells](#)

Mohammed Abdullah Hamdan Alkwedhim, Vahid Pouresmaeil ... Masoud Homayouni Tabrizi

Molecular Biology Reports (2023)

[Quantum studies of the interaction of nanostructured graphene with polymethyl methacrylate for dental applications](#)

Ernesto López-Chávez, Jesús Estrada-Hernández ... Fray de Landa Castillo-Alvarado

Journal of Nanoparticle Research (2023)

[A functionalized graphene oxide with improved cytocompatibility for stimuli-responsive co-delivery of curcumin and doxorubicin in cancer treatment](#)

Fatemeh Yaghoubi, Najmeh Sadat Hosseini Motlagh ... Ali Moradi

Scientific Reports (2022)

Nature Protocols (*Nat Protoc*) | ISSN 1750-2799 (online) | ISSN 1754-2189 (print)