Microwave enabled Direct Fabrication of Graphene Nanosheets for NIR Photoacoustic Imaging Guided Therapy

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Graphene, a single- or few-layered sheet of sp²- bonded carbon atoms, whose discovery won the 2010 Nobel Prize in physics. Potential applications of graphene for nanoelectronics, sensors, and nano composites have been actively pursued [1]. The biological applications of graphene oxide (GO, an oxidized form of graphene) has been explored starting in 2008 and the research in this area is exploded soon after [1,2].

Inspired by its strong near infrared (NIR) absorption, high photo thermal conversion efficiency, and exceptional large surface area, graphene has emerged as a new shining star of nanomaterial for biological applications, especially in the areas of photo thermal therapy including photo thermal enhanced drug, protein, and gene delivery systems [3-7]. Intriguingly, its intrinsic ability to generate near inferred photoacoustic signal allows not only to monitor the distribution of multifunctional drug delivery systems *in vivo*, to evaluate their post-treatment therapeutic outcomes in situ, and most importantly, to track the long term fate of graphene sheets in the human body, but also enables imaging guided therapy [8,9]. These capabilities could largely facilitate their application in practical multifunctional nanomedicine regimes, fighting various diseases. Equally important, although a number of studies have uncovered that pristine graphene and graphene oxide could induce toxicity in biological system, recent studies demonstrate that well pegylated GO or reduced GO (rGO) nanosheets (nanometer in their lateral dimension) are not toxic *in vitro* to cells and *in vivo* to animals, demonstrating great potential for practical clinical applications.

On the other hand, applications of graphene in biomedical applications, such as drug delivery require mono dispersed grapheme nanosheets with lateral dimensions on the order of nanometers (typically <50 nm) [1].

Large effort has been devoted to fabricate nanosized graphene sheets for variety of applications including the field of nanomedicine. The dominant approaches typically rely upon Hummer's or modified Hummer's methods which involve rather tedious and time consuming procedures [10]. In brief, one must first oxidize graphite powder (this step normally takes hours to a few days depending on the specific chemical recipes); exfoliate the oxidized product to form GO suspensions. The as-prepared GO sheets are very poly-disperse, ranging from a few nanometers to tens of micrometers. To reach a predefined nanometer-sized GO sheet, extended oxidation and sonication were applied. The final GO products still have a large distribution of size and shape. To increase the size uniformity of the GO sheets, extensive size separation steps need to be performed. Alternatively, size-controlled GO was synthesized using starting materials which are already small such as graphite nanofibers or carbon fibers [11,12]. Compared to graphene, GO has very low absorption capability in the near infrared (NIR) region, various chemical, electrochemical, or hydrothermal reduction have been explored to produce rGO nanosheets with some fraction of its NIR absorption, photo thermal conversion recovered [7,8,13,14]. Furthermore, even though there were no metal catalyst leftover, the major possible toxicity resources of carbon nanotube (CNT) based delivery systems, are naturally avoided in graphene based delivery systems, trace amounts of metal ions and other chemicals involved in the oxidation and subsequent reduction processes can participate in unwanted toxic reactions and could be detrimental to biological applications. However, purification of GO is difficult due to its gelatin tendency, therefore, extensive purification steps, which requires large amount of solvents and long time of washing, making the production of clean GO and rGO very time consuming [15].
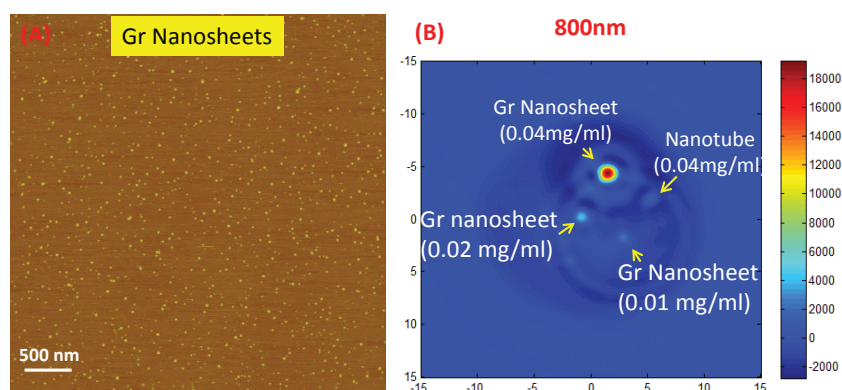

Figure 1: (A) An Atomic Force Microscopy image of the graphene nanosheets fabricated by the microwave enabled direct fabrication approach. (B) Photo acoustic signal of graphene nanosheets with different concentrations and single walled carbon nanotubes.

Recently, a Ph. D student, Mehulkumar Patel in Prof. Huixin He research group at Rutgers-Newark has developed an innovative approach, which was referred to microwave enabled direct fabrication approach, could solve the above mentioned problems (Figure 1). This novel approach allows rapid and direct fabrication of uniform amphiphilic low oxygen containing (meaning low defects) graphene nanosheets [16], which can be used as multifunctional drug delivery carrier. This novel fabrication approach does not involve toxic chemicals and is much easier for cleaning and surface modification to render them physiological stability and biocompatibility. Compared to the commonly used GO, or chemically reduced graphene oxide nanosheets (rGO), the grapheme nanosheets contain much increased graphene domains, which ensures much higher drug loading capability, especially for hydrophobic anticancer drugs. Without the requirement of a post-reduction process, the fabricated grapheme nanosheets exhibits strong NIR absorption, high optical-thermal conversion efficiency for external controlled "on demand' release capabilities [17]. It could also enhance the photo thermal therapeutic efficiency [18]. They also show strong NIR photo-acoustic conversion efficiency, stronger than GO and single walled carbon nanotubes with the same concentrations (Figure 1B), which provides great potential for the future to build *in vivo* imaging capabilities for in-situ evaluation of therapeutic effects and/or imaging guided therapy.

References

1. Sun X, Liu Z, Welsher K, Robinson JT, Goodwin A, et al. (2008) Nano-Graphene Oxide for Cellular Imaging and Drug Delivery. Nano Res 1: 203-212.

2. Liu Z, Robinson JT, Sun X, Dai H (2008) PEGylated Nano-Graphene Oxide for Delivery of Water Insoluble Cancer Drugs. J Am Chem Soc 130: 10876-10877.

3. Yang K, Feng L, Shi X, Liu Z (2013) Nano-graphene in biomedicine: theranostic applications. Chem Soc Rev 42: 530-547.

4. Yang K, Zhang S, Zhang G, Sun X, Lee ST, et al. (2010) Graphene in mice: ultrahigh *in vivo* tumor uptake and efficient photothermal therapy. Nano Lett 10: 3318-3323.

5. Yang X, Zhang X, Liu Z, Ma Y, Huang Y, et al. (2008) High-Efficiency Loading and Controlled Release of Doxorubicin Hydrochloride on Graphene Oxide. J Phys Chem C 112: 17554-17558.

6. Zhang L, Wang Z, Lu Z, Shen H, Huang J, et al. (2013) PEGylated reduced graphene oxide as a superior ssRNA delivery system. J Mater Chem B 1: 749-755.

7. Tian B, Wang C, Zhang S, Feng L, Liu Z (2011) Photothermally enhanced photodynamic therapy delivered by nano-graphene oxide. Acs Nano 5: 7000-7009.

8. Yang K, Hu LL, Ma XX, Ye SQ, Cheng L, et al. (2012) Multimodal Imaging Guided Photothermal Therapy using Functionalized Graphene Nanosheets Anchored with Magnetic Nanoparticles. Adv Mater 24: 1868- 1872.

9. Shen H, Liu M, He HX, Zhang LM, Huang J, et al. (2012) PEGylated Graphene Oxide-Mediated Protein Delivery for Cell Function Regulation. ACS Appl Mater Interfaces 4: 6317-6323.

10. Hummers WS, Offeman RE (1958) Preparation of Graphitic Oxide. J Am Chem Soc 80: 1339.

11. Luo JY, Cote LJ, Tung VC, Tan ATL, Goins PE, et al. (2010) Graphene Oxide Nanocolloids. J Am Chem Soc 132: 17667-17669.

12. Peng J, Gao W, Gupta BK, Liu Z, Romero-Aburto R, et al. (2012) Graphene Quantum Dots Derived from Carbon Fibers. Nano Lett 12: 844-849.

13. Yang K, Wan J, Zhang S, Tian B, Zhang Y, et al. (2012) The influence of surface chemistry and size of nanoscale graphene oxide on photothermal therapy of cancer using ultra-low laser power. Biomaterials 33: 2206-2214.

14. Robinson JT, Tabakman SM, Liang Y, Wang H, Casalongue HS, et al. (2011) Ultrasmall reduced graphene oxide with high near-infrared absorbance for photothermal therapy. J Am Chem Soc 133: 6825-6831.

15. Kim F, Luo JY, Cruz-Silva R, Cote LJ, Sohn K, et al. (2010) Self-Propagating Domino-like Reactions in Oxidized Graphite. Adv Funct Mater 20: 2867-2873.

16. Patel MA, Yang H, Chiu PL, Mastrogiovanni D, Flach CR, et al. (2013) Direct Production of Graphene Nanosheets for Near Infrared Photoacoustic Imaging. Acs Nano 7: 8147-8157.

17. Yang K, Xu H, Cheng L, Sun CY, Wang J, et al. (2012) *In Vitro* and *In Vivo* Near-Infrared Photothermal Therapy of Cancer Using Polypyrrole Organic Nanoparticles. Adv Mater 24: 5586-5592.

18. Taratula O, Patel M, Schumann C, Naleway MA, Pang AJ, et al. (2015) Phthalocyanine-loaded graphene nanoplatform for imaging-guided combinatorial phototherapy. Int J Nanomedicine 10: 2347-2362.