Comment on "Amine-Modified Graphene: Thrombo-Protective Safer Alternative to Graphene Oxide for Biomedical Applications"

■ In a recent issue of *ACS Nano*, we read with great interest the excellent article by Singh *et al.*[1] detailing the fabrication of amine-modified graphene (G-NH_2) and their application potential in biomedicine.[1] However, we found that the authors neglected a key point that the performances of both micro- and nanosized graphene in biological context were essential for guiding their biomedical applications.[2]

In this study, the authors[1] constructed a suspended graphene-amine membrane consisting of single- or few-layer sheets. Flow cytometric analysis showed that identical size distribution of different graphene derivatives was fabricated, and high-resolution transmission electron microscopy (HR-TEM) revealed that the average dimension of different graphene derivatives was 2 μm. Finally, the authors concluded that G-NH_2 was a far safer alternative to GO and was thus potentially safe for biomedical applications in areas such as imaging, drug delivery, as well as photothermal therapy. However, all the graphene derivatives in this study were microsized, and nanosized graphene oxide, a widely studied material with high biocompatibility,[3] was not mentioned as a control group in this article.

As this study mentioned, graphene derivatives were finally developed to act as diagnostic or therapeutic materials. Such materials often required site-specific cellular entry to deliver their payload to subcellular locations hidden beneath cell membranes.[4] Sahay[5] thought that the uptake of those materials was regulated by their size. For example, Raffa's study[6] showed that microsized carbon nanotubes with length longer than 2 μm could hardly enter cells, and Gratton[7] showed that the nanosized particles seemed to enter cells more rapidly than the microparticles. Moreover, the pharmacokinetic profile analysis between the two types (micro- and nanosized) of GO suggested that a material with a small lateral dimension might be more suitable for potential biomedical applications.[8]

Furthermore, microsized graphene derivatives might induce inflammation response, which might not be suitable for biomedical applications such as drug delivery and cancer therapy. Once inside the cells, the microsized (2 μm) GO might form wrinkles and then induce a much stronger inflammatory response with high release of key cytokines than nanosized (350 nm) GO.[2] Yue[2] concluded that the high cytokine level induced by 2 μm GO might be attributed to the strong steric effect of microsized GO. Besides, histological micrographics of mice showed that a large number of mononuclear cells infiltrated subcutaneous adipose tissue, and lipid-filled vacuoles as well as tissue impairment appeared after 2 μm GO injection. In contrast, the inflammation response was weak under the treatment of 350 nm GO.[2] Such observations were in agreement with those by Schinwald,[9] who reported that microsized graphene induced an inflammatory response and granuloma formation in lung and pleural space. Low inflammatory profiles exerted by nanosized GO can be beneficial for applications in drug carrier and cancer therapy, where improved biocompatibility is demanded.[3]

In conclusion, the size of graphene derivatives might be the control factor of biocompatibility of graphene derivatives. Therefore, when investigating the biocompatibility of graphene derivatives, both microsized and nanosized graphene derivatives should be considered. Finally, we would like to congratulate the authors for their contributions to graphene functionalization, which is innovative to biomedical research.

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