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Graphene quantum dots with nitrogen-doped content dependence for highly efficient dual-modality photodynamic antimicrobial therapy and bioimaging

Wen-Shuo Kuo [a, b, c, *, 1], Hua-Han Chen [d, 1], Shih-Yao Chen [e, 1], Chia-Yuan Chang [c], Pei-Chi Chen [b, f], Yung-I Hou [b], Yu-Ting Shao [b, g], Hui-Fang Kao [b, h], Chih-Li Lilian Hsu [f, g], Yi-Chun Chen [i], Shean-Jen Chen [a, c], Shang-Rung Wu [f, j, **], Jiu-Yao Wang [b, f, g, ***]

[a] Advanced Optoelectronic Technology Center, National Cheng Kung University, Tainan 701, Taiwan, ROC
[b] Allergy & Clinical Immunology Research Center, National Cheng Kung University, Tainan 701, Taiwan, ROC
[c] Center for Micro/Nano Science and Technology, National Cheng Kung University, Tainan 701, Taiwan, ROC
[d] Department of Food Science, National Penghu University of Science and Technology, Penghu 880, Taiwan, ROC
[e] Department of Internal Medicine, National Cheng Kung University, Tainan 701, Taiwan, ROC
[f] Institute of Basic Medical Sciences, National Cheng Kung University, Tainan 701, Taiwan, ROC
[g] Department of Microbiology & Immunology, National Cheng Kung University, Tainan 701, Taiwan, ROC
[h] Department of Nursing, National Tainan Junior College of Nursing, Tainan 700, Taiwan, ROC
[i] Department of Physics, National Cheng Kung University, Tainan 701, Taiwan, ROC
[j] Institute of Oral Medicine, National Cheng Kung University, Tainan 701, Taiwan, ROC

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ABSTRACT

Reactive oxygen species is the main contributor to photodynamic therapy. The results of this study show that a nitrogen-doped graphene quantum dot, serving as a photosensitizer, was capable of generating a higher amount of reactive oxygen species than a nitrogen-free graphene quantum dot in photodynamic therapy when photoexcited for only 3 min of 670 nm laser exposure (0.1 W cm^{-2}), indicating highly improved antimicrobial effects. In addition, we found that higher nitrogen-bonding compositions of graphene quantum dots more efficiently performed photodynamic therapy actions than did the lower compositions that underwent identical treatments. Furthermore, the intrinsically emitted luminescence from nitrogen-doped graphene quantum dots and high photostability simultaneously enabled it to act as a promising contrast probe for tracking and localizing bacteria in biomedical imaging. Thus, the dual modality of nitrogen-doped graphene quantum dots presents possibilities for future clinical applications, and in particular multidrug resistant bacteria.

1. Introduction

Photodynamic therapy (PDT) is a type of phototherapy that has established medical applications, involving selectively exposing photosensitizers (PSs) to light of appropriate wavelength and energy. In addition to conventional PSs [1,2], several newly synthesized PSs [3,4] have recently been prepared and applied. With recent developments in nanotechnology, the use of nanomaterials in PDT has served as a potential alternative approach to achieving improved therapeutic efficacy [5,6]. Graphene-based nanomaterial has shown potential in the pursuit of applying new nanomaterials to efficiently perform PDT. Graphene is a two-dimensional monolayer of graphite that bonds carbon (C) atoms, forming a hexagonal lattice. Graphene-based materials have been investigated in numerous fields; they exhibit excellent conductivity as well as mechanical, thermal, and optical properties. Although some studies have reported using graphene-based nanomaterials combined with

* Corresponding author. Advanced Optoelectronic Technology Center, National Cheng Kung University, Tainan 701, Taiwan, ROC.
** Corresponding author. Institute of Oral Medicine, National Cheng Kung University, Tainan 701, Taiwan, ROC.
*** Corresponding author. Institute of Basic Medical Sciences, National Cheng Kung University, Tainan 701, Taiwan, ROC.
E-mail addresses: wenshuokuo@mail.ncku.edu.tw (W.-S. Kuo), z10208056@email.ncku.edu.tw (S.-R. Wu), a122@mail.ncku.edu.tw (J.-Y. Wang).
[1] The authors contributed equally to this work.

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PSs for PDT [7,8], research on the direct use of a graphene quantum dot (GQD) as a PS for generating reactive oxygen species (ROS) in developing PDT has been limited [9–12]. The GQD is a new type of zero-dimensional QD converted from two-dimensional graphene sheets that is emerging as a promising optical nanomaterial; contributions from intrinsic- and defective-state emission, possibly involving photoluminescence (PL) mechanisms and sequential GQDs, could be applied to biomedical research, including PDT research. Furthermore, doping is crucial in semiconductors because the carrier density can be noticeably changed, resulting in optical and electrical properties completely distinct from the intrinsic counterpart. Consequently, nitrogen (N) functionalization and doping is considered to be highly useful in modifying the intrinsic properties of GQDs because the five-valance electrons and incorporated electron-donating N atoms, which have a comparable atomic size to that of C atoms, impart a relatively high positive-charge density to adjacent C atoms in graphene [13]. Thus, given GQDs exhibiting extraordinary quantum confinement and edge effects, N atoms doped with GQDs (N-GQDs) can exhibit enhanced electrocatalytic, electrochemical, and photochemical activities, providing advantages in biomedical and optoelectronic applications [14].

This study employed N-GQDs as PSs to eliminate the gram-negative bacteria *Escherichia coli* (*E. coli*) by using 670-nm laser exposure involving a PDT pathway. A limited quantity (1 μg mL^{-1}) of N-GQDs with an N 1s–C 1s ratio of approximately 5.1%, determined by X-ray photoelectron spectroscopy (XPS), with a 3-min photoexcitation time and low-dose energy irradiation (0.1 W cm^{-2}) led to 100% bacterial elimination, compared with approximately 52% elimination when conducting the same treatment with a GQD. Additionally, in contrast to a lower N content (2.9%), a higher N content in N-GQDs (5.1%) enhanced PDT efficacy and achieved improved bactericidal capability. Furthermore, the photoproperty of emitted PL (Em: 728 nm) in a near-infrared (NIR) region, employing slight scattering, low energy absorption, and optimal irradiation penetration, enabled N-GQDs to serve in this study as promising contrast probes to track and localize N-GQD-treated-bacteria and provide additional data on the irradiated bacterial status. The integration of N-GQDs, dual-modality PDT, and a contrast agent was found to have high potential in simultaneous PDT for eliminating bacteria and tracking targeted bacteria to evaluate the therapeutic effect.

2. Materials and methods

2.1. N-GQD and GQD preparations

Graphene oxide was prepared from the graphite (Bay carbon, SP-1, USA) with a modified Hummers method [15]. Graphite (8.5 M) and $NaNO_3$ (0.6 M) (Merck Germany) were mixed with H_2SO_4 (18 M) (Wako, Japan). $KMnO_4$ (2.0 M) (Merck Germany) was slowly added and kept stirring overnight. Then, the ddH_2O was gradually added and kept stirred. Adding H_2O_2 (35 wt %) (Shimakyu, Japan) to terminate reaction. Washing and centrifugation with ddH_2O several times were addressed and the graphene oxide was collected. The as-prepared graphene oxide was heated to 400–600 °C (400 °C and 600 °C for N-GQD 2.9% and 5.1% of the N(1)/C(1s) ratio for N-GQD determined by XPS, respectively) in the presence of ammonia for several hours, and then were introduced to concentrated HNO_3 (16 M) (Wako, Japan) and stirred overnight. The mixture was put in sonicator and then put it in oven at 160 °C to vaporize all the liquid. Washing and centrifugation (83000 rpm) (Optima TLX Ultracentrifuge, BECKMAN, USA) with ddH_2O several times were addressed. The resulting black suspension was tuned the pH to 7.4 with NaOH (Merck Germany), and it was stayed in a dialysis bag (retained molecular weight: 100 kDa) > 12 h, and N-GQD was obtained. On the other hand, the as-prepared graphene oxide was put in a tube furnace to elevate the temperature to 400 °C for several hours in the presence of argon and then conducted the same treatment for the following steps to prepare GQD.

2.2. Characterization

The samples were then subject to transmission electron microscopy (TEM) and high-resolution TEM (HRTEM) (JEOL 1400 and JEOL 2100F, Japan) observation. The height profile diagram, thickness and size of samples were determined by atomic force microscopy (AFM, multimode 8, Bruker, Germany). The crystalline structures of samples were identified using X-ray diffraction (XRD, Bruker AXS Gmbh, Germany/D2 Phaser) with Cuκα radiation at 30 kv and 30 mA. Fourier transform infrared spectroscopy (FTIR), ultraviolet–visible (UV–vis), zeta potential spectra and dynamic light scattering (DLS) of samples were recorded by the spectrometers: PerkinElmer RX1 USA, U-4100 Hitachi Japan and Malvern Nano-ZS90 UK, respectively. Raman spectroscopy (DXR, Thermo Scientific, USA) was examined the crystallinity of samples with 532 nm laser. XPS (PHI 5000, VersaProbe, USA) was examined the surface chemistry of materials. The PL signal was recorded by the spectrometer (F-7000, Hitachi, Japan).

2.3. Culturing E. coli

E. coli, obtained from our own laboratory were grown on the nutrient agar plate of LB, composed of agar 15 g, sodium chloride 8 g, yeast extract 5 g, tryptone 10 g for per liter and tune pH to 7.5) (Sigma Aldrich Co., USA) and incubated at 37 °C.

2.4. Coating antibody

The absorbance of a quantity of antibody (anti-lipopolysaccharide (LPS) antibody (Ab_{LPS}) or anti-epidermal growth factor receptor antibody (Ab_{EGFR}) (Antagene, USA)) was recorded by UV–vis spectroscopy (Abs: approximately 276 nm). By the electrostatic interaction, the nanomaterials were mixed with the same quantity antibody for 30 min of incubation at 4 °C in the dark and centrifuge (83000 rpm) to remove excess antibody, and then the nanomaterial-Ab_{LPS}, or -Ab_{EGFR} was prepared. On the other hand, keep the supernatant and measure its absorbance. The difference in absorbance between the collected supernatant and the original antibody was estimated. Consequentially, the quantity of the antibody absorbed on the nanomaterials was calculated by Lambert-Beer's law. There was approximately 8.5 μg of Ab_{LPS} absorbed on 0.1 mg of N-GQDs (5.1%), which meant the coating efficiency was approximately 8.5%, whereas 11.7% for Ab_{EGFR}. For N-GQDs (2.9%), the coating efficiency was 8.2% for Ab_{LPS} and 11.7% for Ab_{EGFR}, whereas 8.7% of Ab_{LPS} and 12.1% of Ab_{EGFR}, absorbed with GQDs.

2.5. Biocompatibility assay with colony forming unit (CFU) counting method

Bacteria (OD600 ~0.05) were added with nanomaterials-Ab_{LPS} (0–10 μg mL^{-1}), by 3 h of incubation at 37 °C. After incubation, the mixture was centrifuged and the pellet of bacteria were diluted (OD600 ~0.05). Then, a dilution factor of 10^{-5} to 10^{-8} was conducted in the incubated bacteria with plating on the plates. The plates were stayed in an incubator at 37 °C overnight. The number of surviving bacteria was determined and expressed as a percentage (%) that corresponds to the unit of CFU mL^{-1} after incubation.