Shine bright like a ~~diamond~~ graphene quantum dot: Near-infrared-emissive graphene quantum dots for multiplex bioimaging

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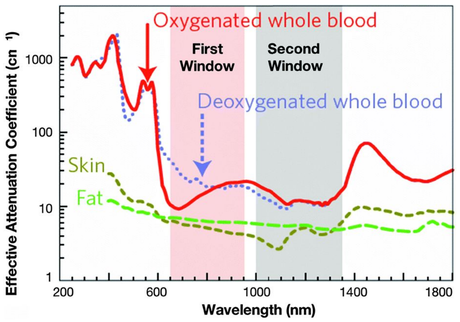
Abstract

Due to high tissue penetration depth and low autofluorescence backgrounds, near-infrared (NIR) fluorescence imaging has recently become an advantageous diagnostic technique used in a variety of fields. However, most of the NIR fluorophores do not have therapeutic delivery capabilities, exhibit low photostabilities, and raise toxicity concerns. To address these issues, we developed and tested five types of biocompatible graphene quantum dots (GQDs) exhibiting spectrally-separated fluorescence in the NIR range of 928–1053 nm with NIR excitation. Their optical properties in the NIR are attributed to either rare-earth metal dopants (Ho-NGQDs, Yb-NGQDs, Nd-NGQDs) or defect-states (nitrogen doped GQDS (NGQDs), reduced graphene oxides) as verified by Hartree-Fock calculations. Moderate up to 1.34% quantum yields of these GQDs are well-compensated by their remarkable >4 h photostability. At the biocompatible concentrations of up to 0.5–2 mg/ml GQDs successfully internalize into HEK-293 cells and enable in vitro imaging in the visible and NIR. Tested all together in HEK-293 cells five GQD types enable simultaneous multiplex imaging in the NIR-I and NIR-II shown for the first time in this work for GQD platforms. Substantial photostability, spectrally-separated NIR emission, and high biocompatibility of five GQD types developed here suggest their promising potential in multianalyte testing and multiwavelength bioimaging of combination therapies.

Introduction

Near-infrared (NIR) fluorescence is an advantageous bioimaging technique as it is: portable, user-friendly, affordable, and non-invasive.



Hemmer, E. et al. *Nanoscale Horizons.* [2016]

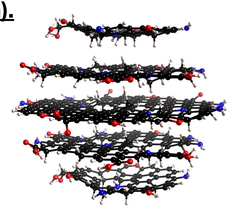
Problem: NIR fluorophores do not have therapeutic delivery capabilities, exhibit low photostabilities, and raise toxicity concerns

Solution: Graphene quantum dots (GQDs) as NIR fluorophores

- have a capability to bind drugs and oligonucleotides;
- can be modified with targeting moieties;
- biocompatible *in vitro* and *in vivo*;
- soluble in water;
- average size <10 nm;

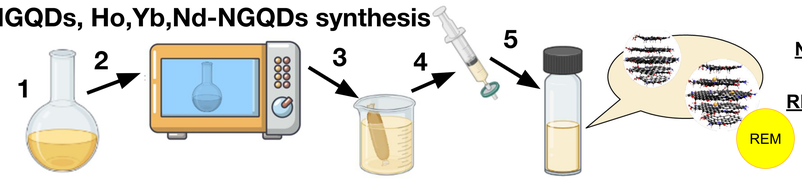
exhibit fluorescence in the

- visible spectral region;
- near-infrared spectral region without doping and after doping with rare-earth metals (REM) (Nd, Ho, Tm).



Synthesis of five types of GQDS

NGQDs, Ho,Yb,Nd-NGQDs synthesis



NGQDs – no metal dopant

REM-NGQDs - REM dopant

- Glucosamine HCl (0.04 M) (and $REM(NO_3)_3 \cdot 6H_2O$ (0.008 M)) were dissolved in water (1), microwaved (2), purified (3) and filtered (4) to get NGQDs and Nd-NGQDs (5).

Cell viability and internalization/excreation study of NGQDs, RGQDs, Ho, Yb, Nd-NGQDs

Concentrations of 0.5 mg/ml for RGQDs and Ho-NGQDs, and 1 mg/ml for NGQDs, Nd-NGQDs, and Yb-NGQDs, were chosen for further internalization/excretion studies in the HEK-293 cells

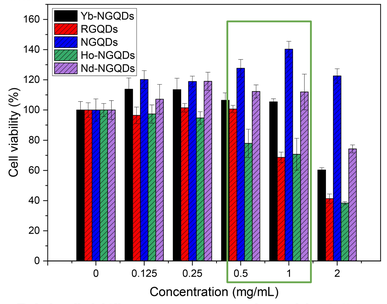


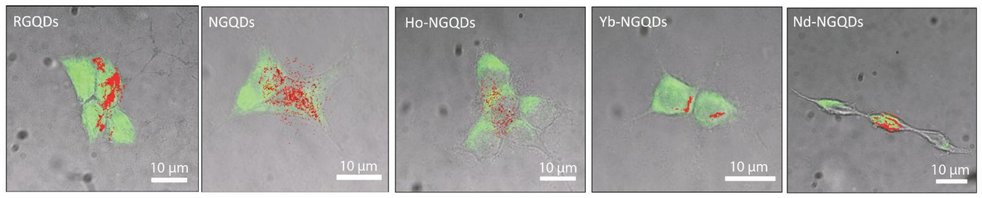
Table 1. Comparative measurements of quantum yield of GQDs and ICG standard.

Sample	Solvent	Excitation	Quantum Yield (%)
Nd-NGQDs	Water	808 nm	0.68
NGQDs	Water	808 nm	0.22
RGQDs	Water	808 nm	1.34
Ho-NGQDs	Water	650 nm	0.09
ICG	Water	808 nm	2.5

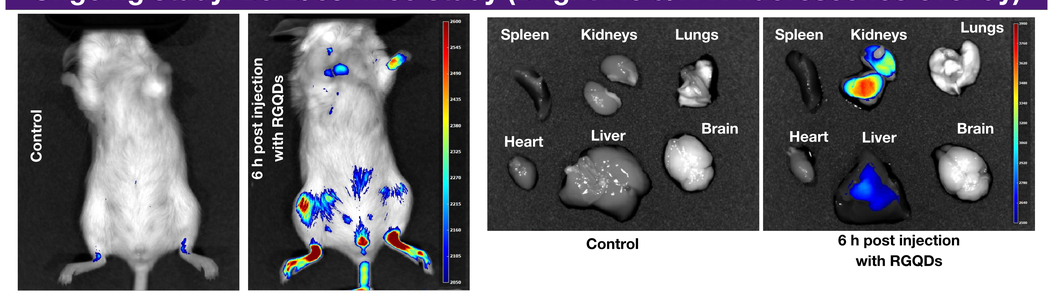
Table 2. Excitation and emission parameters used for internalization/excretion study and multiplex imaging of GQDs in HEK-293 cells based on the NIR fluorescence

Sample	RGQDs	Ho-NGQDs	NGQDs	Yb-NGQDs	Nd-NGQDs
Excitation (nm)	808	650	808	980	808
Emission (nm)	930	970	980	1010	1050

Bright-field/fluorescence overlay with visible and NIR GQD fluorescence collected with visible and hyperspectral near-infrared microscopy. GQD visible fluorescence (green) is excited with 460±20 nm and collected using 535±20 nm filters. Near-infrared GQD fluorescence (red) is excited and collected at the wavelength specified in Table 2 for each GQD type.



Ongoing study: BalB/cJ mice study (Bright Field/NIR fluorescence overlay)

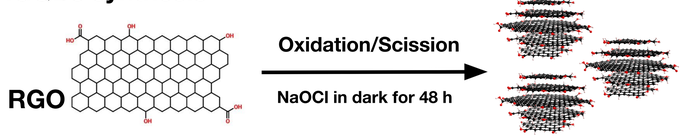


Conclusion

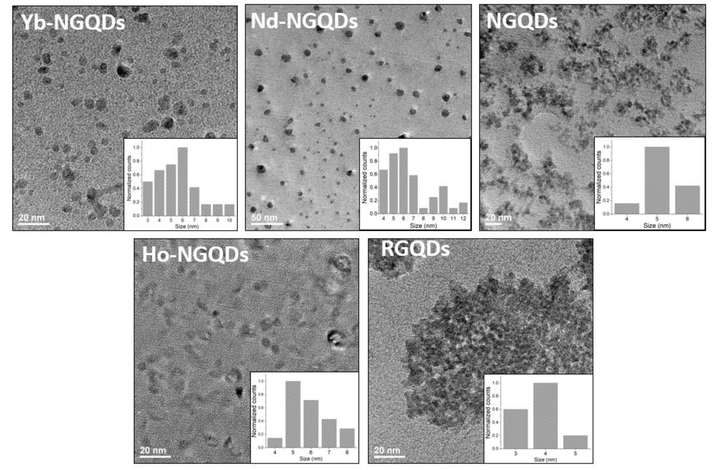
- NGQDs, RGQDs, Ho, Nd-NGQDs exhibit bright NIR fluorescence in the NIR range of 928-1053 nm with NIR excitation and 0.22, 1.34, 0.09, 0.68% of quantum yield respectively;
- NGQDs, RGQDs, Ho, Yb, Nd-NGQDs demonstrate biocompatibility up to 0.5-2 mg/mL and exhibit NIR fluorescence *in vitro* and RGQDs *in vivo*;
- HEK-293 cells treated with five types of GQDs demonstrate the possibility of multiplex imaging;
- REM doping in REM-NGQDs enables deeper tissue near-infrared fluorescence imaging suitable for both *in vitro* and *in vivo* applications.

Synthesis of five types of GQDs

RGQDs synthesis

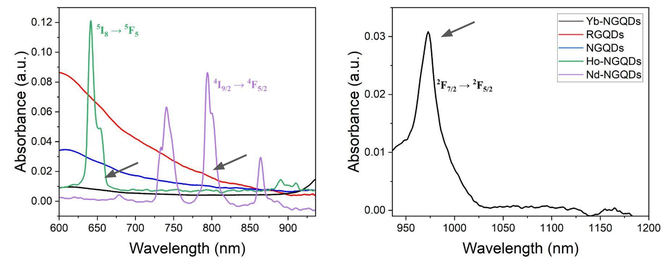


Morphological characterization

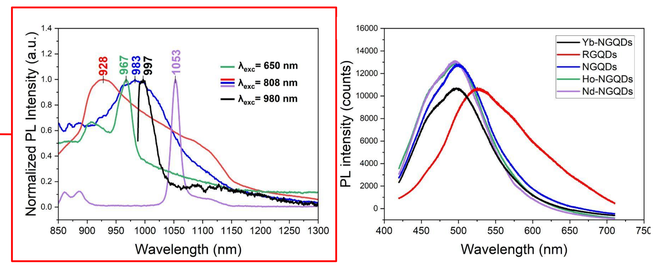


Optical Characterization

Absorbance of GQDs in the NIR spectral region

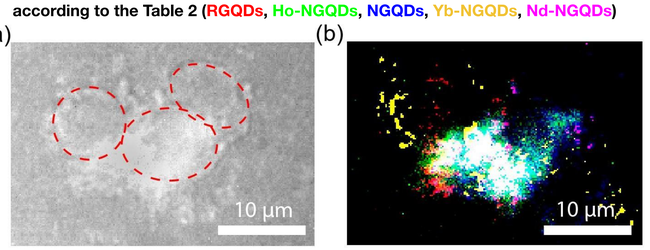


NIR (left) and VIS (right) fluorescence of NGQDs, RGQDs, Ho, Yb, Nd-NGQDs



Multiplex NIR Imaging: HEK-293 cells after treatment with five types of GQDs

(a) Bright field and (b) NIR fluorescence of HEK-293 cells taken at each wavelength separately according to the Table 2 (RGQDs, Ho-NGQDs, NGQDs, Yb-NGQDs, Nd-NGQDs)



Near-infrared (NIR) fluorescence imaging is an advantageous diagnostic bioimaging tool, as NIR light allows higher penetration under the skin than visible light. However, existing NIR fluorophores often lack therapeutic delivery capabilities and have stability and toxicity issues. To overcome these challenges, we developed five biocompatible graphene quantum dots (GQDs) that emit NIR light. These GQDs are tiny particles, that are stable and safe for human cells. Their potential shows promise for the detection of multiple substances and the imaging of various organs simultaneously.