Supplementary Information

Oxygenated graphene quantum dots (GQDs) synthesized using laser ablation for long term real time tracking and imaging

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**Figure S1.** Laser ablation setup of HOPG in beaker containing PEG:water solution in a glass beaker connected to DC rotor (a). Zeta potential measurements show that refluxed GQDs had higher zeta potential compared to control GQDs (b). Dynamic Light scattering measurements of control, 20 min and 1 hr GQDs (c)
Figure S2. Top: AFM images confirm the presence of graphene layers (a) and GQDs (b). Bottom: Centrifuged control GQDs showed the presence of larger graphene sheets (Dotted black circle) and graphene particles (Black circle); SAED pattern confirms that the particles possess hexagonal graphene structure (d).
Figure S3. (a) FT-Raman spectra GQDs had a broad G and D band confirming that the particles possess sp2 and sp3 carbon atoms (b) Wide scan XPS spectrum of GQDs with major peaks of carbon and oxygen. (c) High resolution scanning of C1s (d) and O 1s XPS spectrum of GQDs.

FT-Raman spectrum of pyrolytic graphite had a sharp peak of G band at 1590 cm\(^{-1}\) and D band at 1332 cm\(^{-1}\).\(^1\) G-band is attributed to in-plane vibrations of sp\(^2\) hybridized bonded carbon atoms whereas the D-band indicates sp\(^3\) hybridized carbon atoms due to the presence of structural defects. Broad peak around 1800 cm\(^{-1}\) could bedue to the sp\(^3\) hybridization of polyethylene glycol.\(^2\) Broadening of D-band in GQDs was greatly enhanced by presence -OH and -COOH molecules compared to pure graphite.\(^3\) Furthermore the I\(_D\)/I\(_G\)ratio of GQDs (0.6) was higher when compared to graphene (0.05) suggesting that GQDs had highly disordered structures due to the presence of functional groups at the edges. X-ray photon spectroscopy (XPS) was performed (Fig. S3b-d). The wide spectrum indicates that synthesized GQDs had major peaks corresponding only to Carbon and Oxygen. C 1s spectrum of GQDs was deconvolved into different bands suggesting that the 285, 286.9 and 288.8 eV binding energy values correspond to the sp\(^2\)
carbon chain (C-C), hydroxyl group (C-OH) and carboxyl groups (C=O) respectively. O1s spectrum indicates that the presence of Oxygen in the GQDs was due to the carboxyl groups and hydroxyl groups. These groups were induced due to the refluxing and laser interaction of graphite material with PEG: water solution after ablation.\textsuperscript{3,4}

**pH Stability of GQDs**

To understand the fluorescence emission properties of GQDs (Control GQDs, 20 min and 1 hr), particles were redispersed at a particular concentration in different pH ranging from 1-11 before obtaining fluorescence measurements.

![Figure S4. pH stability study of 20 min (left) and 1 hr sample (right) GQDs](image)

**Quantum Yield of GQDs**

\[
QY = \frac{I}{I_R} \times \frac{A_R}{A} \times \frac{\eta^2}{\eta^2_R} \times QY_R
\]

Quantum yield was estimated for control, 20 min and 1hr GQDs using fluortools software. Where, \(QY_R\) is the quantum yield of the reference compound, \(\eta_R\) is the refractive index of the reference, \(I_R\) is the integrated fluorescence intensity of the reference, \(A_R\) is the absorbance at the excitation wavelength of reference, \(QY\) is the quantum yield of unknown sample, \(\eta\) is the refractive index of the sample, \(I\) is the integrated fluorescence intensity of the sample, \(A\) is the absorbance at the excitation wavelength of the sample.
Figure S5. Fluorescence microscopic images of MCF-7 cells incubated with GQDs for 24 hrs and excited at 365 and 400 nm. The fluorescence images for control, 20 min and 1 hr GQDs are shown. Blue (middle column) and green fluorescence emission (right column) were observed corresponding to excitation at 365 nm and 400 nm respectively. The corresponding bright field images are shown in the left column.
**Figure S6.** Confocal Z-stack images and 3D imaging of GQDs localization in single cell incubated for 48 hours with MCF-7, optically sectioned with a step size of 500 nm (Top).

**Figure S7.** White light image (left), Fluorescence image (middle), and blended fluorescence and white light image of euthanized mouse, subcutaneously implanted with a gel capsule containing GQDs. Imaging was done in trans-illumination mode with excitation wavelength of 530nm using commercial LEDs and the emission filtered at 590nm.
References: