Graphene Quantum Dot Formulation for Cancer Imaging and Redox-Based Drug Delivery

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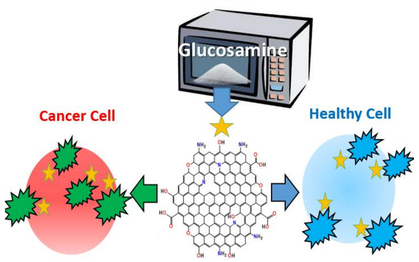
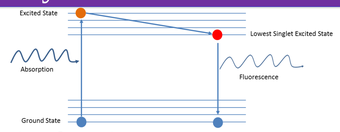
Abstract

Treatment of complex conditions, such as cancer, has been substantially advanced by a field of molecular therapeutics. However, many of these therapies are limited by the dose toxicity and lack the predictive power of tomography-guided approaches. Nanomaterial platforms can address these drawbacks, safely delivering therapeutics, concomitantly imaging their delivery pathways, and presenting sites for targeting agent attachment. Graphene quantum dots (GQDs) possess physical properties that are critical for biomedical applications, including small size (3-5 nm), high quantum yield, low cytotoxicity, and pH-dependent fluorescence emission. Nitrogen doped graphene quantum dots (N-GQDs) are now utilized as a platform for a targeted treatment formulation geared toward cancer therapeutic. Our work utilizes nitrogen-doped GQDs as an emissive platform for covalent attachment of a targeting agent (hyaluronic acid (HA) targeted to the CD44 receptors on several cancer cell types) and oxidative stress-based cancer therapeutic (ferrocene (Fc)). The synthesized multifunctional formulation is characterized and its efficacy evaluated *in vitro*. Elemental mapping indicates that the purified from reactants synthetic product has an average iron content of 0.64 atomic percent, suggesting the successful attachment of the therapeutic, while FFT analysis of TEM images confirms the crystalline structure of the GQDs. Although GQDs alone yield no cytotoxicity as quantified via the MTT assay up to the maximum imaging concentrations of 1 mg/mL, the Fc-HA-GQD formulation exhibits a higher cytotoxic response in the cancer cells (HeLa) targeted by the HA as opposed to healthy ones (HEK-293) that do not overexpress CD44, suggesting cancer-selective targeted efficacy. As Fc induces oxidative stress that is less mitigated in cancer cells, we expect it to also contribute to the observed cancer-selective treatment response. As a result, we propose Fc-HA-GQD formulation as a multifunctional targeted delivery, imaging, and cancer-specific treatment agent further to be studied *in vivo*.

Intro/Theory





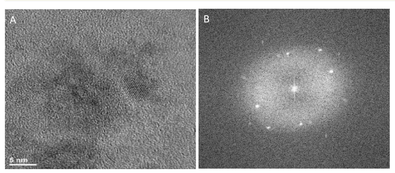
Graphene Quantum Dots:

- Made in a microwave from glucosamine
- Possess graphene ordered structure
- Water soluble and emits fluorescence
- Can be used for:
  -Drug Delivery -Imaging -Sensing

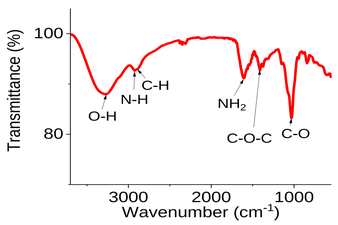
Purpose

The purpose of this experiment is to explore the feasibility of N-GQDs as multifunctional materials for targeted drug delivery and imaging for cancer therapeutics via Fc-HA-GQD Formulation.

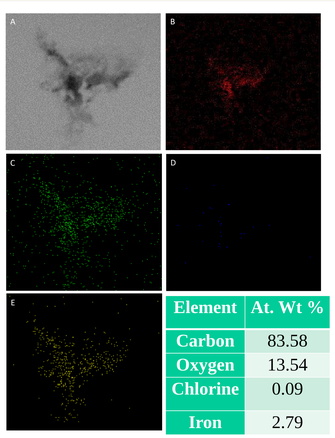
Characterization of Fc-HA-GQD formulation



➢ TEM analysis and FFT of TEM image confirming crystallinity of synthesized sample



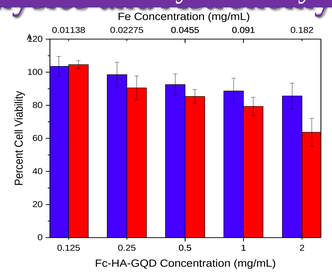
➢ FTIR spectra of Fc-HA-GQD formulation



Element	At. Wt %
Carbon	83.58
Oxygen	13.54
Chlorine	0.09
Iron	2.79

➢ EDX analysis of Fc-HA-GQD sample confirming expected elemental components

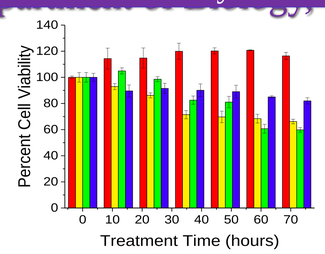
Cytotoxicity and Time Release Study



➢ Fc-HA-GQD formulation shows little toxicity in HEK-293 cells (blue) up to high concentrations: 2 mg/mL!

➢ Fc-HA-GQD formulation shows higher toxicity in HeLa cells (red)

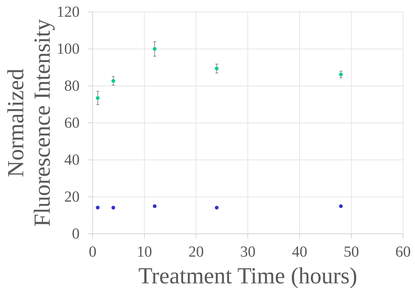
➢ Targeting of cancer cells achieved



➢ Fc-HA-GQD formulation shows little toxicity over time in HEK-293 cells (red), whereas there is higher toxicity in HeLa cells (yellow).

➢ Fc-HA shows little toxicity over time in HeLa cells (blue), whereas there is higher toxicity in HeLa cells (green).

Cellular Internalization



HeLa Cells:

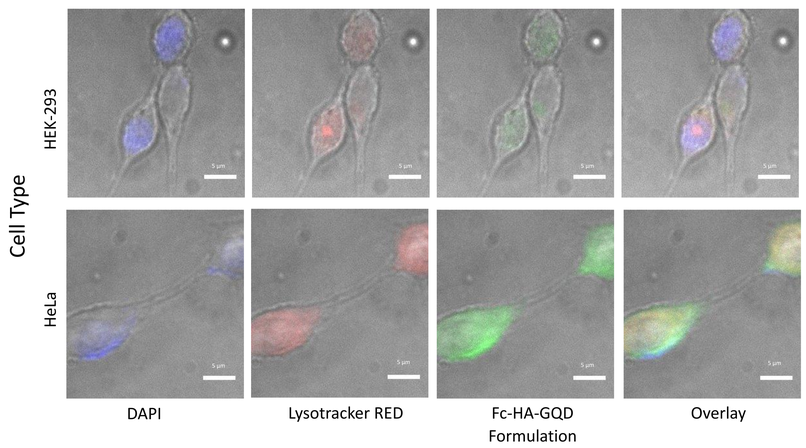
➢ Peak intensity: max internalization at 12 hrs.

➢ Then degradation or excretion is observed

HEK-293 Cells:

➢ Minimal fluorescence suggesting minimal internalization over time

Fluorescence Colocalization Images

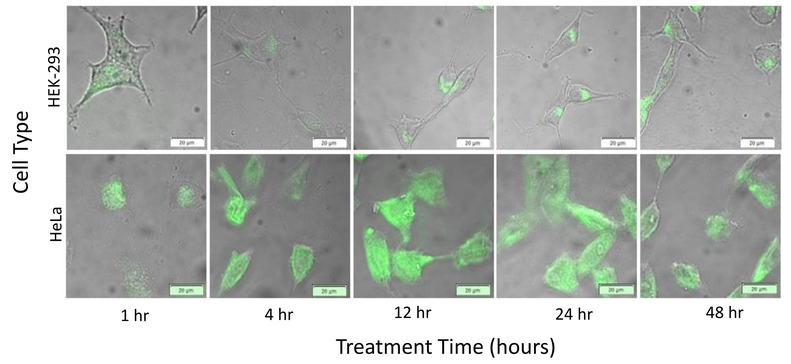


➢ Regular Colocalization performed using dyes:
  ➢DAPI (Blue): Nucleus: excitation: 375 nm emission: 450 nm
  ➢Lysotracker Red: Lysosomes: excitation 540 nm emission 600 nm
  ➢Quantum Dots (Green): excitation 475 nm emission 535 nm

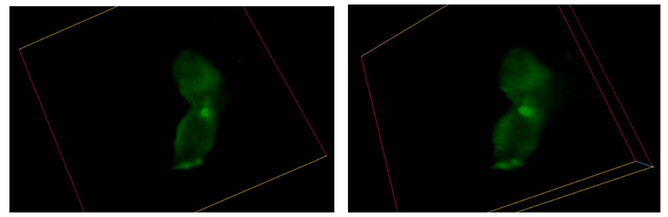
➢ **GQDs localize some with lysosomes, most are in cytoplasm with some in the nucleus**

➢ **GQDs can be utilized for drug delivery**

Imaging



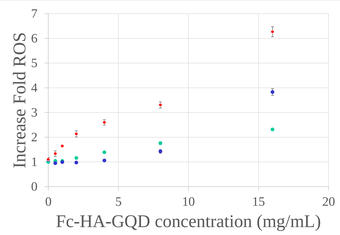
➢ Fluorescence emission of the Fc-HA-GQD formulation in HeLa cells and HEK-293 cells at 1, 4, 12, 24, 48-hour treatment time points. 475 ± 25 nm excitation and 535 ± 20 nm emission filters used. Scale bar = 20 µm.



➢ 3D images of Fc-HA-GQDs in HeLa cells built by z-stack accumulation of confocal microscopy images of GQD emission at different planes within the cells

➢ Collected with 480 nm excitation and 535 nm emission.

Oxidative Stress



➢ DCFH-DA assay of Fc-HA-GQD formulation (red) as compared to ferrocene (blue) and N-GQDs (green) in HeLa cells.

Summary

The Fc-HA-GQD formulation can be used as imaging agent and drug delivery vehicle for cancer therapeutics.

➢ Targeting agent (hyaluronic acid) attached to N-GQDs allows for targeting of CD44 receptors more prominent in cancer cells

➢ Treatment agent (ferrocene) attached to formulation allows for treatment of cancer cells

➢ Fluorescence in the VIS: potential for *in vitro* imaging.

➢ Treatment formulation is non-toxic at high concentration of 2mg/mL in non-cancer cells, but more toxic in cancer cells at same concentrations.

➢ Best internalization occurs at 12h post transfection. Treatment formulation localizes in the cytoplasm, some in the nucleus: potential for drug or gene delivery