Silence is not golden, silence is carbon-based: Graphene Quantum Dots in Gene Silencing Therapy.

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Abstract

While small interfering RNA (siRNA) technology has become a powerful tool in cancer-specific gene therapy, its translation to the clinic is still hampered by the inability of cell transfection by the siRNA alone, poor siRNA stability in blood, and the lack of delivery tracking capabilities. Recently, graphene quantum dots (GQDs) have emerged as a novel platform allowing targeted drug delivery and fluorescence image-tracking in the visible and near-infrared. These capabilities can aid in overcoming primary obstacles to siRNA therapeutics. Here, for the first time, we utilize biocompatible nitrogen and neodymium-doped graphene quantum dots (NGQDs and Nd-NGQDs) for the delivery of Kirsten rat sarcoma virus (KRAS) and epidermal growth factor receptor (EGFR) siRNA effective against a variety of cancer types. GQDs as a delivery platform facilitate successful gene transfection into HeLa cells confirmed by confocal fluorescence microscopy at biocompatible GQD concentrations of 375 μg/mL. While the NGQD platform provides visible fluorescence tracking, Nd doping enables deeper tissue near-infrared fluorescence imaging suitable for both *in vitro* and *in vivo* applications. The therapeutic efficacy of the GQDs/siRNA complex is verified by successful protein knockdown in HeLa cells down to 31-45% comparable with conventional Lipofectamine-mediated delivery. This demonstrates the promising potential of GQDs for the non-toxic delivery of siRNA and genes in general, complemented by multiwavelength image-tracking.

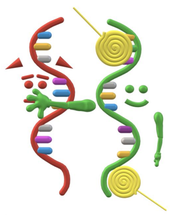
Introduction

Problem: **Cancer** - affects one million people yearly in the USA
- is a genetic disease caused by **mutations in DNA**

Frequently mutated genes: - Kirsten rat sarcoma virus (KRAS)
- Epidermal growth factor receptor (EGFR)

Main issue in chemotherapy against EGFR and KRAS:
drug resistance

Solution: Gene Silencing Therapy →



- **FDA approved**
- **Provide high specificity**
- **Based on the delivery of silencing RNA (siRNA)**

Problem: delivery of siRNA

- **Inability of siRNA to transfect cells on its own**
- **Rapid enzymatic degradation in bodily fluids and tissues**
- **Rapid renal clearance of siRNA**
- **Activation of the immune system**

Solution: Graphene quantum dots (GQDs) as a platform for siRNA delivery

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

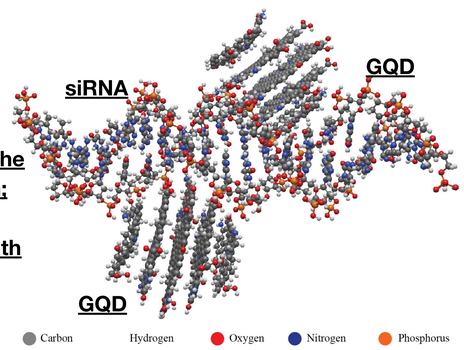
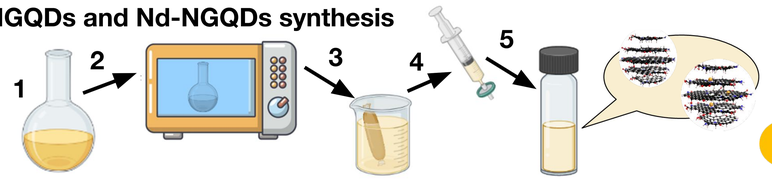


exhibit fluorescence in the
- **visible spectral region;**
- **near-infrared spectral region after doping with neodymium (Nd).**

Synthesis of NGQDs and Nd-NGQDs

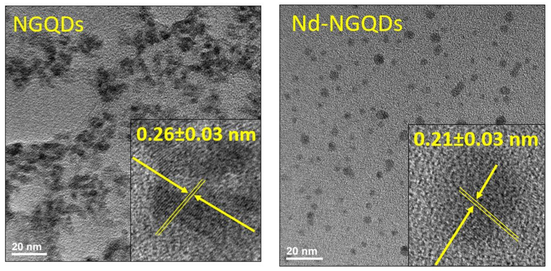
NGQDs and Nd-NGQDs synthesis



NGQDs – no metal dopant
Nd-NGQDs - Nd dopant

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

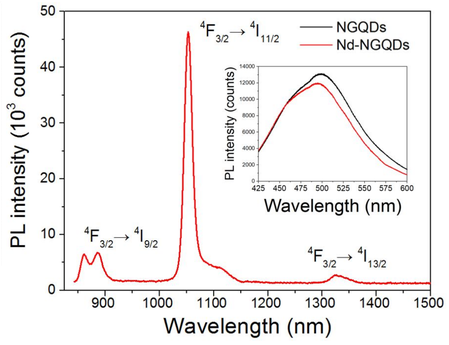
Characterization of NGQDs and Nd-NGQDs



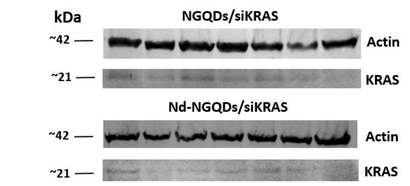
Size 4.0±0.6 nm

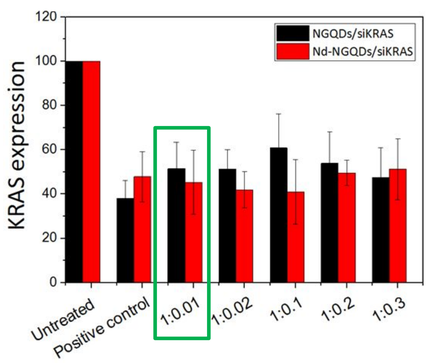
Size 3.8±0.9 nm

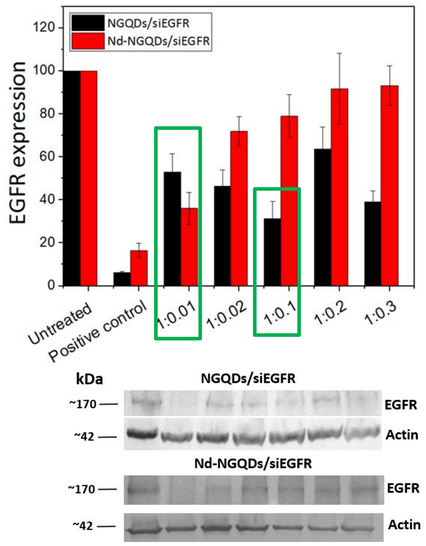
Atomic percentage of elements:
C 76.46 % N 5.41 %
O 17.11 % Nd 1.01 %



Cancer gene silencing: western blot assay



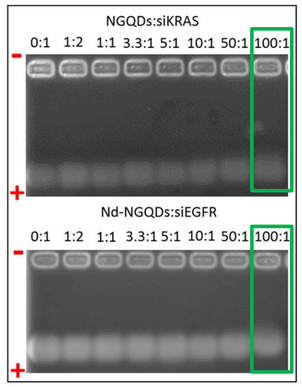


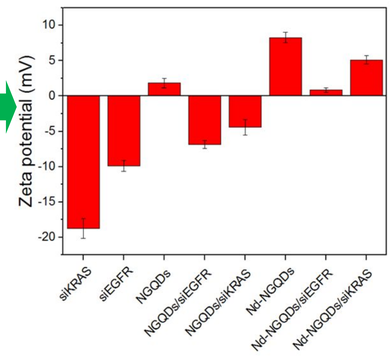


Conclusion

- **NGQDs and Nd-NGQDs successfully attach siRNA for their delivery, the optimal weight ratio is 100/1;**
- **GQDs/siRNA complexes are biocompatible at 375 μg/mL concentration;**
- **NGQDs and Nd-NGQDs deliver siRNA tagged with ROX dye into cancer (HeLa) cells;**
- **Nd doping in Nd-NGQDs enables deeper tissue near-infrared fluorescence imaging suitable for both *in vitro* and *in vivo* applications;**
- **GQDs/siRNA complexes facilitate inhibition of EGFR and KRAS protein expression down to 31-45%;**
- **GQDs/siEGFR and GQDs/siKRAS complexes can address the critical needs of treating cancer caused by mutations in EGFR and KRAS genes.**

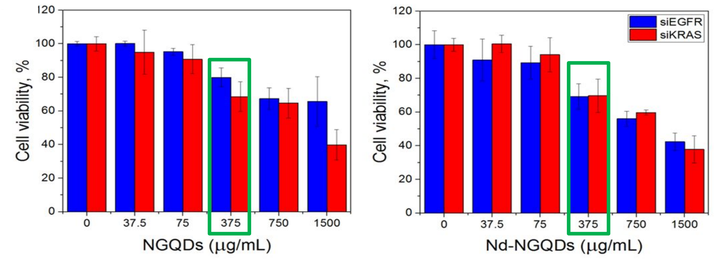
GQDs/siRNA complexation





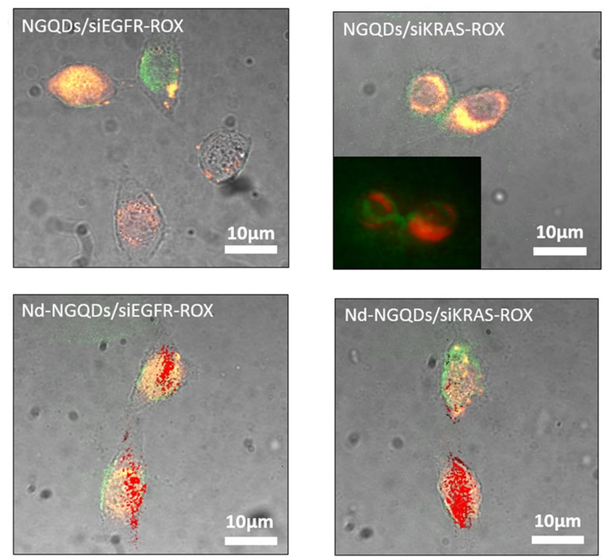
The optimal weight ratio of GQDs and siRNA for their complexation is 100:1

Cell viability



70-80% cell viability at 375 μg/mL

Fluorescence images of GQDs/siRNA complexes in HeLa cells



GQDs/siRNA complexes successfully transfected HeLa cells

With the increasing prevalence of drug-resistant cancers, novel treatments have been developed, and gene silencing therapy has become one of them. This therapeutics is based on the introduction of silencing genes, aka treatment, into cancer cells resulting in their “repair”. However, this technology is hampered by the inability of the silencing genes to be delivered inside cells on their own. Here, we developed tiny biocompatible carbon dots that facilitate gene delivery into cells. Unlike other gene delivery carriers, these carbon dots have the ability to emit light that can be visible through the skin meaning we can track the treatment within an organism and deliver it more precisely. This way, tiny carbon dots can address the critical needs of treating drug-resistant cancers.