Graphene Quantum Dots as Non-Viral Delivery of CRISPR Cas9 RNP for Cancer Therapy

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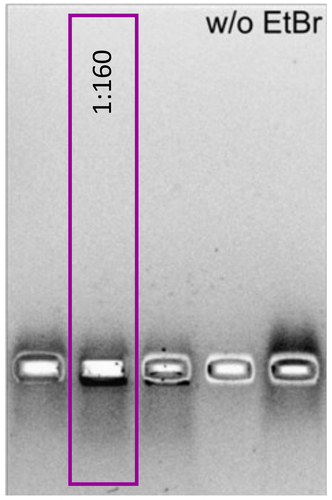
Abstract

CRISPR Cas9 is a programmable single guided RNA (sgRNA) ribonucleic protein (RNP) that has demonstrated their ease and practical use as a gene editing tool for *in vitro* and *ex vivo* applications. For *in vivo* applications of the Cas9 RNP, physiological barriers must be overcome and gene editing to occur transiently, demonstrating the need to develop biocompatible imaging agents to protect and locate Cas9 RNP *in vivo*. Graphene quantum dots (GQDs) are biocompatible carbon-based nanomaterials that have served as delivery and imaging agents for drug and gene medicine due to their ease in synthesis and repertoire of complexation capabilities arising from the choice of precursor materials. In this work, we have synthesized visible and near infrared emitting GQDs with glucosamine HCl and polyethylenimine (PEI) using a bottom-up approach to use them as non-viral delivery vehicles for the Cas9 RNP. PEI increases the net positive charge of GQDs allowing their electrostatic complexation with the net negatively charged RNP. We further demonstrate their complexation with gel retardation assay and TEM. The GQDs+PEI+RNP *in vitro* editing capability is shown by targeting the TP53 414delC frameshift mutation locus present in PC3 cancer cell line for prostate cancer. This form of editing serves as a guide for future cancer therapy using GQDs as non-viral delivery of Cas9 RNP to mutant TP53 genes overexpressed in about 50% of cancers.

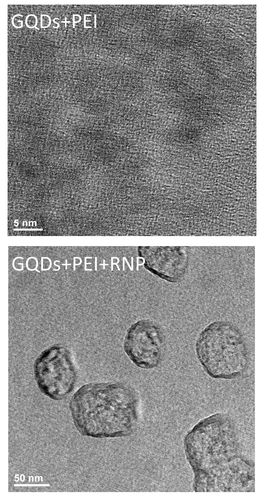
Material Characterization

Gel Retardation Assay



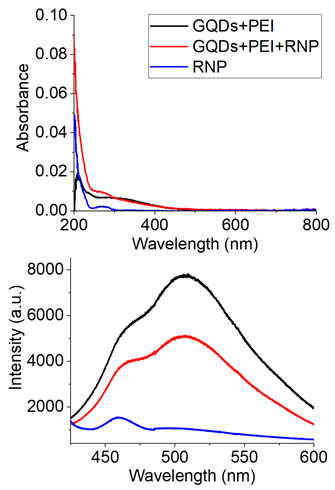
- Optimal ratio of RNP and GQDs+PEI for their electrostatic complexation

TEM



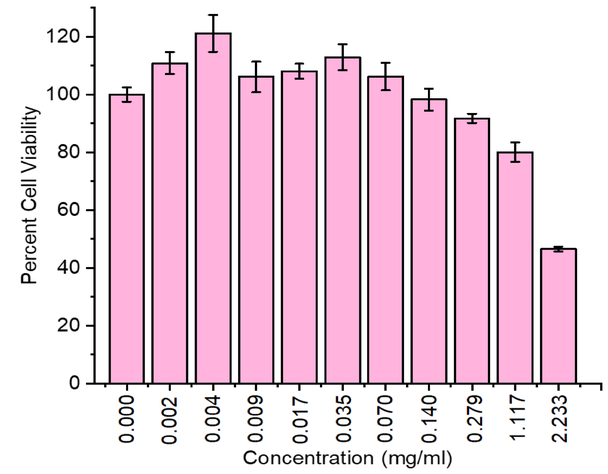
- Morphological change and size increase

Absorption/Fluorescence



- Fluorescence decrease of GQDs+PEI when complexated with RNP

Cell Viability



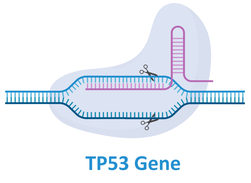
- Cell Titer-Glo luminescent cell viability assay.
- 80% cell viability of HeK293 (healthy cells) for concentrations up to 1 mg/ml of GQDs+PEI.

Overview

TP53 gene, the guardian of our genome

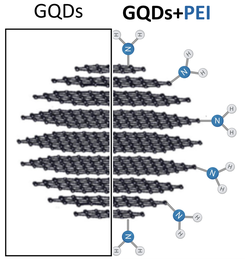
- Gene responsible for transcriptional activation of apoptosis, cell cycle arrest, DNA repair, and senescence related genes [1].
- Mutations on the TP53 gene lead to impairment or loss of p53 protein function.
- TP53 414delC mutation in prostrate cancer (PC-3) cells.

CRISPR Cas9 RNP as a gene editing tool



- CRISPR is an adaptive immune system for prokaryotes to fight against viruses.
- CRISPR Cas9 is the simplest type of CRISPR requiring a single protein and a **programmable synthetic RNA** to target genes [2]; in this case the TP53 gene.

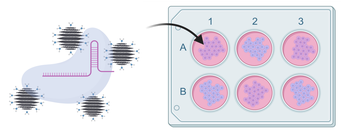
Graphene Quantum Dots (GQDs) as delivery vehicles for CRISPR Cas9



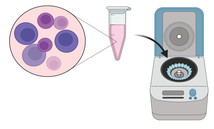
- GQDs are derivatives of graphene
- Synthesized using the bottom up approach with the use of a microwave oven
- Precursor materials: glucosamine HCl and **PEI**
- Electrostatic complexation of GQDs+**PEI (positively charged)** and CRISPR Cas9 RNP **(negatively charged)**.

In Vitro Mismatch Cleavage Assay

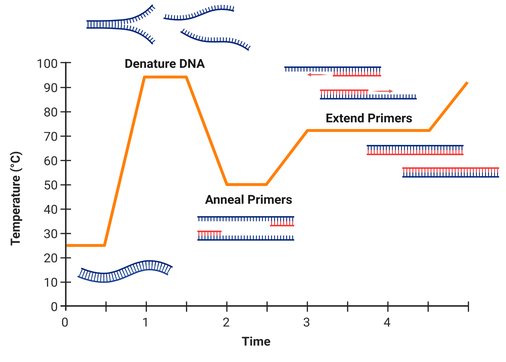
1 Transfection onto PC3 cells



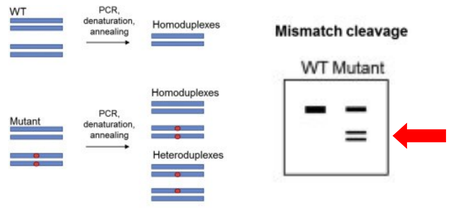
2 Genomic Extraction



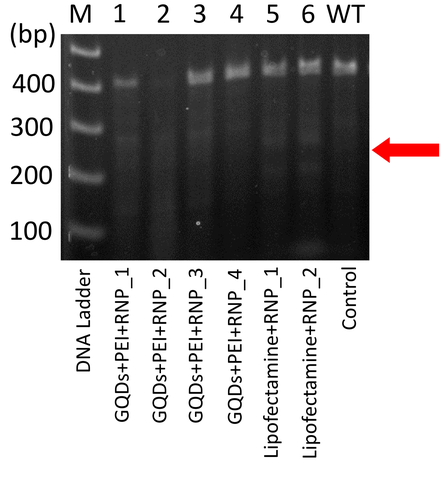
3 Polymerase Chain Reaction (PCR)



4 Mismatch Cleavage Assay



5 Gel Electrophoresis



Conclusion and Outlook

- Optimal complexation ratio of GQDs+PEI and RNP is 1:160.
- From the TEM, there are morphological changes and size increase of GQDs+PEI after being complexated with RNP.
- Visible fluorescence present in GQDs+PEI for fluorescence applications.
- In vitro mismatch cleavage assay demonstrates editing by GQDs+PEI+RNP.
- Use of restriction enzyme to validate homology directed repair with a repair template of TP53 gene complexated with GQDs+PEI+RNP.
- Confocal cell imaging using 1 mg/ml of NGQDs+PEI with a CRISPR Cas9 RNP **GFP** terminated tag.

Acknowledgements

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References

[1] Batır, M. B., Şahin, E., & Çam, F. S. (2019). Evaluation of the CRISPR/Cas9 directed mutant TP53 gene repairing effect in human prostate cancer cell line PC-3. *Molecular Biology Reports*, *46*(6), 6471-6484.

[2] Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., & Charpentier, E. (2012). A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. *science*, *337*(6096), 816-821.

Our genetic story is written in the form of DNA. However, damages to DNA can occur and they are like typos in a story. These damages lead to mutations that form cancers. Fortunately, we have our own molecular guardian known as TP53 protein that recognizes cells with DNA damage and protects us. However, the very genetic makeup of these guardians can also be damaged, and it has been shown that over 50% of cancers already possess this damage. Finding alterations within a gene on its base pairs have become equal to finding a needle in a haystack from the 3 billion base pairs in the human genome. With the developments of gene editing technology, CRISPR, an ancient immune system from bacteria that has been used to fight viruses till today, has been repurposed to find and edit genes of interest; similar to a Word document's find and replace function. To deliver CRISPR into our body to fix our damaged guardian for cancer treatment, we have synthesized graphene quantum dots, carbon-based nanomaterials made from biodegradable sources, to not only protect CRISPR from degradation in its perilous journey into our body, but also track their location within cancer cells and our body via their glow.