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The endocytotic fate of a mesoporous silica nanoparticle supported lipid bylayer CRISPR delivery vehicle

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What is CRISPR?
Clustered, Regularly Interspaced, Short Palindromic Repeat
- Bacterial derived DNA editing technology that allows segments of DNA to be cut
- Two part system that includes a customized guide RNA, and an endonuclease Cas9 which cleaves the DNA
- CRISPR components are generally packaged in plasmids, adenovirus associated viruses or ribonucleic proteins
- CRISPR research has skyrocketed since its' gene editing applications were suggested by Doudna and Charpentier

Current Limitations of CRISPR:
- CRISPR technology can't be directly ingested or injected so delivery poses a complex engineering problem
- Very few clinical application studies conducted in humans
- High incidences of off target gene editing effects can't be ignored

Vehicle Internalization
Our CRISPR delivery vehicle is internalized in Hela and A549 cells via clathrin and actin mediated endocytosis. To elucidate these internalization mechanisms for our vehicle, flow cytometry was used to analyze the amount of fluorescence in the cell samples. This is correlated to the amount of particles that were internalized under aberrant genetic sequences. In order to overcome drawbacks associated with AAV-CRISPR, the establishment of an effective non-viral CRISPR delivery vehicle has become a primary goal for nanomaterial scientists. Herein, we introduce the first monolysed lipid-coated mesoporous silica nanoparticle (LC-MSN) delivery vehicle that enables loading of CRISPR components (11% wt RNP) with efficient release within cancer cells (~70%). With a low toxicity and a clathrin-mediated endocytotic internalization pathway, the gene editing efficiency in a reporter cell line was up to 10% using ribonucloprotein (RNP) complex (Cas9/gRNA) and a CRISPR plasmid. The structural and chemical versatility of the silica core and the lipid coat along with their biocompatibility make LC-MSN a promising vector towards safe CRISPR components delivery and enhanced gene editing.

Benefits of Using Silica Based Nanoparticle:
- Enhanced biocompatibility
- Fluorescent conjugation allows for in vitro tracking
- Stability of CRISPR components is increased
- Ability to precisely engineer tailored aspects of vehicle

Clathrin Colocalization
To confirm that our vehicle is indeed internalized via clathrin mediated endocytosis, clathrin antibodies were used. A primary heavy chain mouse DyLight 488 antibody. The second fluorescent antibody was used to track the location of clathrin in cells using confocal microscopy and Hela cells are those that show the most effects from chemical inhibition. Confocal microscopy was used to qualitatively ensure that the trends seen in flow cytometry can be reproduced visually.

Abstract
The CRISPR vehicle is composed of a mesoporous silica nanoparticle (MSN) core. The core's porosity is tailored to the CRISPR cargo specifications. The core is encapsulated in a lipid bilayer to enhance stability and specific interactions within biological environments. The CRISPR delivery vehicle was assessed by utilizing reporter versions of the A549 and Hela cell lines. These reporter cell lines were genetically changed to express a red fluorescent protein. We target this gene and induce a double stranded break, which causes the cell to now express a green fluorescent protein.

Gene Editing Results
The gene editing abilities of our CRISPR delivery vehicle were assessed by utilizing reporter versions of the A549 and Hela cell lines. These reporter cell lines were genetically changed to express a red fluorescent protein. We target this gene and induce a double stranded break, which causes the cell to now express a green fluorescent protein.

Vehicle Design
The CRISPR vehicle is composed of a mesoporous silica nanoparticle (MSN) core. The core's porosity is tailored to the CRISPR cargo specifications. The core is encapsulated in a lipid bilayer to enhance stability and specific interactions within biological environments.