Optimizing Gene Silencing Using siRNA-Loaded Porous Silicon Nanoparticles

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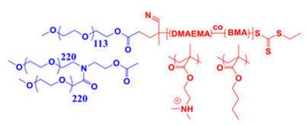
Introduction

Short-interfering RNA (siRNA)
- Suppresses genes by interfering with messenger RNA (mRNA)
- Rapidly cleared and degraded
- Unable to penetrate cells or escape endolysosomal pathway

Porous Silicon Nanoparticles (PSNPs)
- Large surface area-to-volume ratio for packing siRNA
- Biodegradable and low toxicity



Poly[(ethylene glycol)- block -(2-(dimethylamino)ethyl methacrylate- co- butyl methacrylate)] (PEG-DB) polymer
- Can be used to coat the surface of PSNPs
- Hydrophilic PEG facilitates circulation
- DB disrupts acidic endosomes to releases siRNA into the cytoplasm

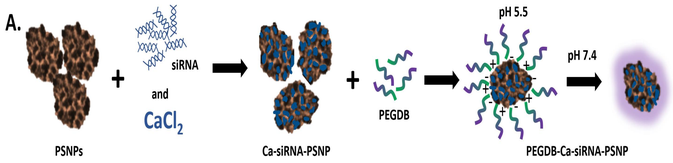
Hypothesis: Loading siRNA into porous silicon nanoparticles coated with PEG-DB polymer will demonstrate improved knockdown of target genes

Motivation and Methods

Current methods for producing gene-silencing pSiNPs require heavy chemical complexation (2). Our aim was to produce these same, if not more efficient, gene-silencing vehicles but make them chemically simplistic, allowing for efficient mass generation of these specifically modified particles.

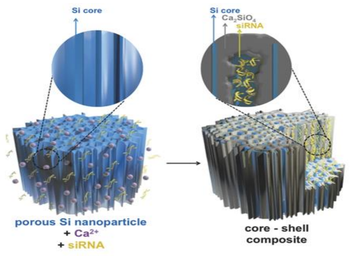
Objectives:

1. Optimize siRNA loading efficiency within PSNPs.
2. Ensure elongated stability of pSiNPs for sustained drug release
3. Optimally coat PEG- DB onto the particles



Formulation of the completed PEG-DB-Ca-siRNA-PSNP complex, created from the electrostatic attraction between the negative surface charge of PSNPs, the negatively charged siRNA molecule and the divalent cation calcium from the salt solution. These steps are followed by a thorough PEG-DB coat of the particles.

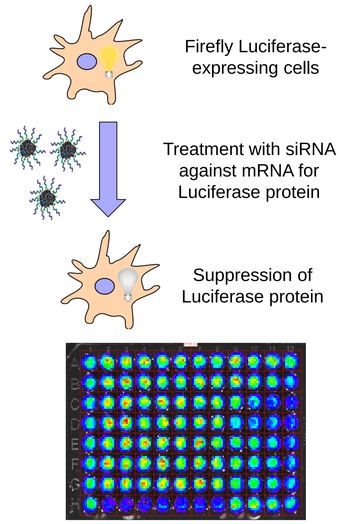
(Divalent) Calcium ions
Forms electrostatic linkage between negative surface charge of pSi and siRNA



Methods

In Vitro Silencing Assay:

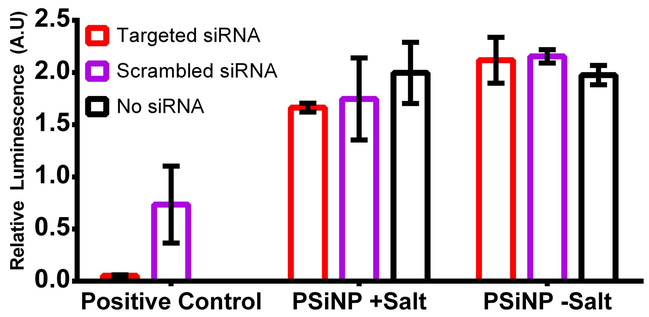
1. Firefly Luciferase expressing MDA MB 231s breast cancer cells were seeded into 96 well plates.
2. Treatment and control groups were loaded into 1.5 mL DNA LoBind tubes.
3. To each tube, 0.2 mg/mL PSNPs, 5 uM siRNA against Firefly Luciferase or a scrambled control, 3M Calcium salt solution and sterile, molecular biology grade (MBio) water was added.
4. After overnight incubation, samples were washed with MBio Water, 1.5 mg/mL PegDB solution, 5% Ethanol, and Opti-MEM, followed by a centrifugation step.
5. Appropriate treatment was added to each cell well.
6. After overnight incubation, the Luciferin substrate was added and measurements of luminescent knockdown were observed at 24- and 48-hour time points.



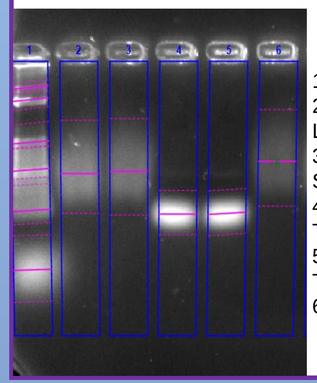
Representative image of cells with or without treatment

Results

48 hr In Vitro Luciferase Knockdown



The results from the PSiNP-siRNA gene knockdown assay demonstrate that there does not appear to be significant differences in knockdown between targeted and scrambled siRNA delivered by the porous silicon nanoparticles.

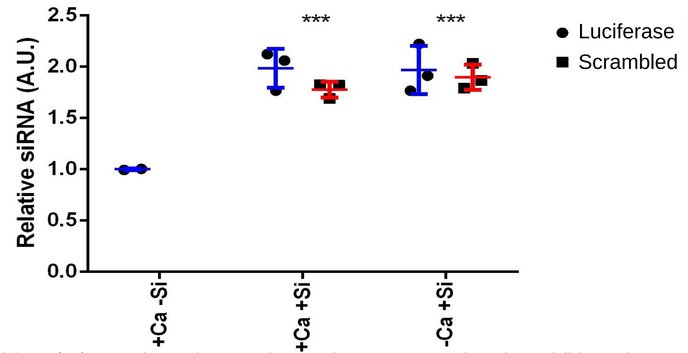


1. Ladder
2. PSNP Treatment with Luciferase (LZ) siRNA
3. PSNP Treatment with Scrambled siRNA
4. PSNP Luciferase Treatment (No Salt)
5. PSNP Scrambled Treatment (No Salt)
6. No Silicon

Gel electrophoresis of siRNA remaining in the supernatant demonstrates that the groups without salt show the same siRNA content as those with calcium, even though the brightness is dimmer and spread out over a vast area.
Interestingly, the lowest content is demonstrated in the samples without PSiNPs, potentially signaling at calcium-siRNA sequestration.

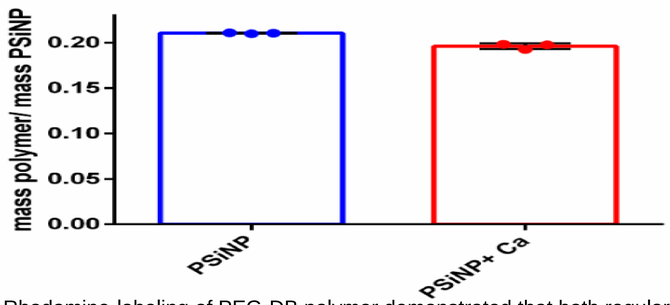
Results

siRNA Remaining in Supernatant



This gel electrophoresis experiment demonstrates that the addition of calcium to porous silicon nanoparticles minimally affects siRNA loading levels. Calcium may have negatively affected the ability of the siRNA to remain on the particle.

PegDB coating of PSiNPs



Rhodamine-labeling of PEG-DB polymer demonstrated that both regular PSiNPs and PSiNPs + calcium groups can be coated successfully with polymer with little effect from the addition of the salt.

Conclusions & Future Work

From our luciferase assay results, greater luminescence and lower knockdown was shown by siRNA loaded into porous silicon nanocomposites compared to PEG-DB.

We theorize that too little porous silicon and too much salt is being utilized. On a nanoscale, porous silicon is encapsulating enough siRNA but the calcium concentration is too high, causing the calcium to compete more;' with the porous silicon to bind siRNA.

Future work will include reevaluation of concentrations used in the protocol to achieve optimal loading.

Acknowledgements & References

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