Nanoparticle Development For siRNA Delivery To Treat Osteoarthritis (OA)

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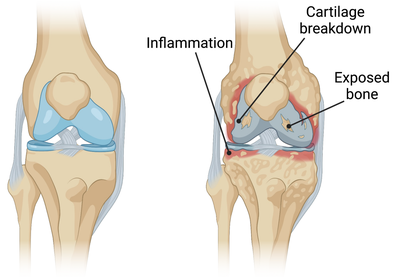
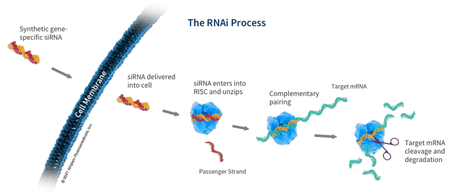
Background

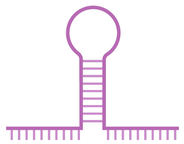
- Osteoarthritis (OA) is a degenerative joint disease that affects over 32 million U.S. adults
- OA causes painful cartilage breakdown in joints and currently has no cure
- The gene MMP13 plays a key role in cartilage degradation





- Small interfering RNA (siRNA) against MMP13 silences the gene by cleavage of mRNA, reducing cartilage breakdown and inhibiting disease progression

- siRNA delivery is limited *in vivo* due to endosomal escape issues and kidney clearance
- Encapsulating siRNA in polymeric nanoparticles (si-NPs) can help overcome delivery challenges



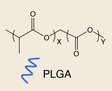
Objective

To develop a polymeric nanoparticle formulation to optimize siRNA delivery

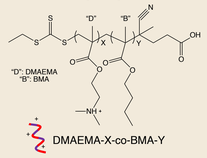
Nanoparticle Composition

si-NP Components:

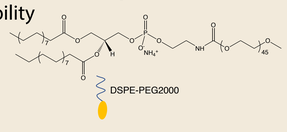
(1) **Poly(lactic-*co*-glycolic acid (PLGA)** for stability



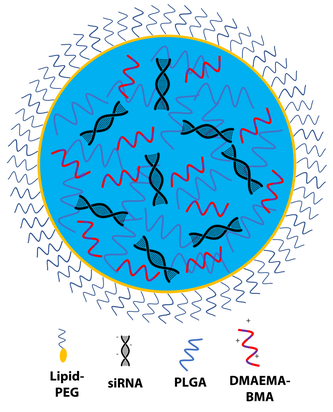
(2) **50:50 DP 100 DMAEMA-*co*-BMA (DB)** for endosome escape



(3) **Surfactant- DSPE-PEG (Lipid-PEG)** for biocompatibility



(4) **siRNA** for gene silencing



siRNA Used to Test for Gene Silencing:

Luciferase (Luc)- fluorescent firefly gene

Scrambled (Scr)- control

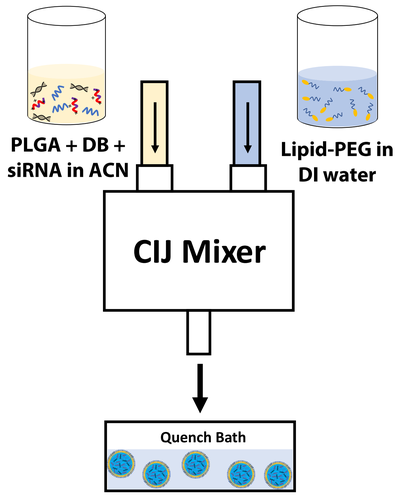
Nanoparticle Preparation

Formulation Optimization Parameters:

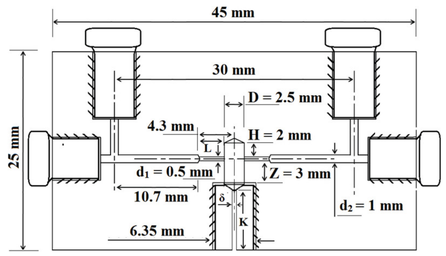
(1) **PLGA + DB concentration** 1-6 mg/mL

(2) **Percent DB** 25, 50, and 75%

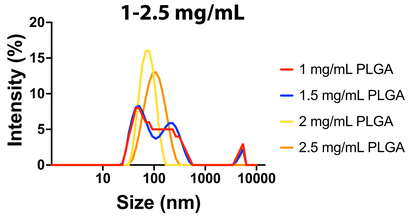
(3) **Amines to Phosphates (N:P) Ratio** 5 and 10

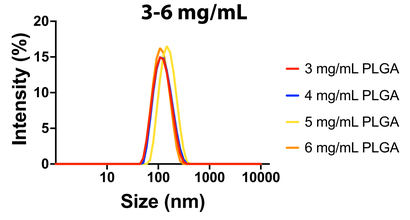


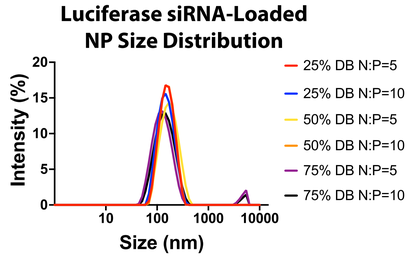
CIJ Mixer Internal Design:

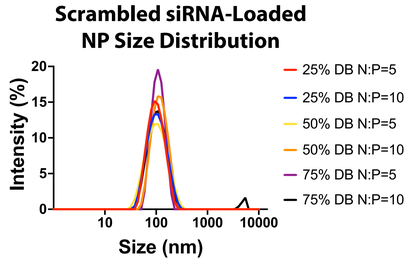


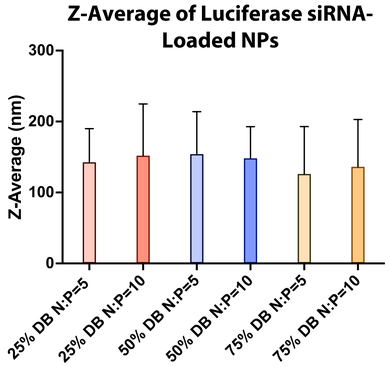
Results- Dynamic Light Scattering (DLS)





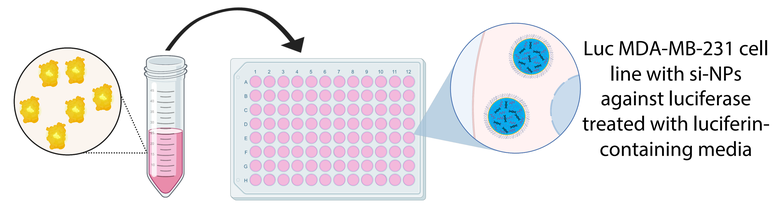


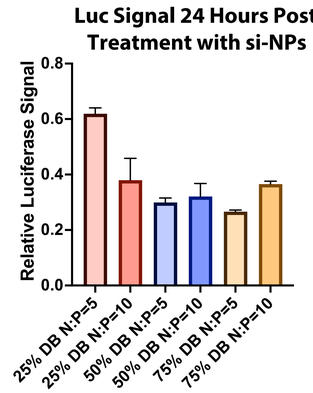


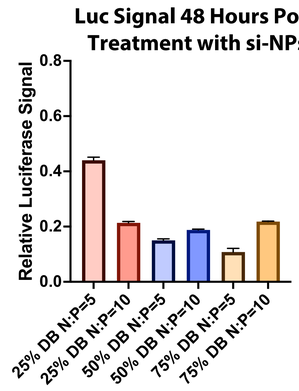


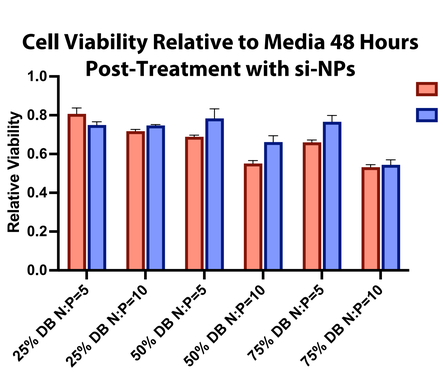
si-NPs with **3 mg/mL** DB and PLGA in the solvent stream and a core concentration of **25-50% DB** were the most uniform.

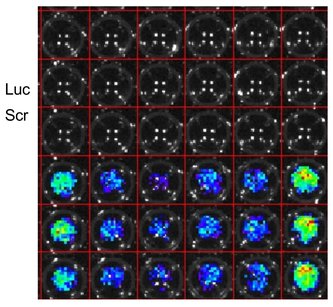
Results- *In Vitro* Luciferase Knockdown/Cell Viability











Luciferase knockdown in si-NP containing MDA-MB-231 cells 48 hours after treatment with luciferin. Top three rows contain luc siRNA, bottom rows scr siRNA.

Luciferase si-NPs with a core composition of **50% DB** and an **N:P ratio of 5** were most uniform, viable, and effective at luciferase knockdown.

Future Work

Main Challenge During Formulation

- Aggregation of si-NPs

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- Test more si-NP formulations
- Vary si-NP dose *in vitro*
- Quantify siRNA loading
- Test novel DMA surfactants

Acknowledgements

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- Figures created with BioRender.com

References

1. Alnylam, 2023.
2. Gulati et al., 2022.
3. Han et al. National Library of Medicine, 2012