Evaluating lipid-based nanoparticle delivery of dsDNA agonists for activation of cGAS-STING pathway

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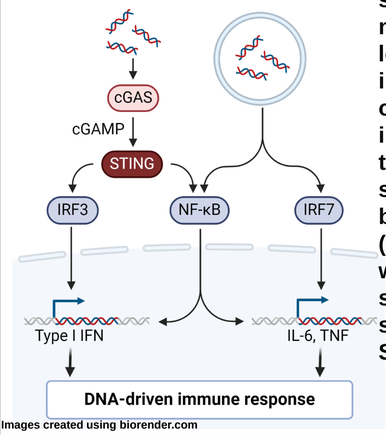
INTRODUCTION

In eukaryotes, DNA is primarily found in the nucleus and mitochondria. The presence of cytosolic DNA is typically a sign of internal dysregulation or presence of pathogens.[1] The cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) pathway is responsible for detecting cytosolic DNA and eliciting an innate immune response to protect the cell.[2] Activation of this pathway plays an important role in cancer immunotherapies.[3] To activate this pathway, exogenous double-stranded DNA (dsDNA) must be delivered into the cytosol of a cell to stimulate cGAS. When cGAS detects dsDNA, cGAMP is produced, which activates STING. In response, type I interferons are produced, eliciting an immune response, such as dendritic cell maturation, which can lead to more adoptive immune cell activity, and overcoming an immunosuppressive tumor environment. This study looks to use lipid-based nanoparticles (LBNPs) encapsulated with a dsDNA agonist to safely and effectively stimulate the cGAS-STING pathway.

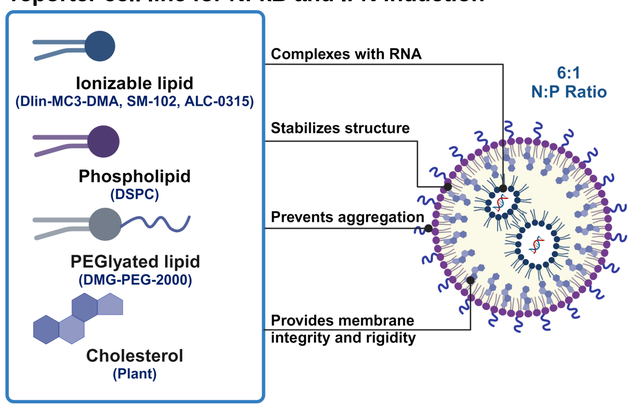


Images created using biorender.com

References:
[1] Li, A. J Hematol Oncol (2019).
[2] Oh, J. Royal Society of Chemistry (2020).
[3] Motwani, M. Nat Rev Genet (2019).

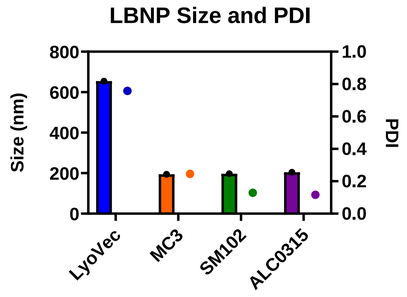
METHODS

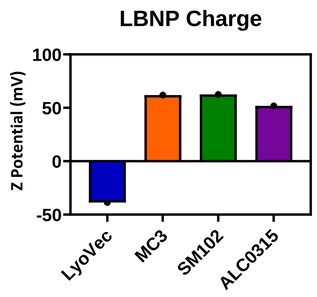
- LNPs were synthesized via pipette mixing and each encapsulates G3YSD, a Y-form dsDNA agonist. Encapsulation efficiency assessed by Quant-iT RiboGreen RNA Assay
- Particle size and charge determined by dynamic light scattering (DLS)
- cGAS-STING pathway activation assessed in THP-1 reporter cell line for NFkB and IFN induction

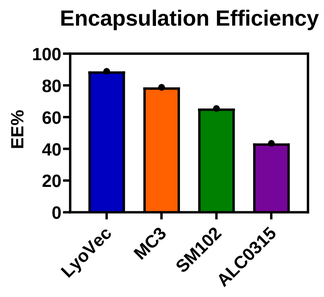


RESULTS

1. All LBNP formulations formed small, monodisperse particles that successfully encapsulated G3YSD.

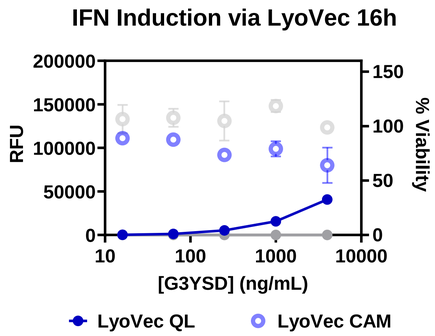


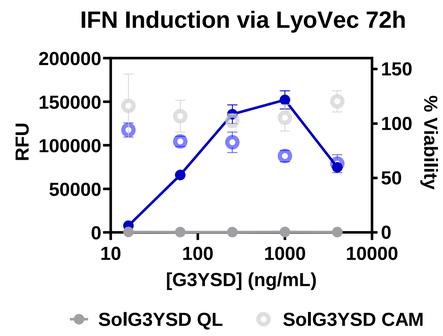


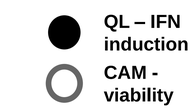


Figures (above): LBNP diameter, PDI, and charge determined via dynamic light scattering. Encapsulation efficiency determined via Quant-iT RiboGreen RNA assay.

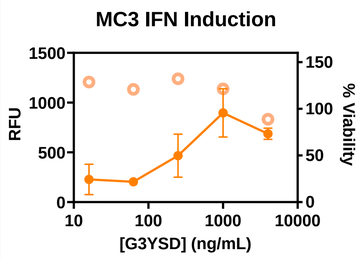
2. LyoVec-G3YSD stimulated the most interferon production without compromising viability.

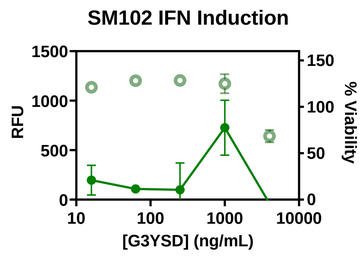


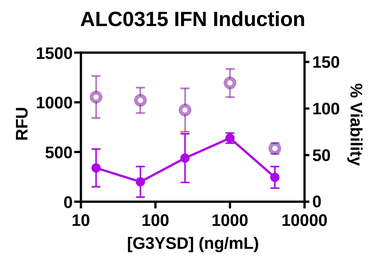




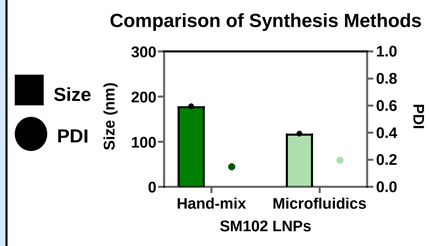
Figures (left): IFN induction of LyoVec and free G3YSD after 16 and 72 hours of incubation. Figures (below): IFN induction via LNP formulations after 72 hours.

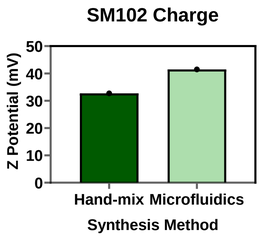


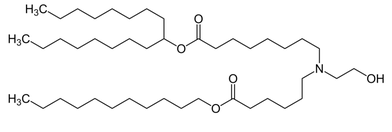




3. LBNP production can be scaled up using a microfluidic system.





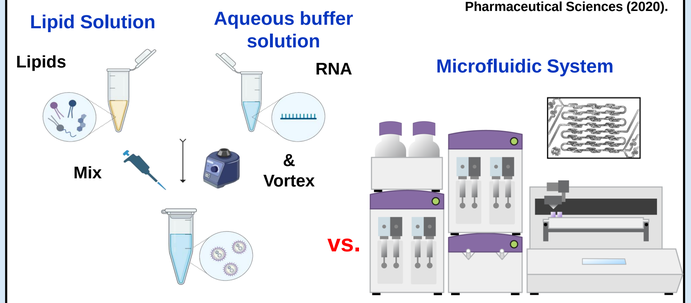


Figures (left): Diameter, PDI, and charge of SM102 LNPs synthesized by hand-mixing or microfluidics.
Figure (above): Chemical structure of SM102 lipid.

DISCUSSION

- Despite having a large size and a negative charge, LyoVec encapsulated the most G3YSD.
- LyoVec performed the best in vitro, successfully activating the cGAS-STING pathway after 72 hours of incubation, likely due to charge interactions with the dsDNA.[4] Higher concentrations for each LNP affected the viability of the cells.
- LNPs may require higher dosage but could become trapped in an endosome.
- LBNPs can be synthesized by hand-mixing or via microfluidic-based chaotic mixing.

Reference:
[4] Rahimmanesh, I. Research in Pharmaceutical Sciences (2020).



WHY IS THIS ALL IMPORTANT?

- cGAS-STING activation can stimulate innate immune system
- Can be used as a cancer vaccine adjuvant
- Investigate LNP-cell interaction and DNA internalization mechanisms

CONCLUSIONS

- Hand-mixing MC3, SM-102, and ALC-0315 formed small, monodisperse, positively charged nanoparticles.
- Each LBNP formulation encapsulated G3YSD, with LyoVec and MC3 being the most successful.
- LyoVec stimulated the most interferon production, suggesting cGAS-STING activation.
- There is a threshold concentration for maximum interferon stimulation.
- High concentrations of G3YSD impacted viability.
- LBNPs can be synthesized on the ANP for high throughput.

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