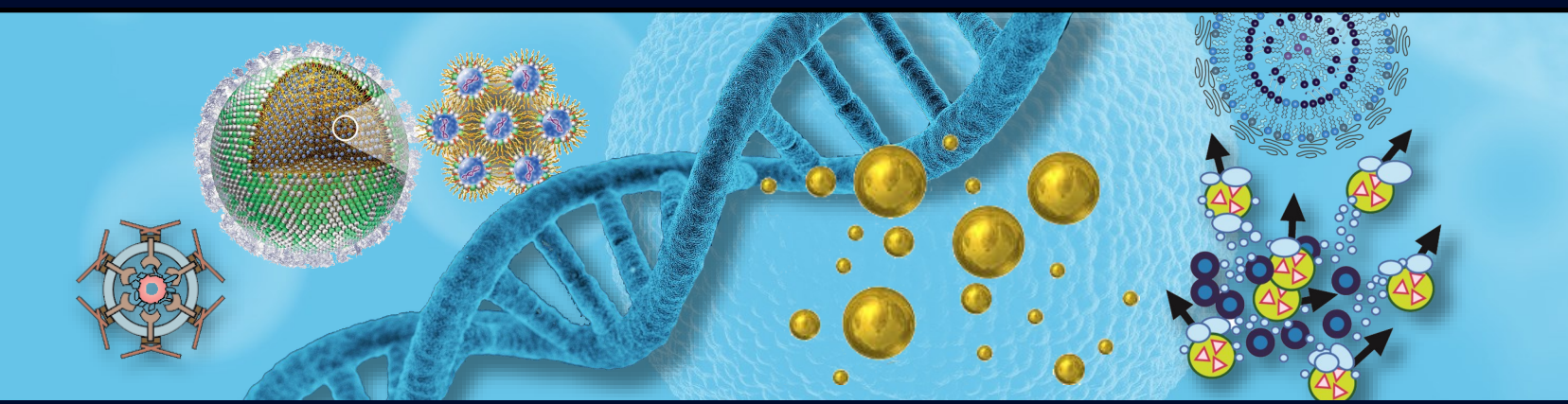
ABSTRACTS

Non-Adjudicated Poster Abstract Compendium



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Abstract #38

Development of an ROS-sensitive and ROS-scavenging nanoparticle system for anti-inflammatory therapies

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Background

Inflammation is a normal biological response involving molecules such as reactive oxygen species (ROS). Due to their high reactivity, ROS levels are generally well regulated by our endogenous antioxidants and enzymes. Unfortunately, diseases may cause dysfunctional ROS regulation resulting in tissue damage and persisting inflammation. This occurs in chronic inflammatory diseases which often present with abnormally elevated ROS levels. Despite the close relationship between ROS and inflammation, no currently used therapeutic approach modulates ROS levels for anti-inflammatory purposes.

Methods

In this study, we are developing optimized, self-assembling, ROS-sensitive and ROS-scavenging nanoparticles (NP) as a prospective therapeutic avenue for chronic inflammatory diseases. RAFT polymerization methods enabled synthesis of boronic acid polymers, the basis of the system's ROS-sensitivity which relies on boronic ester bonds between these polymers and polyphenol catechol groups. Our NPs are synthesized using nanoprecipitation and microfluidic methods and characterized by size and surface charge. TEM imaging and UV-Vis and fluorescence spectroscopic studies are performed to confirm NP complexation and ROS-sensitivity. ROS-Glo H_2O_2 and DCFDA assays will confirm ROS-scavenging in macrophages and microglial cells. Flow cytometry will confirm our NP's entry in cells and microscopy will enable observation of its mitochondrial localization. ELISA will be performed to monitor pro-inflammatory cytokines ensuring our ROS-scavenging translates to reduced inflammation.

Results

Two boronic acid polymers were synthesized and their structure confirmed by NMR. These were used to develop PLGA-based and acrylate-based ROS-sensitive and ROS-scavenging NPs. NP variations with different degrees of ROS-scavenging and boronic ester bond potential were made, ranging from 60-200nm in size. Spectroscopic and NMR studies showed successful formation of boronic ester bonds and their ROS-sensitivity. NPs were imaged by TEM and in vitro ROS-sensitivity and ROS-scavenging assays are underway.

Conclusions

We hope the developed NP system can reduce ROS levels back to their homeostatic ranges, enabling an amelioration of chronic inflammatory diseases whilst ensuring ROS' natural biological effects remain at a reasonable level. Our versatile NP system could serve as a multipurpose tool to be harnessed for various inflammatory conditions, thus helping pave the way to a new set of treatments for chronic inflammatory diseases.

Abstract #39

Safety of Genome Editing: A Novel Fluorescent Model System to Investigate Reducing Off-Target Genome Edits by Base Editors

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Background

Gene editing is a potentially curative approach that could be applied to treat thousands of different genetic diseases. However, current gene editing technologies remain limited in their efficacy and safety. Standard CRISPR-Cas9 approaches install genotoxic double-stranded breaks in the genome, lack precision, and are significantly limited by the low efficiency of homology-directed repair [1]. Newer generations of gene editors such as CRISPR/Cas9-derived base editors have improved specificity by precisely and enzymatically converting a target base to another [2]. However, base editors are also capable of creating unwanted mutations, known as off-target edits, which could potentially lead to tumourgenesis [3]. The difficulty of detecting these base changes has resulted in a poor understanding of factors involved in off-target edits.

Methods

We developed two human cell fluorescent model systems using a mutated Green Fluorescent Protein (*GFP*) reporter gene. Upon correction with a base editor, these cells glow green, and can be analyzed by flow cytometry to precisely quantify rates of editing at a single-cell level. To simulate single guide RNA (sgRNA)-dependent off-target editing, a series of mismatched sgRNAs were designed with intentional mismatches in the target the *GFP* sequence. These mismatched sgRNAs mimic the true *GFP* sgRNA matching incorrectly to sequences similar to *GFP* in the genome.

In this study, we first investigated the on-target and off-target editing efficiencies of the new base editor ABE8e. ABE8e is known to exhibit promiscuous off-target editing activity. Secondly, as a potential strategy to reduce off-target editing, we inserted a specific mutation (V106W) in the ABE8e base editor. **We hypothesized that ABE8e^{V106W} will have reduced off-target genome editing compared to ABE8e.**

Results

Our new cell models were shown to efficiently quantify on- and off-target editing using high-throughput flow cytometry. We were also able to demonstrate via Sanger sequencing that ABE8e^{V106W} exhibited a significant two-fold reduction in nearby off-target edits ($p<0.0001$) compared to ABE8e.

Conclusions

ABE^{V106W} was shown to be able to reduce off-target edits compared to ABE8e. This research will help determine the safety of base editors and if we can reduce off-target edits while maintaining on-target edits in the cell genome.

References

1. Yin H, Song CQ, Dorkin JR, Zhu LJ, Li Y, Wu Q, Park A, Yang J, Suresh S, Bizhanova A, Gupta A, Bolukbasi MF, Walsh S, Bogorad RL, Gao G, Weng Z, Dong Y, Koteliansky V, Wolfe SA, Langer R, Xue W, Anderson DG: **Therapeutic genome editing by combined viral and non-viral delivery of CRISPR system components in vivo.** *Nat Biotechnol* 2016, 34:328-33.

2. Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR: **Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage.** *Nature* 2016, 533:420-424.

3. Buchumenski I, Roth SH, Kopel E, et al. **Global quantification exposes abundant low-level off-target activity by base editors.** *Genome research* 2021, 31:2354-2361.

Abstract #40

Intracellular Dynamics of LNP-mediated mRNA Delivery: A Single-Cell Exploration Toward Enhanced Therapeutics and Vaccine Developments

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Background

The use of lipid nanoparticles (LNPs)-mediated mRNA delivery systems has gained significant traction in recent years, particularly in the field of vaccine development. LNPs are the enabling technology behind the messenger RNA-based COVID-19 vaccines and therapies. These delivery vehicles protect the mRNA cargo following injection or infusion, and facilitate uptake into target cells[1,2]. Variances in LNP trajectory within cells, their fusion and trafficking dynamics with other molecules or organelles, and mRNA release patterns critically dictate the efficacy of LNP-mediated therapies and vaccines. However, a comprehensive understanding of these complexes remains a challenge. Addressing this gap is vital for optimizing the efficacy and safety of mRNA-based therapeutics. This study aims to unravel the mechanisms of action, intracellular trafficking dynamics, release of LNP/mRNA complexes at the single cell level, and sequencing the transcriptome of single cells using advanced imaging techniques and omics tools.

Methods

LNPs will be formulated using molar ratios based on Moderna and Pfizer formulations, FDA approved LNP/mRNA vaccine formulations. Live single Huh7 cells will be confined in an array of 20 µm circular diameter, 5 µm depth microwells using Convex Lens-induced Confinement (CLiC) imaging technique developed for high-throughput and quantitative imaging at the single-cell level. CLiC enables direct imaging of single cells with precision and control, long observation times and allows reagent exchange during observations[3]. The live single cells will be treated with LNP/mRNA formulations and organelle staining including early endosome, late endosome, and lysosomes. Simultaneous fluorescence and label-free tracking of intracellular dynamics as well as precise measurements of LNPs/mRNA sizes and quantities will be done using confocal fluorescence, Differential Interference Contrast (DIC), confocal interferometric scattering microscopy (iSCAT) and super-resolution microscopy. Additionally, multichannel imaging will be used for colocalization of LNPs/mRNAs with various stained organelles. Using laser ablation technique, live cells will be isolated and transferred onto smart chips for transcriptome library construction.

Results

The imaging techniques will allow for precise sizes, number and tracking of LNP/mRNA complexes within the single cells. Confocal iSCAT imaging provides exceptional details in visualizing live recordings of endocytosis and intracellular trafficking of these complexes, especially in the nucleus and near protein expressions[4]. Quantitative analysis of colocalization events will reveal significant interactions between the LNPs/mRNA and various cellular organelles, including early and late endosomes, and lysosomes. The imaging data together with genomics and proteomics will provide insights into the cellular pathways involved in the uptake and release of LNP/mRNA, highlighting the efficiency of endosomal escape and subsequent release of mRNA that encodes for different proteins.

Conclusion

This study offers novel insights into the intracellular behavior of LNP/mRNA complexes, demonstrating the potential of single-cell imaging techniques in understanding the mechanisms of mRNA delivery and release in live cells. The findings have practical impact on the development and optimization of LNP-based delivery systems in mRNA therapeutics and vaccine development.

References

(1) Pardi, N., et al. mRNA vaccines – a new era in vaccinology. *Nature Reviews Drug Delivery,* 2018. **17**(4):261-279.
(2) Mo, Y. et al. Light-Activated siRNA Endosomal Release (LASER) by Porphyrin Lipid Nanoparticles. *ACS Nano*, 2023. **17**(5):4688-4703
(3) Leslie, R.S., Fields, A.P., and Cohen, A.E. Convex lens-induced confinement for imaging single molecules. *Analytical Chemistry*, 2010. **82**(14): p. 6224.
(4) Küppers, M., et al. Confocal interferometric scattering microscopy reveals 3D nanoscopic structure and dynamics in live cells. *Nat Commun* 2023, **14**, 1962.

Abstract #41

Lipid Nanoparticle-Mediated *In Situ* Gene Editing for the Treatment of Monogenic Skin Diseases

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Background

Genodermatoses such as autosomal recessive congenital ichthyosis (ARCI) and recessive dystrophic epidermolysis bullosa (RDEB), dramatically affect the patient's quality of life and, in certain cases, life expectancy. Patients suffering from ARCI develop severe skin conditions characterized by aberrant keratinization due to mutations in various genes. Notably, *TGM1* mutations, particularly c.877-2A>G, account for roughly one-third of all *TGM1* mutation alleles and lead to a premature stop codon. RDEB, another lifelong genodermatose, causes blistering, wounding, and scarring. It is often caused by frameshift mutations in *COL7A1*, which encodes collagen VII (C7), an essential component of anchoring fibrils. While current treatments for ARCI and RDEB focus on temporary symptomatic relief, they do not address the underlying cause of the disease.

Methods

Recent gene-editing advancements present the possibility of repairing the mutations underlying skin diseases. However, the delivery of gene editing tools to the basal cells remains challenging due to the skin's restrictive barrier, even in diseased states. To overcome these obstacles, we established human *in vitro* skin models and developed an *in situ* gene therapy strategy that combines physical modulation of the skin barrier, lipid nanoparticle (LNP)-mediated drug delivery, and CRISPR-Cas9-based gene editing.

Results

In order to address the *TGM1* c.877-2A>G mutation, we employed NG-BE4max, a cytosine base editor to induce a G-to-A conversion at the mutant site. We assessed the safety and efficacy of our approach in human skin models and achieved *in situ* editing rates of ≥10% in excised human skin and skin model without inducing pro-inflammatory cytokine release. In ARCI patient cells, our gene-editing tool, specifically designed for the patient's mutation, led to approximately 20% base editing rates. Notably, emerging evidence suggests that correcting 5-10% of disease-causing mutations may be sufficient to alleviate the symptoms of severe genetic diseases such as genodermatoses, indicating efficient correction of disease-causing mutations with our approach. Similarly, LNPs loaded with CRISPR/Cas9 nuclease mRNA effectively restored type VII collagen (C7) expression in RDEB patient cells, addressing the *COL7A1* frameshift mutation c.6081delC.

Conclusion

In conclusion, our approach aims to correct disease-causing mutations *in situ*, offering an effective treatment and a potential cure for rare skin diseases.

Abstract #42

Metformin-based supramolecular nanoparticle platforms as an atherosclerosis therapy

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Background

Atherosclerotic plaque development begins with cholesterol accumulation, leading to foam cell formation, in the arterial lamina. By activating the ATP-binding cassette transporter A1 (ABCA1) in foam cells, cholesterol can be expelled, and plaques can regress. Previous studies used GW3965 (GW) to upregulate liver X receptors (LXRs); which upregulated ABCA1. Metformin increased AMP-activated protein kinase (AMPK) activation, which also increased cholesterol efflux. However, poor in vivo stability and plasma half-lives of drugs has hampered their potential as effective atherosclerotic therapeutics. In addition, the LXR activators led to a downstream of liver-derived diseases, such as hypertriglyceridemia. In this project, we developed metformin-based gold nanoparticle (NP) systems for synergistic multidrug delivery. After encapsulating GW, the Au-Met-GW NPs can be targeted to atherosclerotic plaques where they synergistically upregulate ABCA1. The dual drug NP will protect the drugs from degradation & minimize off-target effects, making the system suitable for future animal or clinical studies, enhancing its therapeutic index.

Methods

Metformin derivatives were synthesized when metformin was chemically conjugated to hydrocarbon chains of increasing lengths. By increasing metformin's hydrophobicity, it better allowed for its incorporation into the backbone of the self-assembly citric acid-capped gold NPs (Au-Met-GW). NPs of varying size, polydispersity index, surface charge, encapsulation efficiency & stability were produced by mixing gold, metformin conjugates, DSPE-PEG & GW. Then, RAW 264.7 macrophages were treated with appropriate controls & the said Au-Met-GW NPs. rtQPCR was performed 24 hours post drug treatment or 48 hours post Au-Met-GW treatment. Expression levels of ABCA1 mRNA was determined and normalized against β-actin.

Results

Synergistic drug ratios (10 μM Metformin: 1 μM GW) that cause an upregulation of ABCA1 mRNA in RAW 264.7 cells were determined. The dual drug system increased ABCA1 expression more than the individual monotherapies. NP sizing was found to be between ~111-208nm, depending on the metformin-conjugate used. Furthermore, cell treatment with this dual drug NP system revealed an even greater increase in ABCA1 expression.

Conclusions

Thus far, we have synthesized a dual drug NP system, which can be useful for any disease where metformin is main therapeutic agent. Future work will involve testing the Au-Met-GW particles in atherosclerotic mice models.

Abstract #43

A novel liposomal Topotecan formulation with increased copper retention for the treatment of lung cancer.

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Background

Lung cancer is a leading cause of death worldwide. 1 in 16 people are diagnosed with it, and it kills 3 times as many people as prostate and breast cancer. Topotecan is a poorly soluble but highly potent drug that is approved for use after failure of first-line treatment. Topotecan results in severe hematoxicity. A novel liposomal formulation would be a significant leap for the safe treatment of small-cell lung cancer and may even allow it to be used in earlier stages and for other cancers in combination with checkpoint inhibitors. Liposomal formulations allow for passive targeting of drugs via the Enhanced Permeability and Retention (EPR) effect, which increases the concentration of drugs at the tumour and reduces delivery to normal tissue, resulting in a more effective drug with less severe side effects. Despite this, Topotecan does not have an approved liposomal formulation due to its insolubility.

Methods

We created a novel high Topotecan concentration formulation using Ammonium Sulfate to create the pH gradient. This will retain more copper inside the liposome to trap more Topotecan and deliver more copper to the tumor site. The novel formulation is characterized. The formulation is then compared to free drug in various assays. It is also tested against various immune cells and tumor cells from A549 and CT26 tumors. The treatment will also be tested in vivo mouse models. This treatment also holds potential for combination with immunotherapy drugs like checkpoint inhibitors.

Results

We were able to load Topotecan at much higher drug to lipid ratio when compared to earlier methods utilizing ionophores. The new formulation was also more stable when purifying and concentrating and in long term storage.

Liposomal Topotecan has shown lower toxicity against BMDC and BMDM cells when compared to free topotecan and when compared to A549 and CT26 tumor cells in vitro.

Conclusions

The new formulation utilizing a novel pH gradient generation method allows a higher drug to lipid ratio. It also is safer when compared to free drug in vitro and similar result is hypothesized for in vivo assay.

Abstract #44

Quantitative visualization of lipid nanoparticle fusion as a function of formulation and process parameters

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Background

Lipid nanoparticles (LNPs) have been shown to be effective and safe delivery vehicles for RNA-based therapeutics. Common RNA payloads include short interfering RNA (siRNA) for silencing pathological genes or messenger RNA (mRNA) for the expression of therapeutic proteins (1). LNP formulations contain ionizable lipids, which become protonated/de-protonated as a function of buffer pH, resulting in subsequent dynamic changes to the LNP structure. These changes are fundamental to their delivery mechanisms, allowing 1) encapsulation of the anionic RNA drug during LNP formation (at low pH, ~4); 2) Remaining neutral once administered and in circulation (at physiological pH, ~7.4); and 3) allow the release of the payload through their interaction with maturing endosomes in the target cell (at low pH, ~5) (2). A quantitative understanding of these dynamic processes is required for the design of improved LNP vaccines and therapeutics.

Methods

LNPs were formulated using molar ratios based on OnpattroTM, an FDA-approved siRNA- LNP formulation. Fusion dynamics were measured using single-particle CLiC (convex-lens induced confinement) microscopy in combination with Förster resonance energy transfer (FRET) measurements to investigate LNP dynamics as the buffer pH is increased. Fusion processes were measured as a function of lipid concentration, buffer conditions, drug loading fraction and lipid composition. CLiC data was also supported by cryogenic transmission electron microscopy (CryoTEM) and dynamic light scattering (DLS) studies.

Results

First, buffer pH and ionic strength were investigated dependently and independently of each other. Holding pH constant while increasing ionic strength resulted in a slow fusion process, while the opposite held true for increasing pH while holding ionic strength constant. Interestingly, increasing both pH and ionic strength resulted in the highest degree of fusion. Second, as drug loading increased, it took longer for fusion to complete, with low fusion events observed at the highest loading ratio, likely due to the saturation of RNA-ionizable lipid interactions. Lastly, LNP fusion was shown to correlate with the apparent pKa values of the LNPs, as well as the chemical structure of the ionizable lipids used.

Conclusion

CLiC imaging and FRET were successfully used to study LNP fusion with single-particle resolution. The degree and kinetics of fusion was investigated as a function of lipid composition, buffer and drug loading which correlated well with traditional LNP characterization methods (CryoTEM and DLS).

Abstract #45

Lipid nanoparticle delivery of adenine base editors as a potential treatment for inherited retinal diseases

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Background

Over two million people worldwide suffer from inherited retinal diseases (IRDs) with most classified clinically as retinitis pigmentosa (RP). Over 30-40% of autosomal dominant cases of RP are caused by mutations in the rhodopsin gene. Although significant progress has been made in the application of gene therapy for the treatment of some IRDs, there is a need for the development of new and improved therapeutic strategies based on emerging technologies that overcome the limitations of previous gene therapy. The purpose of this study is to explore the use of genome base editing in combination with a lipid nanoparticle (LNP) delivery system to correct a nonsense mutation in the rhodopsin gene.

Methods

HEK293T cells stably expressing C-terminus EGFP-tagged rhodopsin with or without a Q344Ter mutation were treated with pNG-ABE8e (addgene #138491) and sgRNA that place the target base in position A3 or A6 using either polyethylenimine (PEI) or LNPs as transfection agent. LNPs were synthesized by rapid mixing of ionizable lipids, phospholipid, cholesterol, PEG lipid, pNG-Abe8e, and sgRNA. Editing efficiency was determined 72 hours post- transfection by restoration of EGFP signal as quantified by flow cytometry with non-transfected cells used as negative control. Base editing was also evaluated by immunofluorescence (IF), Western blotting (WB), and DNA Sanger sequencing.

Results

The *Rho* gene was successfully edited using pNG-ABE8e and sgRNA in position A6. PEI transfection of pNG-Abe8e resulted in 18% editing for A6-sgRNA and 3% editing for A3-sgRNA (n=5). In contrast, the delivery of pNG-Abe8e and A6 sgRNA using LNP resulted in 81% editing efficiency (n=3). WB and IF confirmed the restoration of EGFP signal after treatment with pNG-ABE8e and A6 sgRNA. No editing was detected when a scrambled control sgRNA was used. Sanger sequencing confirmed editing at the target nucleotide and the absence of off-target edits within the editing window.

Conclusions

This data demonstrates the efficiency of LNP transfection and the ability to successfully edit the Rho c. 1030C>T (p.Q344Ter) mutation. These findings provide the foundation for the pairing of LNP and base editing technology for potential in vivo rescue and survival of photoreceptors.

Abstract #46

Simultaneous, single-particle measurements of size and loading give new insights into the structure of drug-delivery nanoparticles

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Background

Nanoparticles are a promising solution for delivery of a wide range of medicines and vaccines. Optimizing their design depends on being able to resolve, understand, and predict biophysical and therapeutic properties, as a function of design parameters. While existing tools have made great progress, gaps in understanding remain because of the inability to make detailed measurements of multiple correlated properties. Typically, an average measurement is made across a heterogeneous population, obscuring potentially important information.

Methods

In this work, we develop and apply a method for characterizing nanoparticles with single-particle resolution. We use convex lens-induced confinement (CLiC) microscopy to isolate and quantify the diffusive trajectories and fluorescent intensities of individual nanoparticles trapped in microwells for long times. First, we benchmark detailed measurements of fluorescent polystyrene nanoparticles against prior data to validate our approach. Second, we apply our method to investigate the size and loading properties of lipid nanoparticle (LNP) vehicles containing silencing RNA (siRNA & mRNA), as a function of lipid formulation, solution pH, and drug-loading.

Results

By taking a comprehensive look at the correlation between the intensity and size measurements, we gain insights into LNP structure and how the siRNA is distributed in the LNP. Next, we apply these measurements to quantify subpopulations of loaded and unloaded LNPs in various formulations of mRNA loaded LNPs. Beyond introducing an analytic for size and loading, this work allows for future studies of dynamics with single-particle resolution, such as LNP fusion and drug-release kinetics.

Conclusion

The prime contribution of this work is to better understand the connections between microscopic and macroscopic properties of drug-delivery vehicles, enabling and accelerating their discovery and development.

Abstract #47

Minimum Information for RNA Experiment: An RNA production guide for lab-scale studies

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Background

The widespread success of mRNA has sparked new investments and initiatives by academic and industry groups alike. Furthermore, modalities such as self-amplifying RNA (saRNA), circular RNA (circRNA), and small RNA approaches offer exciting, potent alternatives to mRNA. However, many groups insufficiently appreciate the challenge with producing RNA modalities in high quality, purity, and consistency. Without proper QC, experimental inconsistencies, insufficient activity, or unexplainable failures remain common. We are therefore approaching this problem in two ways: (1) developing the **m**inimum **i**nformation for **R**NA **e**xperiments (MIRE), which provides a guide to the affordable lab-scale production of highly quality, reproducible RNA modalities; and (2) offering a non-profit core facility to improve accessibility of quality custom RNA production for research groups.

Methods

Endotoxin-free plasmid preparations were sequenced using Oxford Nanopore full plasmid sequencing. Routine lab-scale linearization and purification protocols were optimized to balance time and affordability for µg to mg DNA/RNA preparations. Capping approaches were modality dependent: mRNA was co-transcriptionally capped, saRNA was post-transcriptionally capped, and no capping was performed for circRNA (produced via autocataltyic circularization). All RNA was purified by DNase I treatment and LiCl precipitation. Batch-to-batch consistency and quality was evaluated, and *in vivo* functionality was compared against commercial suppliers. A case study was also used to exemplify the need for strict controls.

Results

High yield, batch-to-batch consistently, and quality was observed for mRNA produced in-house. Our mRNA performance was on-par with commercially available mRNA. Additionally, our case study used capillary electrophoresis to identify multiple IVT products in a sample, despite passing traditionally employed lab-scale quality controls.

Conclusion

mRNA synthesized using our optimized methods offers confidence in the products while leveraging affordable, yet robust protocol modifications. Whether using our methods or our services, high batch consistency and quality, matching that of world-class facilities, helps unlock accessible RNA production for research labs.

Abstract #48

Property-structure-function analysis of complex LNPs using integrative biophysical, molecular-, and cell-based assays

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Background

The complexity of lipid nanoparticles (LNPs) for the delivery of nucleic acids presents a developability challenge. Fit-for-purpose and complementary analytical tools are required to design successful formulations, and to inform robust production of stable, safe, and efficacious products.

Methods

In this study, different mRNA-LNP formulations and batches were compared using biophysical and cell-based assessments. Two Fluc mRNA-LNP formulations were compared based on size, particle concentration and zeta-potential with nanoparticle tracking analysis (NTA), dynamic and electrophoretic light scattering (DLS, ELS) [1]. Differential scanning calorimetry (DSC) was used along with cell-based transfection efficiency assay to compare higher order structure (HOS) and functional activity of the two batches of Fluc RNA-LNP2 formulation.
The size of eGFP RNA-LNP formulations (Batch 1-4) was analysed by DLS before determining mRNA encapsulation efficiency using a RiboGreen RNA Assay Kit to measure total and free mRNA concentrations. mRNA transfection efficiency was assessed in human embryonic kidney (HEK 293) cells using live cell fluorescence 48 hours after mRNA-LNP treatment.

Results

Differences in physicochemical profiles of Fluc mRNA-LNPs were well-correlated and robustly resolved with DLS, NTA and ESL. Poor HOS comparability of LNP2 batches was corroborated in the functional assay. For the eGFP mRNA-LNPs, Batch 2 and 4 did not show significant differences in size, while Batch 1 and 3 exhibited a shift to larger sizes, with the aggregation apparent in Batch 1 likely explaining the poor mRNA encapsulation efficiency (<20%). Batch 2 – 4 were smaller and similar in size, which correlated well with encapsulation efficiency data (>90%). There was also exceptional agreement with cell-based transfection efficiency data showing that LNP aggregation and poor encapsulation efficiency of Batch 1 led to low transfection efficiency compared to Batches 2 – 4.

Conclusions

Taken together, these data suggest that biophysical characterization of LNP vectors combined with cell-based assays to assess LNP transfection efficiency are key for informing the association between particle properties, HOS and functional performance. Therefore, systematic physiochemical and functional analyses are key to guiding formulation screening, quality control, and stability studies.

References

1. Markova N., Cairns S., Jankevics Jones H., Kaszuba M., Caputo F., Parot J., **Biophysical Characterization of Viral and Lipid-Based Vectors for Vaccines and Therapeutics with Light Scattering and Calorimetric Techniques** (2021) Vaccines **10**, 49 Vaccines | Free Full-Text | Biophysical Characterization of Viral and Lipid-Based Vectors for Vaccines and Therapeutics with Light Scattering and Calorimetric Techniques (mdpi.com)

Abstract #49

Strategies for producing clinical and commercial RNA-LNP drug products

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Background

The RNA-lipid nanoparticle (LNP) vaccines for the SARS-CoV-2 pandemic highlight the impact of genomic medicines deployed at scale. RNA-LNP applications also include gene editing, oncology, and rare diseases. Despite this growing momentum, developing RNA-based vaccines and therapeutics still faces significant manufacturing challenges. The mixing process to encapsulate RNA within LNPs is among the most difficult unit operations to scale-up to commercial throughput rates and batch sizes, while maintaining critical quality attributes (CQAs) such as size and biological potency.

Methods

We have developed the new NanoAssemblr® commercial formulation system (CFS) for commercial-scale production of RNA-LNPs. This system utilizes the same NxGen™ mixing technology deployed across the complete NanoAssembr suite of instrumentation to enable rapid scale-up and production. To demonstrate the scale-up capability of the suite, we produced and characterized a SARS-CoV-2 saRNA-LNP vaccine candidate under increasing flow rates and batch volumes to mimic the drug development process from discovery through to commercial production. The formulation flow rate was stepwise increased from 12 mL/min using the NanoAssemblr® Ignite+™ through to 800 mL/min using the new NanoAssemblr CFS. The LNP quality was evaluated by measuring size, polydispersity index (PDI), nucleic acid encapsulation efficiency, saRNA integrity, *in vitro* expression, and *in vivo* expression.

Results

The saRNA-LNPs were of similar quality with sizes ranging from 61 – 83 nm, PDI < 0.18 and RNA encapsulation efficiency > 94%. ANOVA testing showed no significant differences in size, PDI, or encapsulation efficiency when comparing instruments or mixers. *In vitro* potency assays showed similar dose response curves between samples and consistent EC_{50} values between 1.3 to 3.6 ng/mL. Finally, *in vivo* immunization studies showed a robust SARS-CoV-2 specific IgG response in all instrument-mixer combinations with titers varying between $1.94x10^5$ to $2.83x10^6$ ng/mL.

Conclusions

This work demonstrates that the production of nucleic acid-LNPs with consistent CQAs can be achieved on a wide range of scales using NanoAssemblr instruments and NxGen mixers. The NxGen 48 L/h and NanoAssemblr CFS can prepare RNA-LNPs at throughputs needed to meet commercial manufacturing goals. The introduction of these new instruments will simplify the production of RNA-LNP drug products for a wide range of applications.

Abstract #19

Development of novel and robust LNP-RNA vaccines

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Background

mRNA vaccines represent a novel and clinically validated approach to provide rapid responses with scalable solutions to ongoing and future diseases. Lipid nanoparticles (LNP) have enabled success of mRNA-based vaccines by providing an appropriate carrier and incorporating adjuvant features, such as innate immune activation, potent antibody production, and T cell responses to mRNA-LNP vaccines. This has been demonstrated in the historically successful clinical trials run by Pfizer/BioNTech and Moderna. Critically, the approved formulations represent repurposed formulations that were initially designed for hepatocyte gene silencing. These formulations in turn resulted in successful vaccines that were rapidly translated to the clinic for use in the COVID-19 pandemic. We aimed to develop fit-for-purpose mRNA vaccines to achieve robust immune responses. Here, we demonstrate that our novel ionizable lipids and proprietary LNP formulations can stimulate comparable and/or improved immune responses, such as increased antibody production, to clinically approved mRNA-LNP vaccines.

Methods

Our LNP formulations consisting of an ionizable lipid, helper lipid, cholesterol, and PEG-DMG at differing molar ratios containing mRNA encoding for the SARS-CoV-2 Spike protein were used to screen our formulation library for an enhanced immune response. The mRNA-LNPs were delivered intramuscularly into mice at days 0 and 21 at a low dose of 1 mg, and serum SARS-CoV-2 Spike IgG antibodies were measured using the mesoscale discovery platform along with other immune response parameters.

Results

Through screening of our LNP library, we found that several of our proprietary ionizable lipids and formulations resulted in similar or enhanced serum levels of total SARS-CoV-2 Spike IgG antibodies compared to clinically approved lipids and formulations. Specifically, NanoVation proprietary long circulating LNPs (lcLNPs) demonstrated up to 2-fold enhancement in serum titers at a low dose of 1 ug, 2 weeks post boost immunization when compared to clinically approved benchmark formulations.

Conclusions

Our ability to develop lipids and tailor formulations to specific needs provides additional tools to develop potent and effective vaccines for infectious diseases beyond SARS-CoV-2.

Abstract #33

Long circulating lipid nanoparticles (lcLNP) enhance transfection of hematopoietic stem and progenitor cells in the bone marrow

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Background

Hematopoietic stem and progenitor cells (HSPCs) are the precursors of most immune cells. Specifically, haematopoietic stem cells (HSC) possess the ability to self-renew and to give rise to downstream differentiated cell populations. These features make HSPCs of particular interest for gene editing in order to treat and, potentially cure, rare genetic diseases. HSPCs mainly reside within the bone marrow of adult mice and humans, and therefore designing lipid nanoparticles (LNPs) capable of delivering to extrahepatic tissues remains an important hurdle to effectively manipulate HSPC populations *in vivo*. While hepatocytes are the primary cell target of traditional LNPs, we have developed the long-circulating LNP (lcLNP), which are LNPs specifically designed to deliver to extrahepatic tissues, including the bone marrow.

Methods

In this project, various lcLNP systems were prepared to increase activity of a reporter construct in the bone marrow. These LNP formulations consisted of an ionizable lipid, helper lipid, cholesterol, and PEG-DMG at various molar ratios. These LNPs encapsulated mRNA encoding for enhanced green fluorescent protein (eGFP) and were injected *intravenously* (i.v.) into mice. 24-hours post-injection, the BM was isolated and HSPCs were assessed for potency via flow cytometry.

Results

After screening our lcLNP libraries, it was determined that NanoVation LNPs are capable of extrahepatic enhanced transfection and potency compared to a benchmark LNP with the composition 50/10/38.5/1.5 (ionizable lipid/DSPC/cholesterol/PEG-DMG in mol%) administered i.v. Specifically, we observed significantly increased reporter expression in HSPCs in the bone marrow compared with NanoVation lcLNPs compared to the benchmark mentioned above.

Conclusions

These studies show that a combination of NanoVation proprietary lipids and compositions result in significant improvements of delivery to HSPCs in the bone marrow compared to clinically approved LNPs. These results demonstrate a new generation of LNPs capable of enhanced HSPC delivery, suggesting an important development in the field of gene therapy.

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