Thermal stabilization and immunogenicity of mRNA vaccine delivered by single-use microneedle patches

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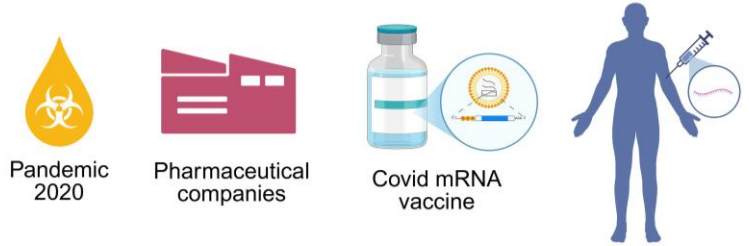
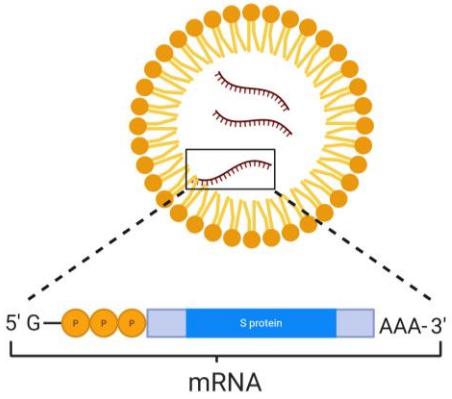
NEXT-GENERATION DELIVERY INNOVATIONS

Introduction of mRNA Vaccine

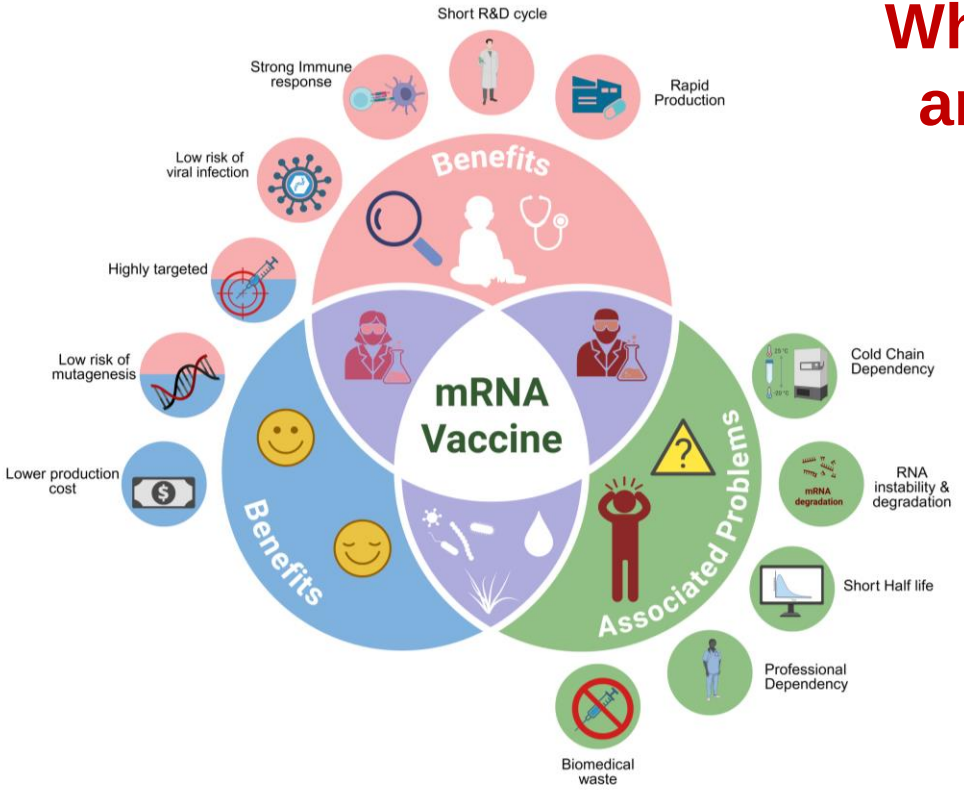
- Innovative platform, which mimics natural infection by instructing cells to make viral proteins
- Strong immune response with **both humoral and cellular activation**
- Safer and faster to manufacture than traditional vaccines
- Requires cold-chain storage due to thermal instability **(a major limitation)**
- Approved e.g: Pfizer-BioNTech and Moderna COVID-19 vaccines

Commercial mRNA vaccine





Platform: LNP-encapsulated mRNA encoding S protein.



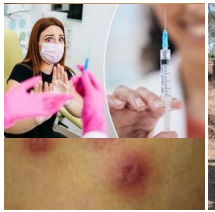
Why mRNA Vaccine Delivery and Thermal stabilization?

- mRNA is inherently unstable and degrades at room temperature.
- Cold chain logistics are costly and limit global access.
- Thermal stabilization enables broader distribution, especially in low-resource settings.
- Stabilized mRNA allows stockpiling and easier transport without deep freezing.

INJECTIONS OF COMMON VACCINES AND THEIR PROBLEMS

Live/attenuated vaccines	Inactivates, subunits, vectors, VLPs, mRNA etc.	Storage Condition
Possible one shot - Risk of viral reversion & cross-infection	Multiple injections over a long period > 2-3 Booster shots (NewYork Post)	-80^{0}C , -20^{0}C, 4^{0}C

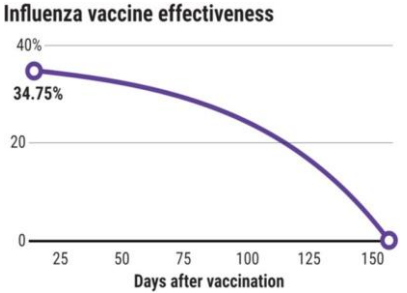
❑ Biohazard



❑ Pain+Fear

❑ Transport

❑ Cold chain

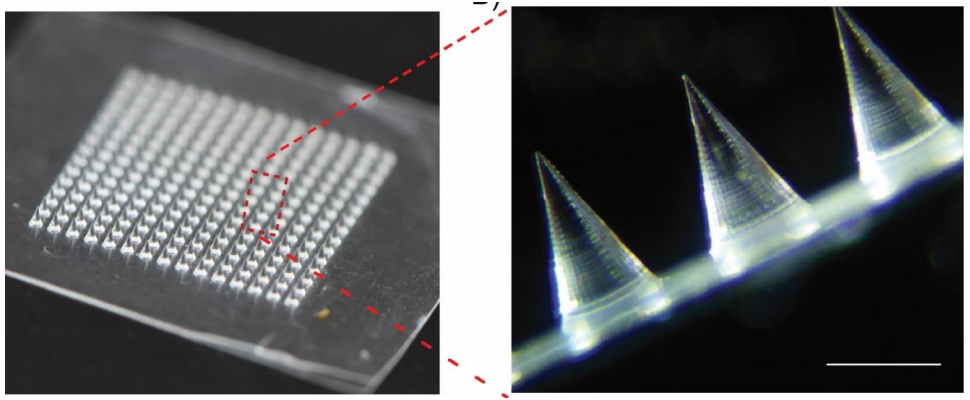


(Science, 2018)
Antibody decline
Booster Shot Needed

Microneedle Delivery system

- Minimally invasive and painless
- Easy self-administration
- Increases patient compliance
- Better absorption
- Targets immune cells in the skin (good for vaccines)
- No cold chain needed with stabilized formulations
- No sharps waste (especially dissolvable types)
- Reduces infection and needle-stick risk
- Allows precise, controlled dosing

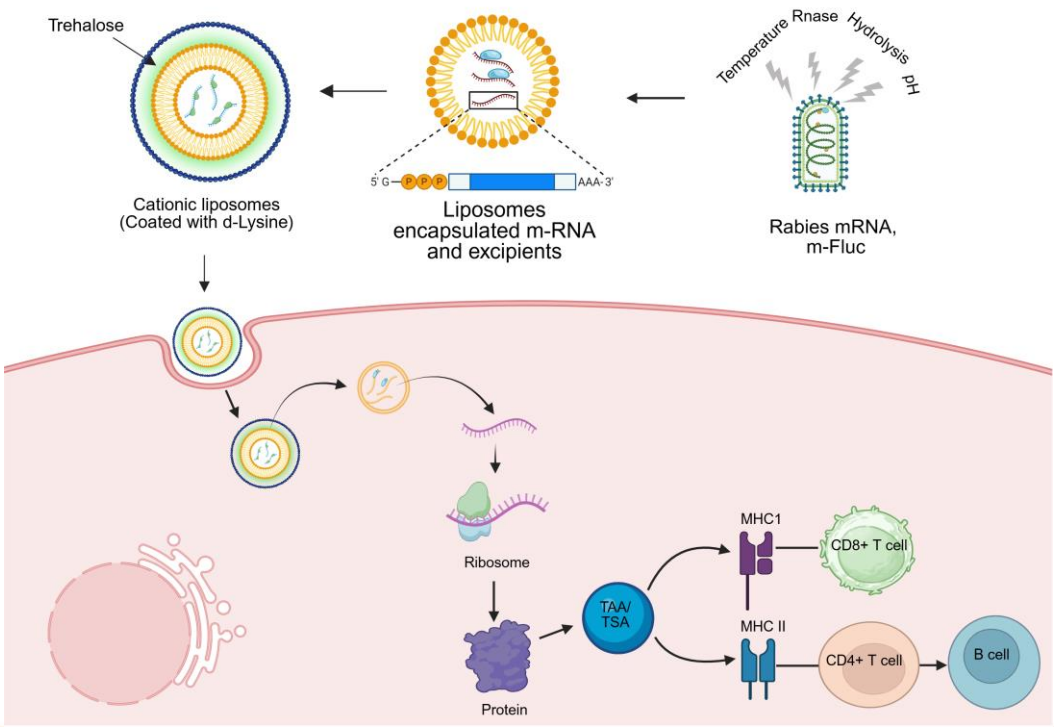
Simple Microneedle (Burst Release Microneedle)



Our Approach for Thermal stabilization ; Cationic Liposomes

Why Cationic Liposomes?

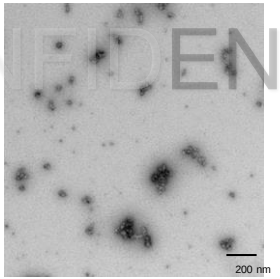
- Promotes strong electrostatic interaction with negatively charged cell membranes
- Enhances cellular uptake via endocytic pathways
- Facilitates endosomal escape (proton sponge effect)
- Improves intracellular delivery of nucleic acid cargo
- Requires charge optimization to avoid cytotoxicity



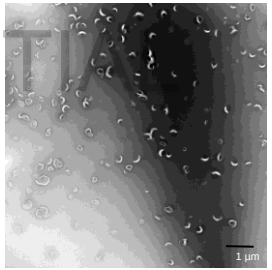
Structural Characterization of mRNA-Encapsulated Formulation by TEM & DLS

Parameters	Layer mRNA formulation
N^+/P^- ratio	3
Z-Ave (nm ± S.D.)	265.2 ±5.7
PI	0.264 ±0.035
Zeta Potential (mV ± S.D.)	64.8 ± 2.76

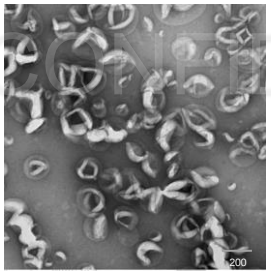
mRNA + Excipients



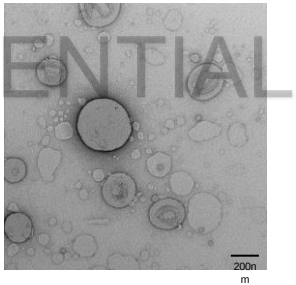
Liposomes only



Liposomes (mRNA + Excipients)



Cationic liposomes



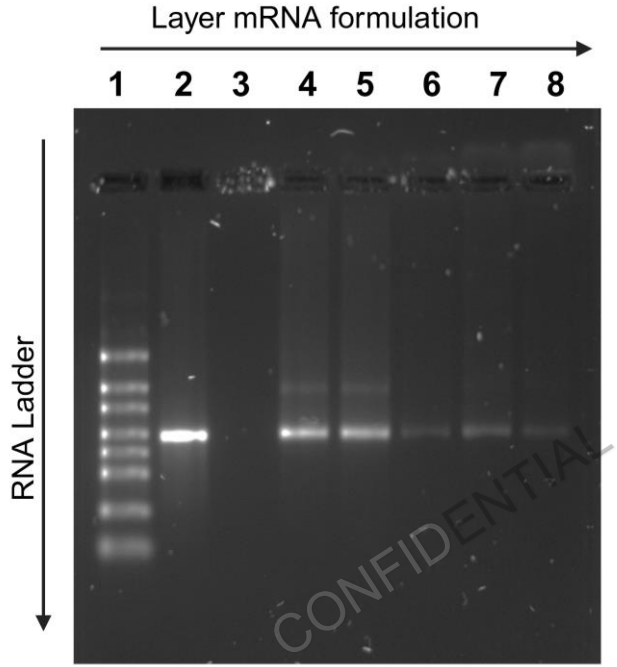
The Structural configuration of liposomes loaded with mRNA called layer formulation has been observed, showing the core-shell structure

(Mocket et al., 2007)

Gel retardation assay

Triton X100 application and Heparin Relase

1. RNA Ladder 3 uL
2. Free mRNA 250 ng
3. mRNA (250 ng)- **e**xcipients -liposome
4. mRNA (250 ng)- **e**xcipients -liposome + TritonX-100 %0.1 + Heparin 5 mg/mL
5. mRNA (250 ng)- **e**xcipients-liposome + TritonX-100 %0.25 + Heparin 5 mg/mL
6. mRNA (250 ng)- **e**xcipients -liposome + TritonX-100 %0.5 + Heparin 5 mg/mL
7. mRNA (250 ng)- **e**xcipients -liposome + TritonX-100 %1 + Heparin 5 mg/mL
8. mRNA (250 ng)- **e**xcipients -liposome + TritonX-100 %2 + Heparin 5 mg/mL

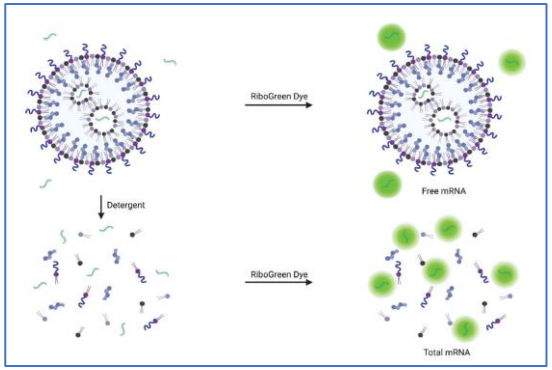


Conclusion: The formulation achieved an encapsulation efficiency of ~82% ± 1.3, as determined semi-quantitatively through band intensity analysis.

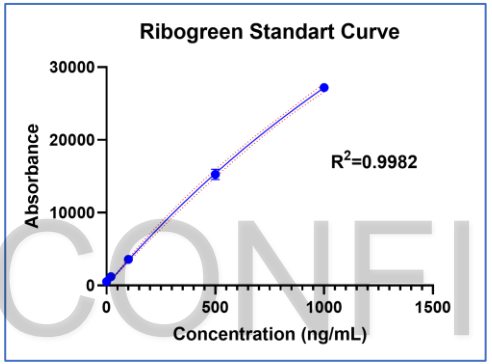
(McKenzie et al., 2023)

Encapsulation efficiency (EE%) of Formulation

- It is calculated by taking the ratio of encapsulated RNA to total RNA (accessable + released)



Principle of Ribogreen Assay



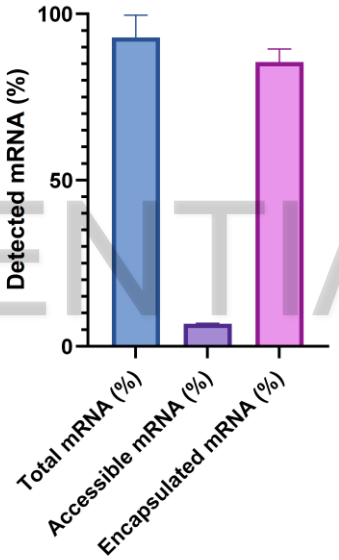
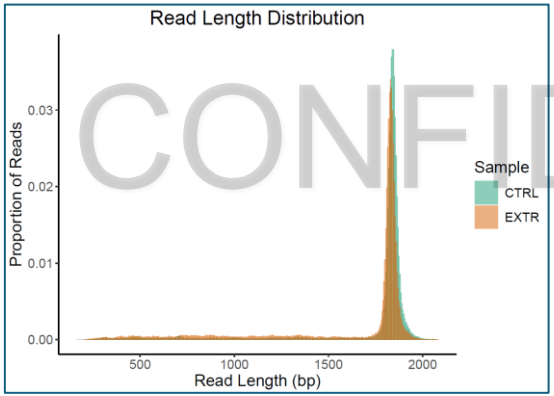


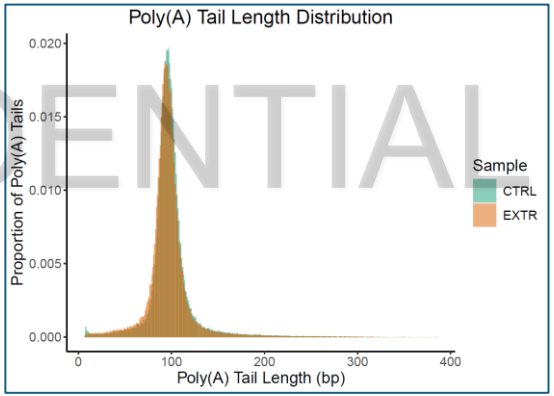
Table. Physicochemical properties of Cationic Liposome Formulation

Sample	***N⁺/P⁻ ratio***	*Z-Ave (nm ± S.D.)*	*PI*	*Zeta Potential (mV ± S.D.)*	***EE (% ± S.D.)***
Complete Liposomal Formulation	**3**	162.2 ±5.7	0.284 ±0.038	67.8 ± 2.66	**86.47 ± 0.59** 85.53 ± 3.86 84.33 ± 7.58

mRNA integrity using NGS technique

LAYER formulation was fabricated afterwards mRNA (Fluc-mRNA) was extracted for integrity test.
Control: Original mRNA from Trilink





	Control	Extracted
Average read length	1644	1593
Average poly(A) length	98	96

Conclusion: High-quality sequencing data was obtained. mRNA ORF and poly(A) tail length are similar and no fragmentation was observed during manufacture, storage and extraction.

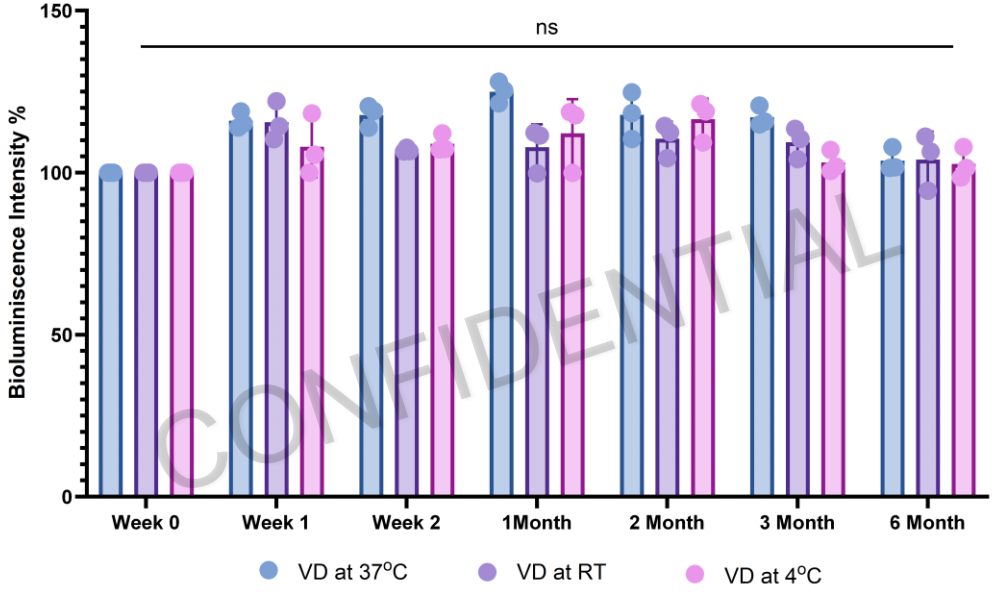
Analysis of Error Profiling & Consensus Sequence Variation

	Control	Extracted
Average Match Rate	92% (max: 100%, min 4%)	92% (max: 100%, min: 3%)
Average Mismatch Rate	6% (max: 91%, min: 0%)	6% (max: 92%, min: 0%)
Average Insertion Rate	1% (max: 39%, min: 0%)	1% (max: 39%, min: 0%)
Average Deletion Rate	2% (max: 56%, min: 0%)	2% (max: 51%, min: 0%)

Conclusion: Across several integrity metrics, the “control” and “extracted” samples are qualitatively similar. Mismatch, insertion and deletion levels are the same for the control and extracted mRNA samples when compared to the original sequence.

In vitro stability data for mRNA (m-Fluc)

RT; Room Temperature



Conclusion: Luciferase-based analysis confirmed sustained bioactivity of the mRNA formulation over 6 months, indicating its long-term stability under the tested storage conditions.

In vitro/in vivo stability data for mRNA

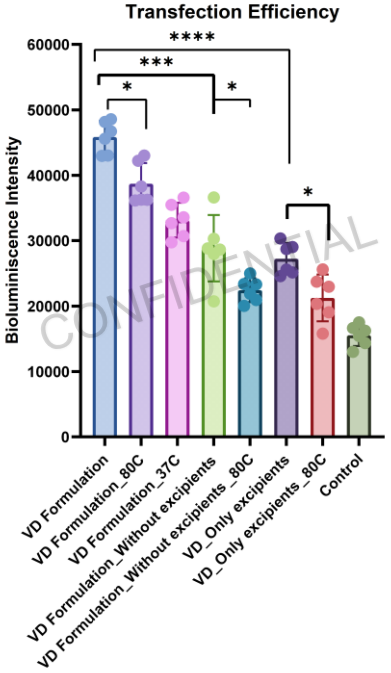
Experimental details:

- **m-RNA** = m-FLUC
- **Detection method:** Microplate reader (one-glo-luciferase Solution)
- **Transfection period**: 24hours

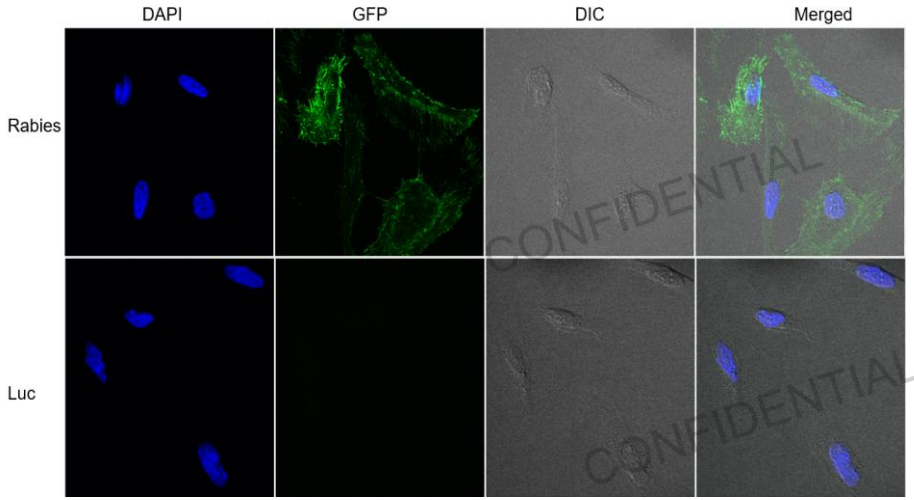
Abbreviations:

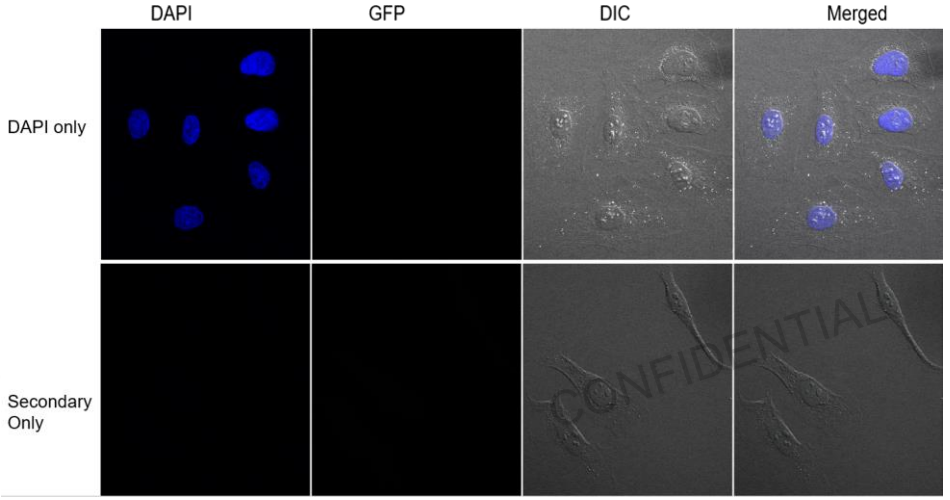
RT; Room Temperature
VD; Vacuum Dried
VD; only excipients; Formulation without liposomes

Conclusion: The stability of our formulation is significantly better than without excipient group



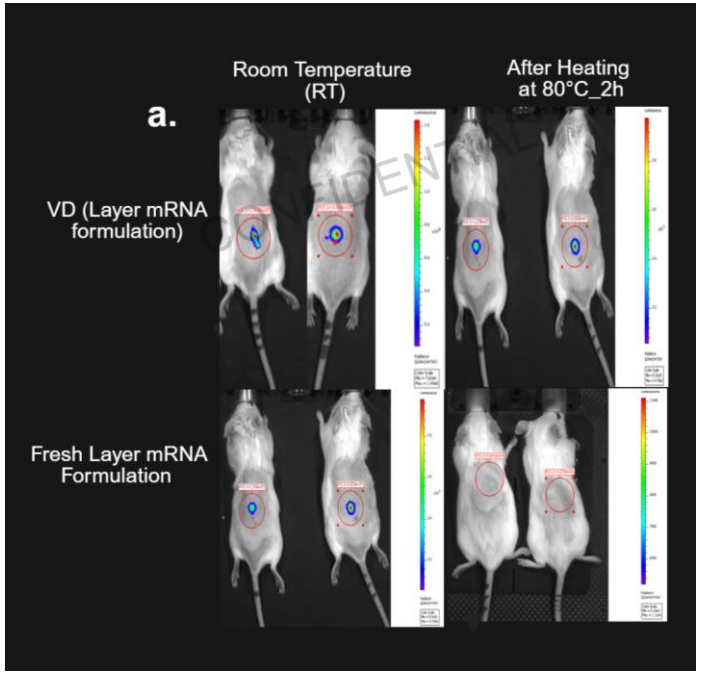
Expression of Rabies glycoprotein using rabies mRNA

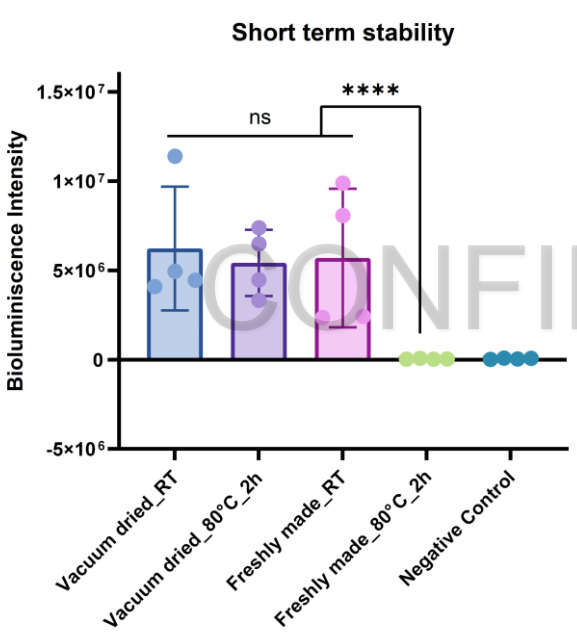


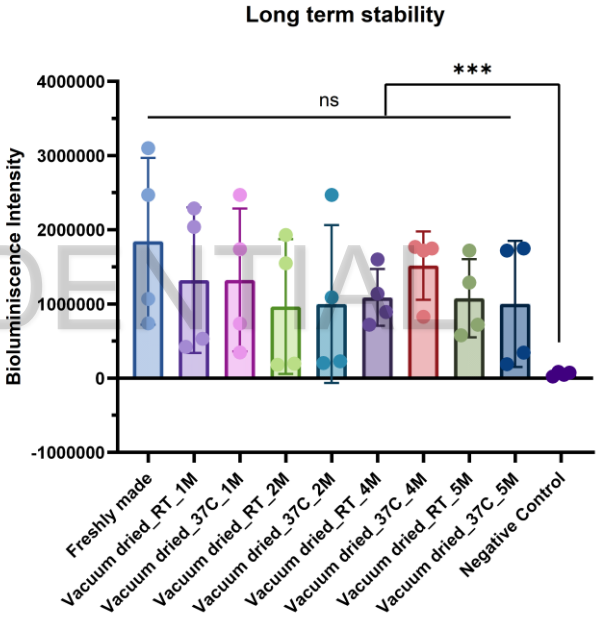


The confocal images are showing the efficient transfection efficiency of rabies mRNA-GFP on HeLa cells after 24 hrs

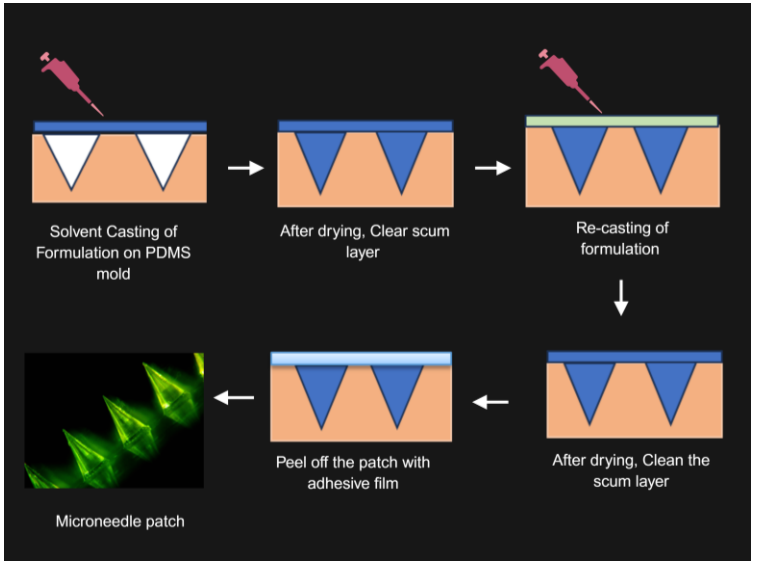
In Vivo Stability Assessment via Bioluminescence Imaging (IVIS)



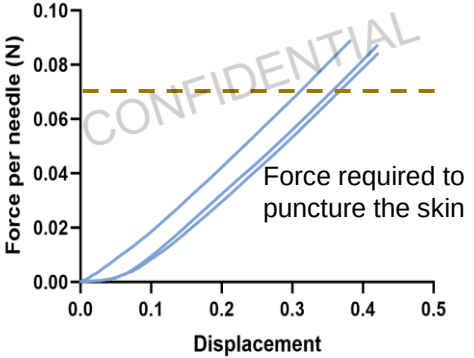




Microneedle Fabrication & its Characterization



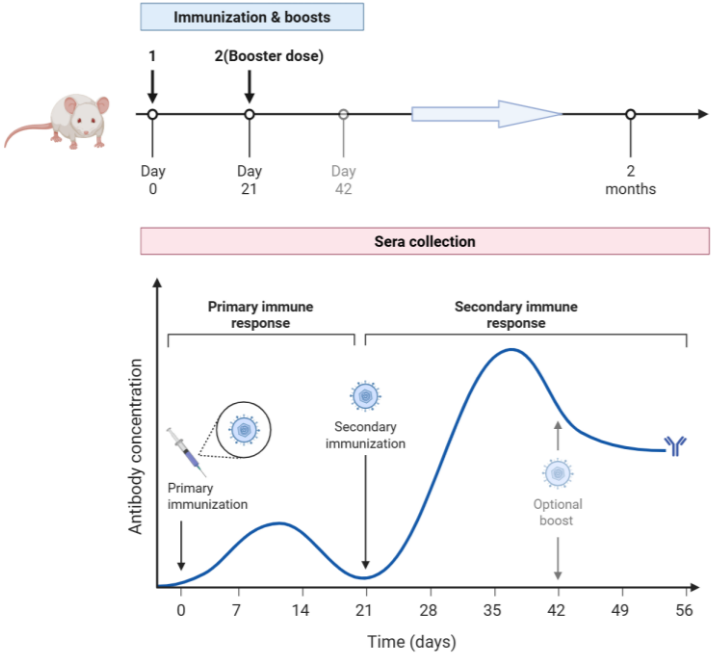
Mechanical testing &Loading Capacity

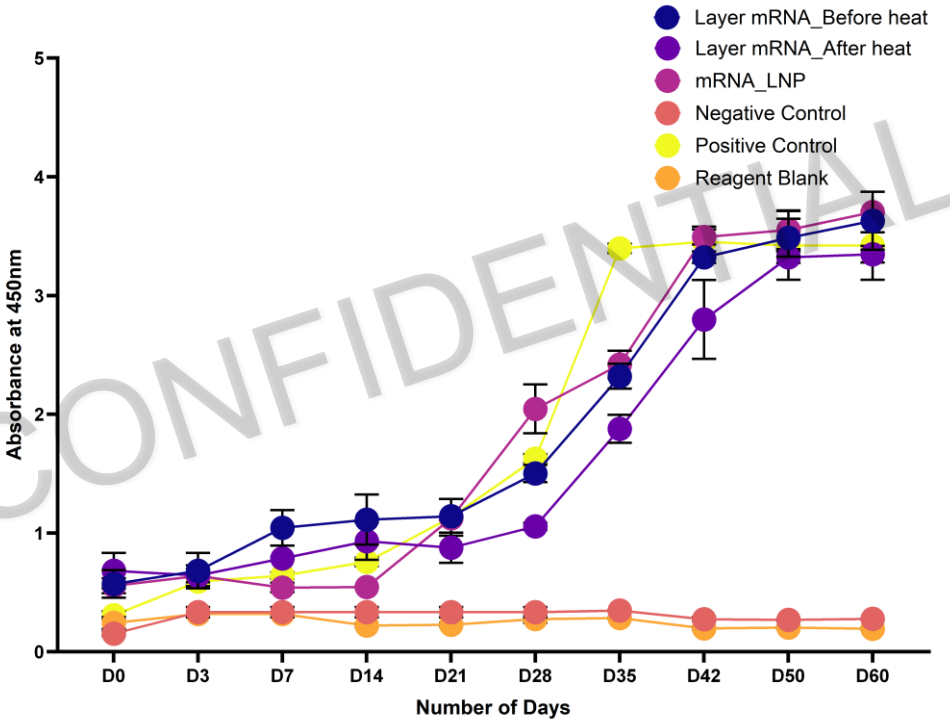


MN patch Conc.	Fluorescence Intensity PVP based MN	Total concentration/ patch (ug/patch)
MN1	2172.66	11.46
MN2	2165.66	11.35

Conclusion: The microneedles exhibited sufficient mechanical strength (0.7 N) and effective loading efficiency (11.46 µg/patch), supporting their potential for transdermal vaccine delivery.

In Vivo study for Anti-Rabies antibodies





Summary

•Achieved effective **in vitro thermal stabilization** of mRNA vaccines using single-use microneed patches.

•Demonstrated **preserved mRNA integrity and expression** post-stabilization.

•**In vivo immunogenicity studies** are in progress to assess efficacy over time including **Viral titer assays** are being performed

•Preliminary observations indicate **good biocompatibility** of the microneedle system.

•The Ongoing work includes reaffirming the data and conducting **safety assessments** include evaluation of local tissue response and systemic toxicity.

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NIH Trailblazer
RO1 EB036011;
RO1 AI186784
RO1 NS131310;
RO1 AR080102;
R21EB024787; R21AR075196;
R21AR074645; R21AR075133;
R21NS116095; R21AR076646;
R21AR078744; R21AR081508;
R43OH012495;
R21AR074645;
USDA-ARS 58-8064-9-011
61268 NCRA MERCK
Gates INV-061798
USDA 58-3022-3-012
Gates INV-076019

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