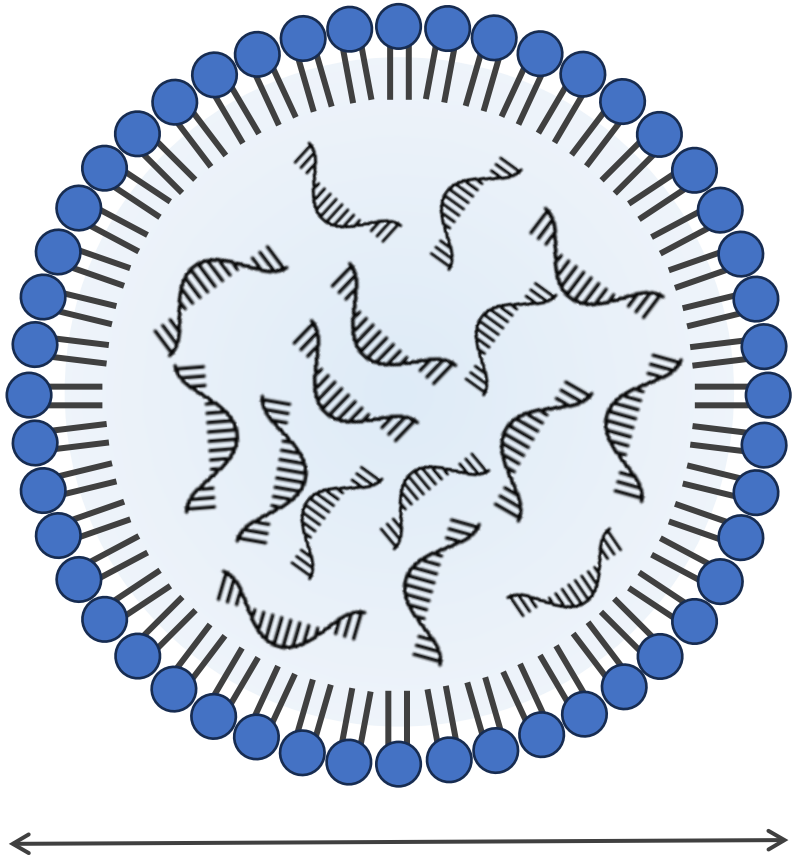
What are lipid nanoparticles？（LNP）

LNP（Lipid Nano Particle） [1※]
＝LNPs are nanocarriers comprised of lipids that safely deliver pharmaceutical ingredients to target cells.



Particle diameter: 50～100nm

Hydrophilic head
Hydrophobic Tail

Lipid

Nucleic acid(e.g., mRNA、siRNA) or small-molecule

The lipid layer encapsulates the nucleic acid or small molecule

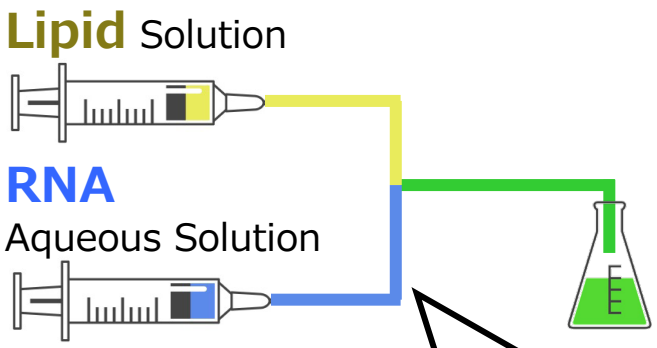
Usage

- ✓ mRNA vaccines
- ✓ Gene therapy
- ✓ Anti-cancer treatments

Etc.

1 – J. Pharm. Sci. Technol. Jpn., 82(2), 63-70 (2022).

Mechanism of LNP formation



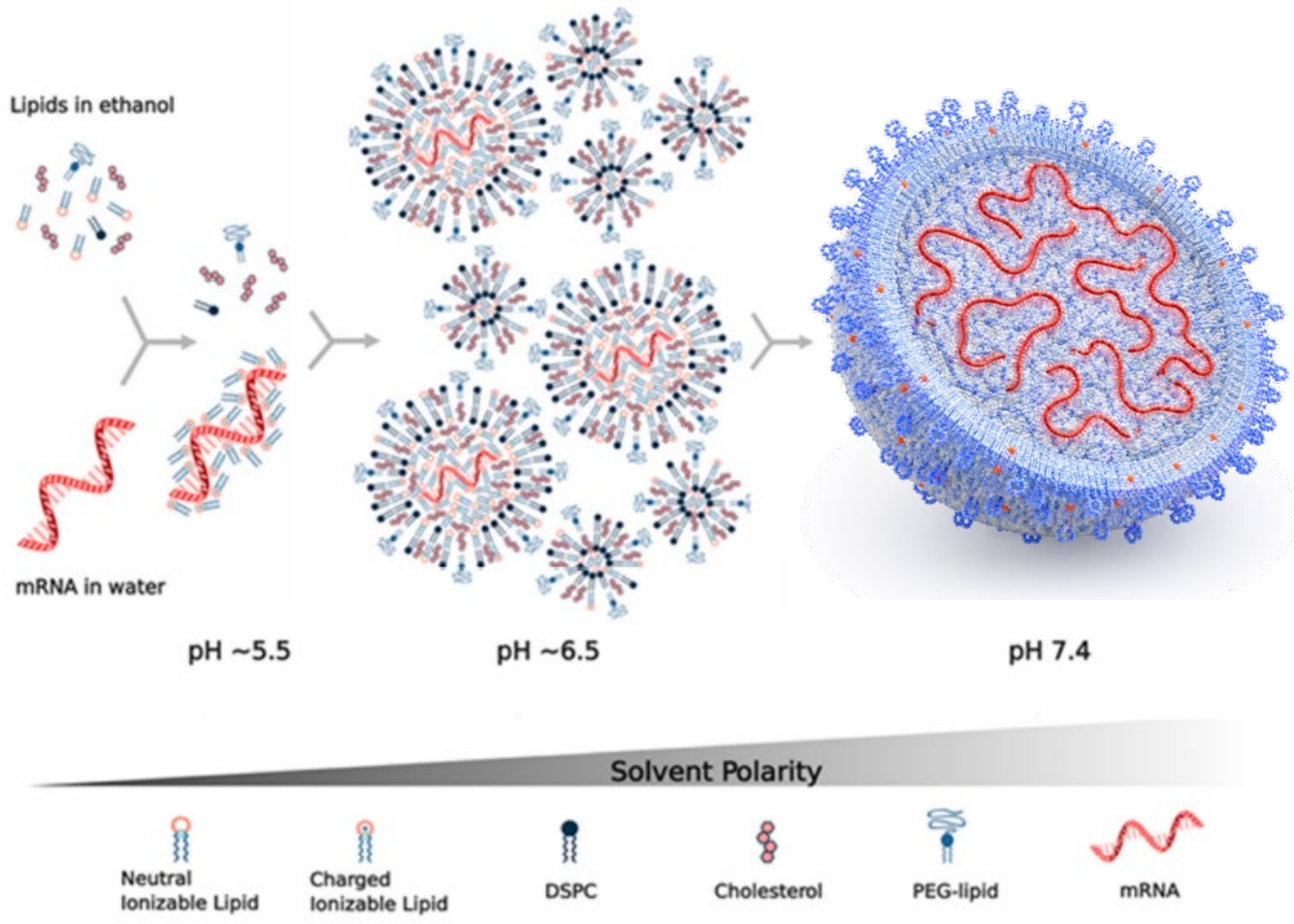
Rapid Mixing changes the solvent polarity, causing lipids to self-assemble.

↓

Under acidic conditions, ionized lipids interact electrostatically with mRNA.

↓

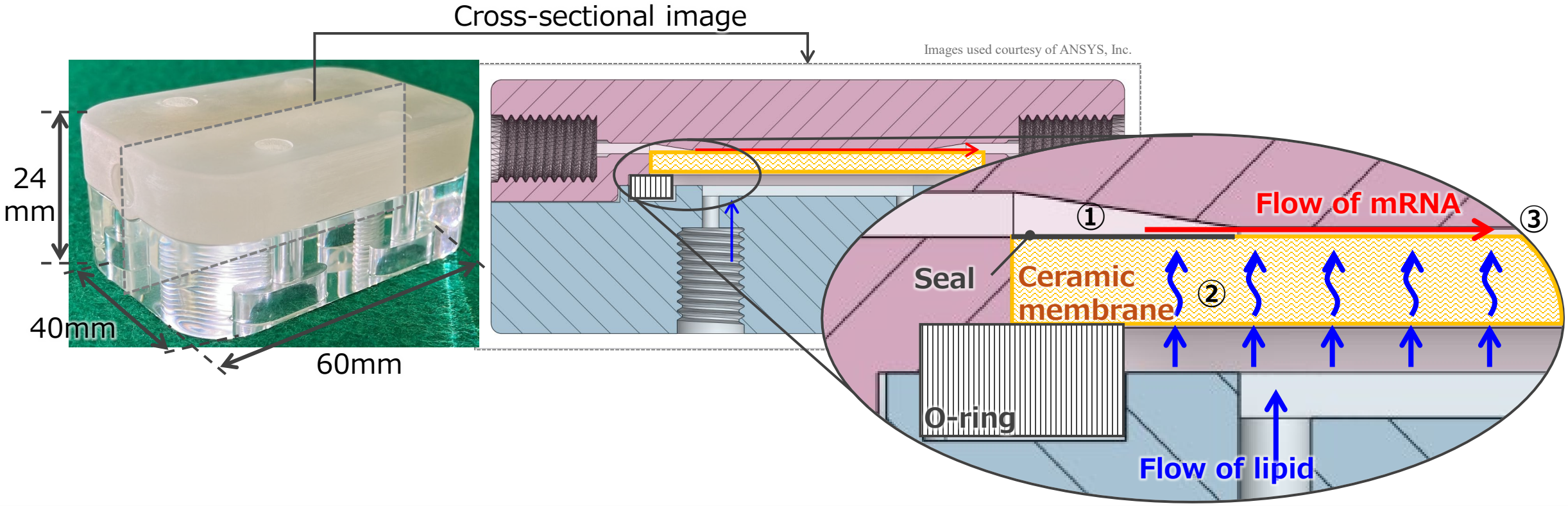
At neutral pH, the particle surface stabilizes, forming mRNA-containing LNPs.



Buschmann, Michael D., et al. "Nanomaterial delivery systems for mRNA vaccines." Vaccines 9.1 (2021): 65. より引用・一部改変

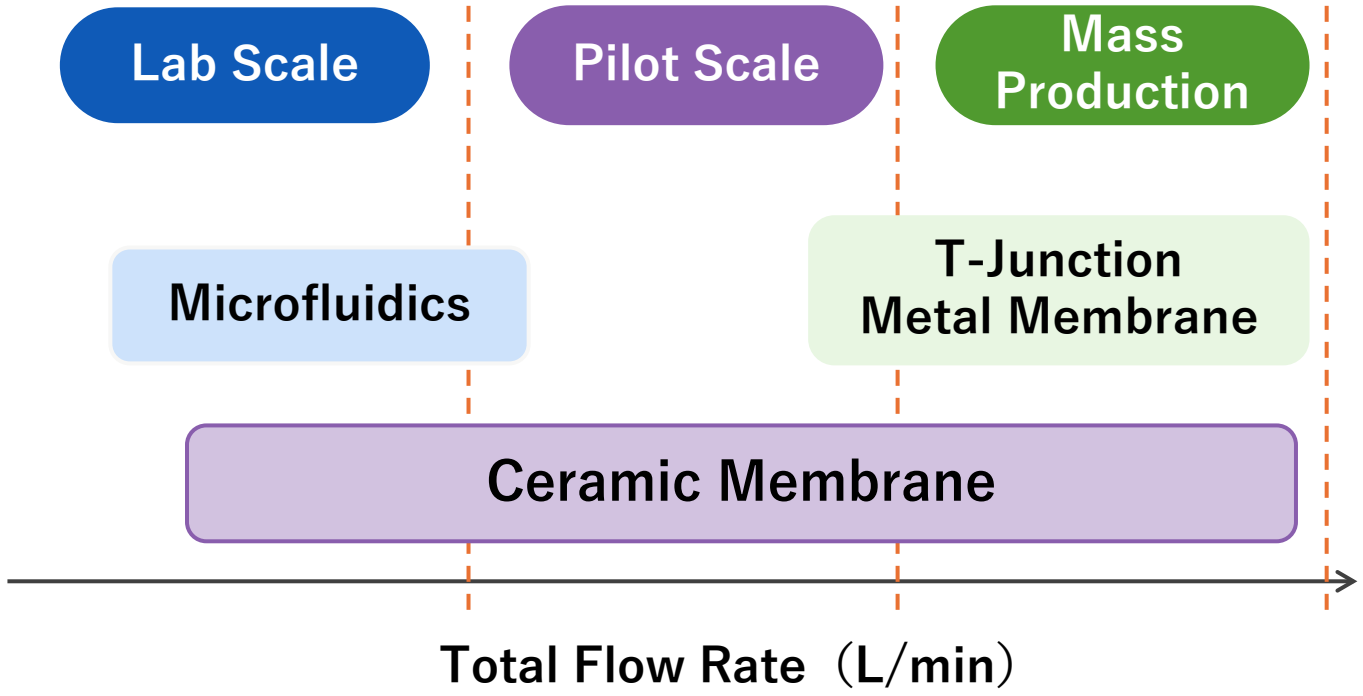
provided by TAIYO HOLDINGS CO., LTD.

NGK's Ceramic Membrane-Based LNP Manufacturing Device



① The API solution(i.e. mRNA solution) flows across the surface of the ceramic membrane from the left side of the device.
② The lipid solution is fed through the bottom of the device and permeates through the ceramic membrane towards the top.
③ As the lipid solution permeates through the numerous pores of the membrane it mixes with API solution flowing across the membrane surface leading to LNP formation. The LNPs are collected from the outlet on the right side of the device.

Scalability: Issues with current methods and their solution

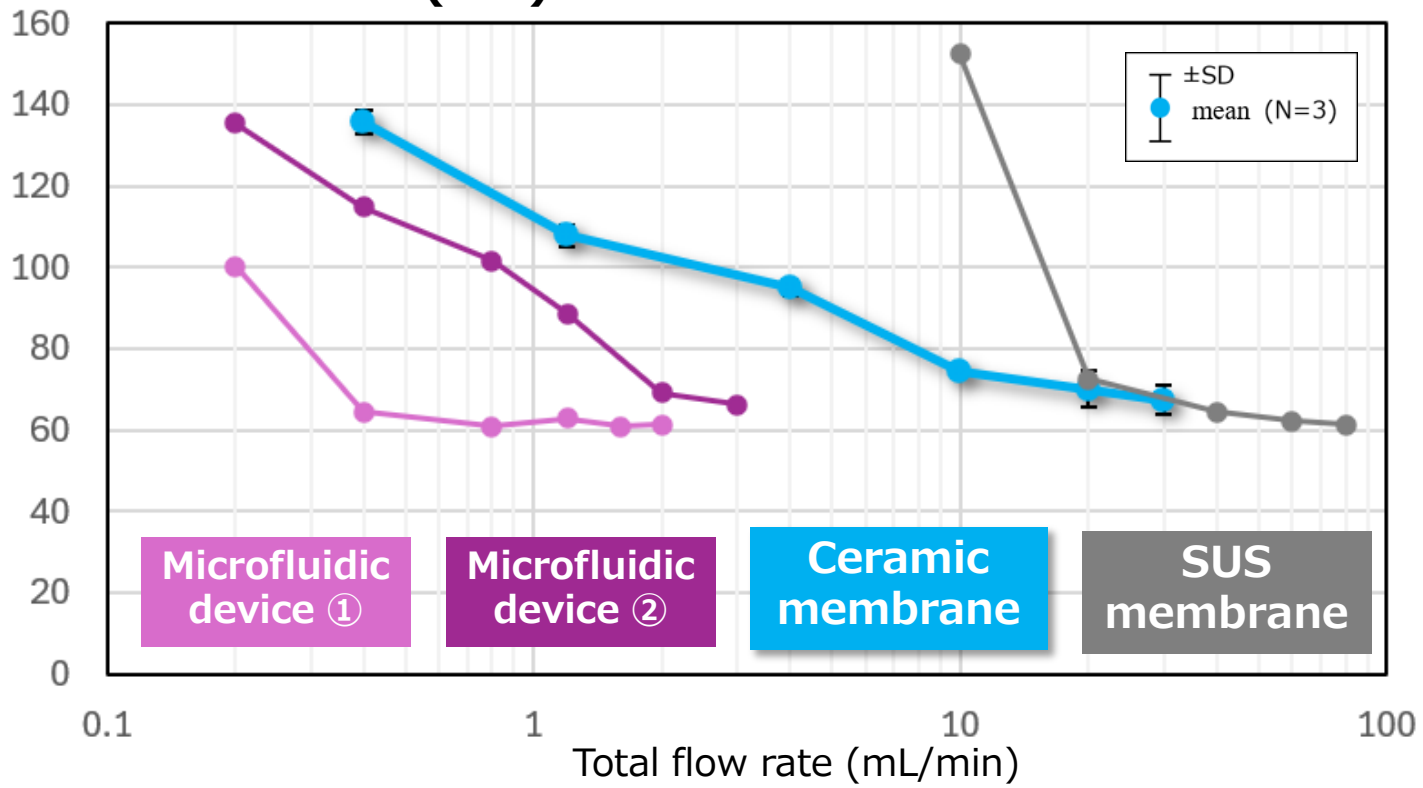


Microfluidics : Difficult to scale up due to LNP aggregation making high flow rates difficult
Metal Membrane : Difficult to scale down as limitations in pore diameters makes forming LNPs at low flow rates difficult.
T-Junction: Difficult to scale down as LNPs are formed by rapid mixing at high speeds makes forming LNPs at low flow rates difficult.
Ceramic Membrane : Adjusting membrane area and porosity enables scalability from laboratory development to commercial production.

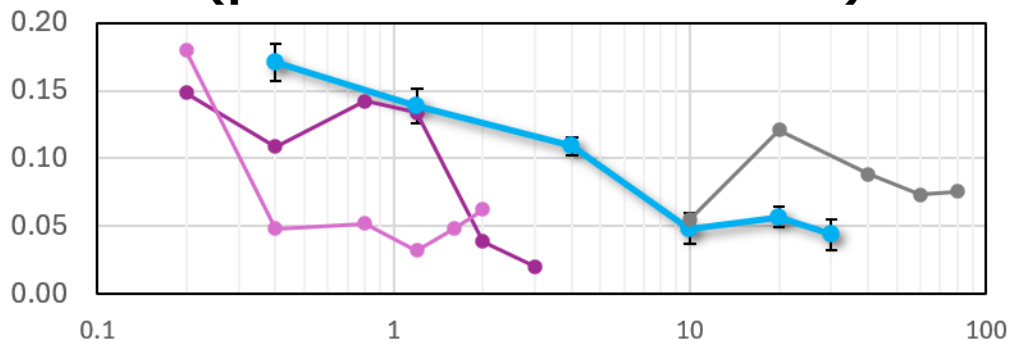
The solution to the issue of scalability

Evaluated by TAIYO HOLDINGS CO., LTD.

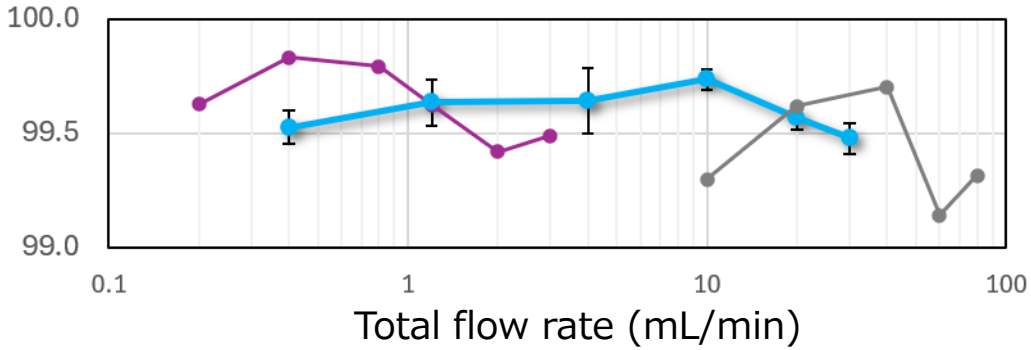
Particle size(nm)



PDI(particle size distribution)



Encapsulation efficiency(%)

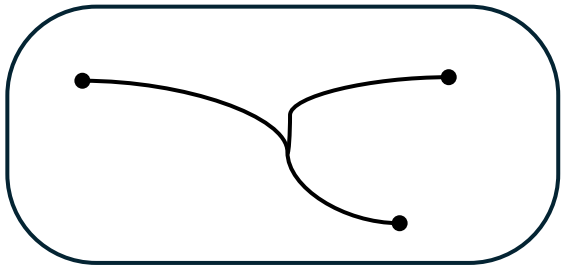
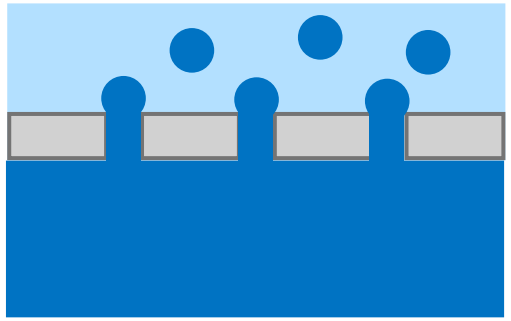
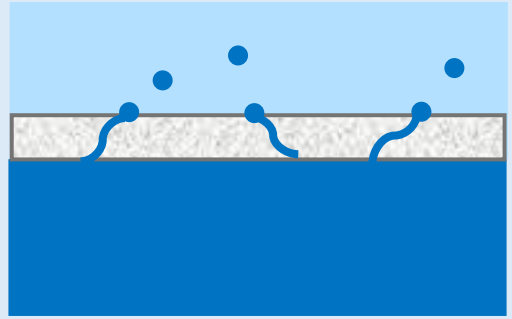


Microfluidic device ① : iLiNP-1.0S
Microfluidic device ② : iLiNP-1.0SW
SUS membrane : AXF-mini

Ceramic membrane technology maintains particle characteristics across a broad operating flow-rate range.

In this evaluation, a Spikevax-type lipid formulation and polyA as an mRNA substitute was used.

Single-Use Operation, Chemical resistance: Issues with current methods and their solution

	Microfluidics	Membrane Emulsification ～Metallic membrane～	Membrane Emulsification ～Ceramic membrane～
			
Single-Use Operation	Single usage considering the cost and replicability of microchip	Single-use operation is challenging due to membrane cost.	Replaceable membrane design enables single-use operation.
Chemical Resistance	If the material is plastic certain solvents may cause channel deformation, altering mixing conditions.	High chemical stability due to the material being metal.	High chemical stability due to the material being ceramic.

In vitro evaluation between conventional method and NGKs method

Evaluated at the Faculty of Pharmaceutical Sciences, Kyushu University,

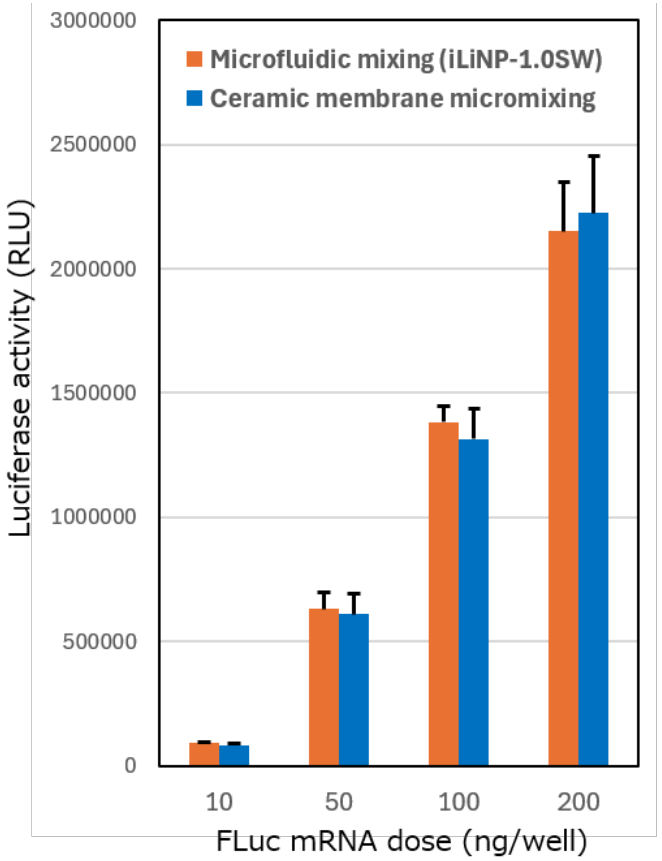
LNP Characterisitcs		Microfluidic (iLiNP-1.0SW)	Ceramic Membrane
Particle size	nm	84.5	84.1
PDI	–	0.131	0.077
Zeta potential	mV	+2.8	+2.5
Encapsulation efficiency	%	95.0	95.9

Concentration : 10μg FLuc-mRNA/mL
Dosage : 1, 5, 10, 20μL/well
No. of cells : 2×10^4 cells/50μL/well
Measurement : 24hrs
N : n=5

The LNPs prepared using the ceramic membrane emulsification method showed *in-vitro* FLuc expression comparable to conventional microfluidic systems

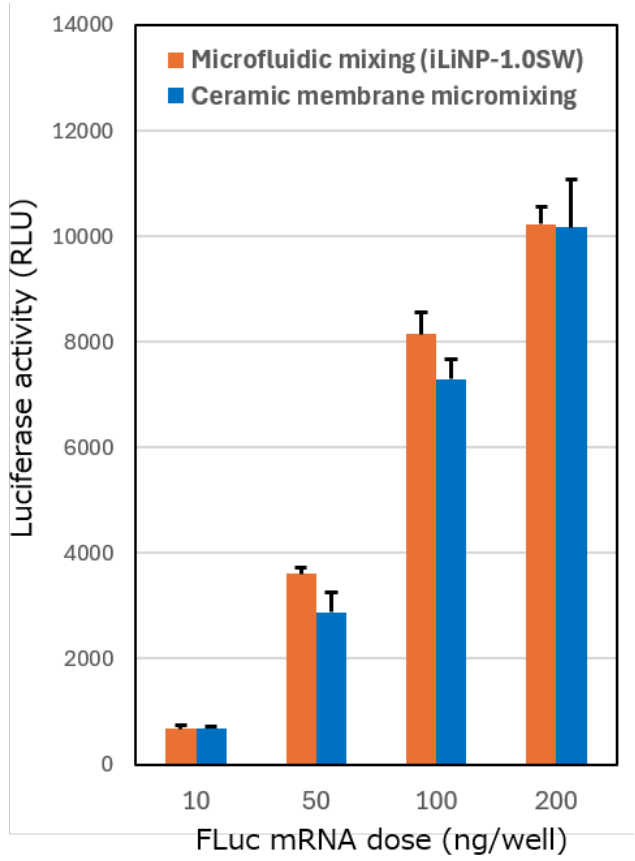
RAW264.7 (Mouse macrophage-like cells)

mean ± S.D.



THP-1 (Human monocytic cells)

mean ± S.D.

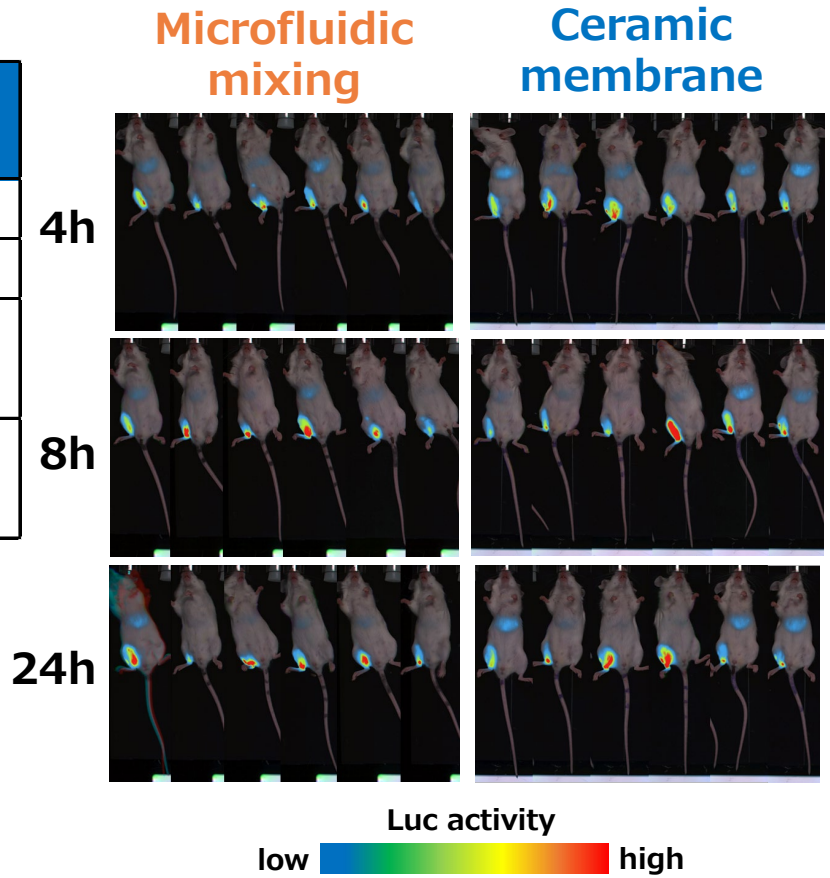


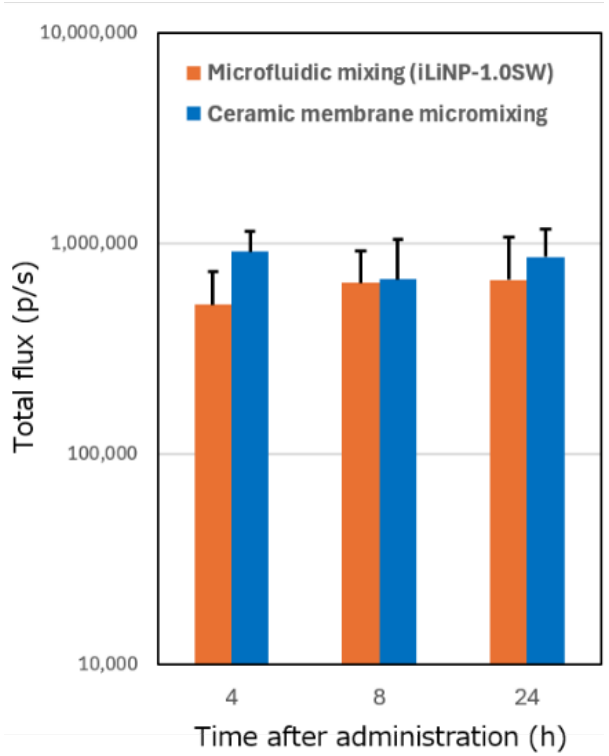
In vivo evaluation: FLuc expression activity

Evaluated at the Faculty of Pharmaceutical Sciences, Kyushu University,

LNP Characterisitcs		Microfluidic (iLiNP-1.0SW)	Ceramic Membrane
Particle size	nm	84.5	84.1
PDI	–	0.131	0.077
Zeta potential	mV	+2.8	+2.5
Encapsulation efficiency	%	95.0	95.9

LNP conc. : 10μg FLuc-mRNA/mL
Mouse : BALB/c Female, 6 weeks of age
Dosage : 0.1μg mRNA/10μL/mouse
Injection site : calf muscle
Measurement : 4h, 8h, 24h
n : n=6



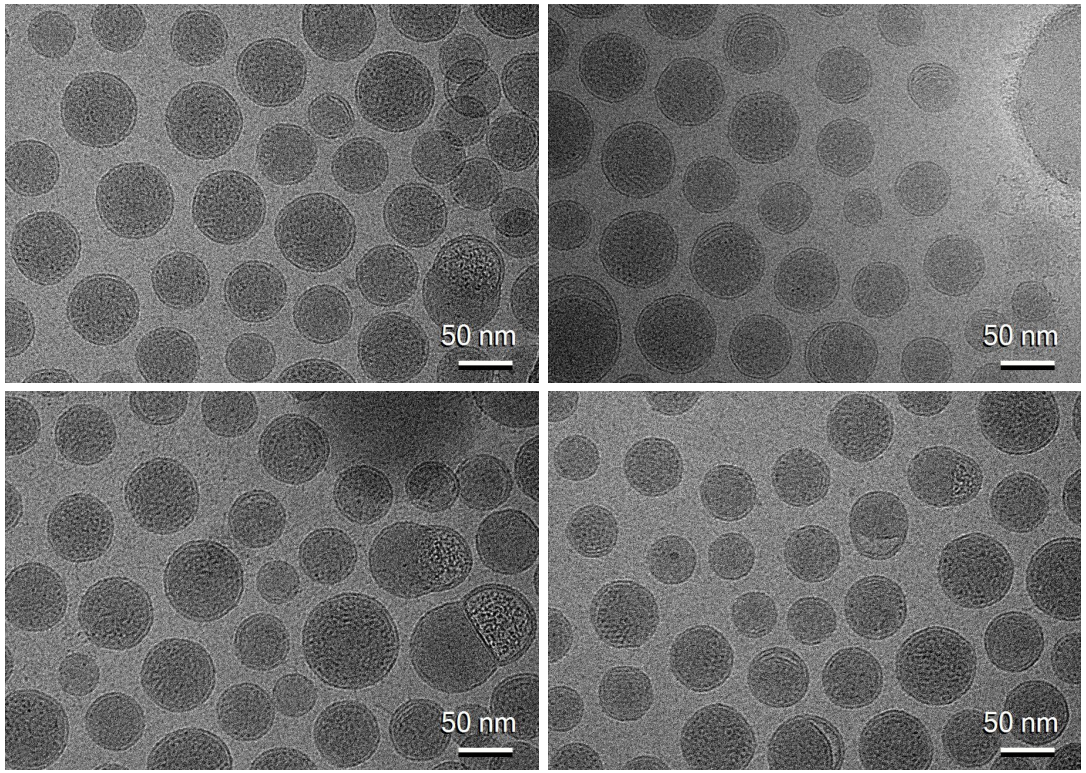


The LNPs prepared using the ceramic membrane emulsification method showed *in-vivo* FLuc expression activity comparable to conventional microfluidic systems.

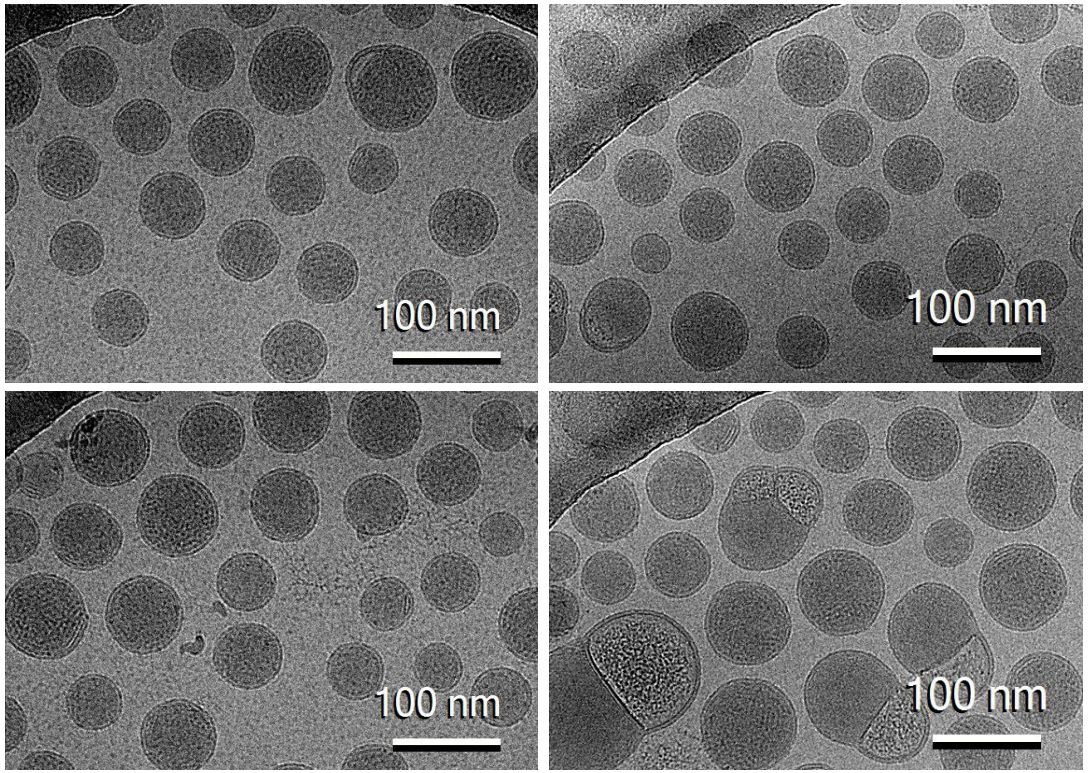
Cryo-TEM Analysis of FLuc mRNA-LNPs

Evaluated at the Faculty of Pharmaceutical Sciences, Kyushu University,

Microfluidic (iLiNP-1.0SW)



Ceramic Membrane



LNPs prepared using the ceramic membrane exhibited structural characteristics comparable to those produced using conventional microfluidic systems.