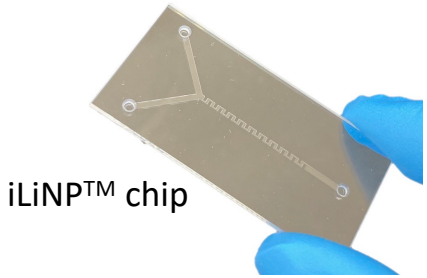
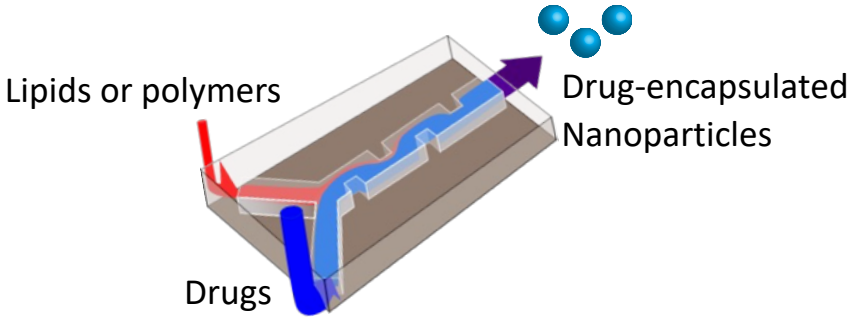
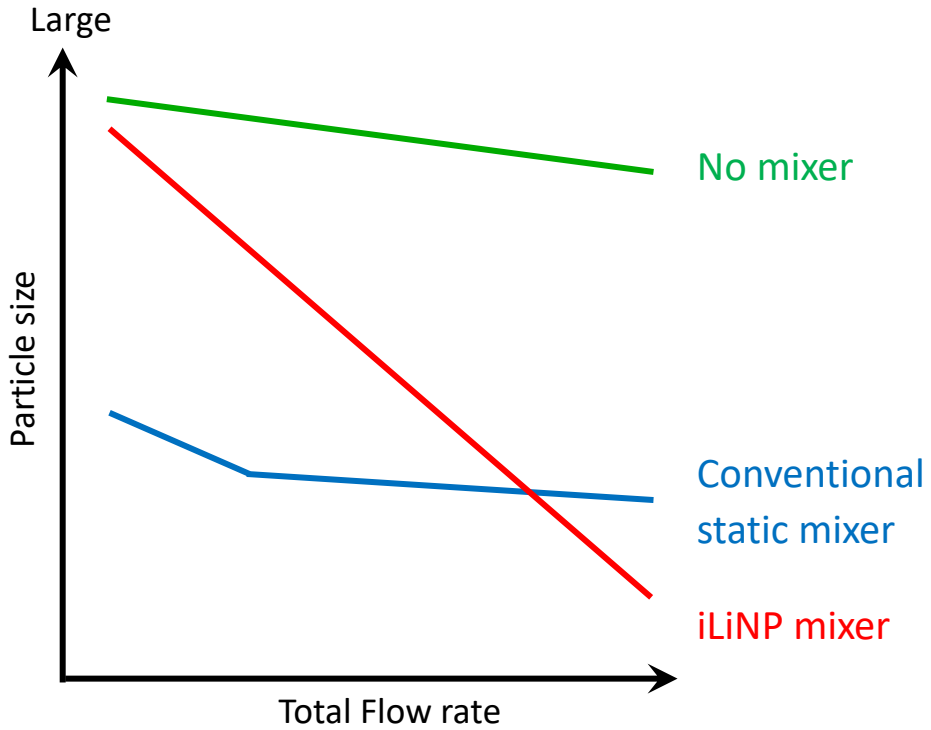
Oct 2023

iLiNP™ Microfluidic Products and Systems for Development and Manufacturing of Innovative Nanoparticle Medicines/Vaccines

The iLiNP™ Microfluidic Technology for manufacturing of nanoparticles



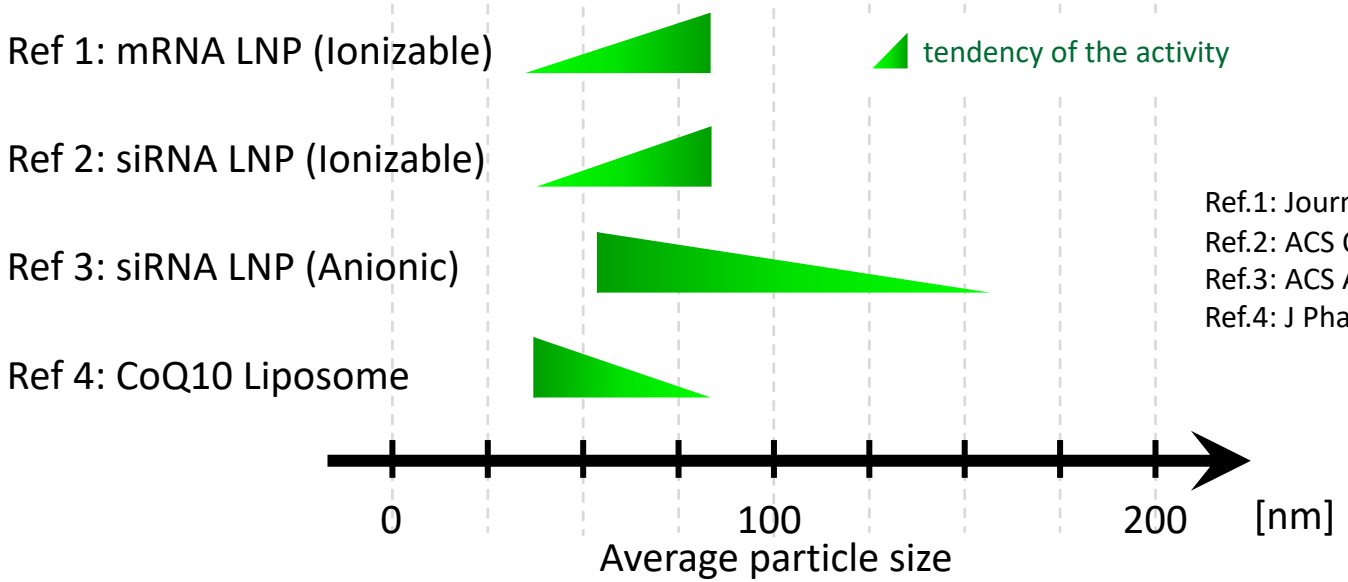




- The iLiNP is a kind of static mixer to make lipid or polymer nanoparticles by mixing its ingredients. (dissolved in water-miscible organic solvent) with drug (dissolved in water). Such a mixing method is currently accepted by pharmaceutical companies as a manufacturing method of non-clinical and clinical-grade nanoparticles medicines and vaccines.
- The iLiNP's unique microchannel structure enables us to control the particle size widely by changing the flow condition. The size controllability is enhanced when preparing Liposomes. Please visit our website (https://global.lilacpharma.com/eng/ilinp_technology_eng/) for the details of the mechanism.
- The patent covering the channel structure is allowed/issued in Japan, USA, Europe (Germany, France, UK, Swiss), Canada, China and Taiwan.

Importance of Particle Size Control

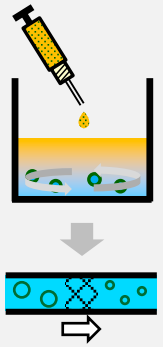
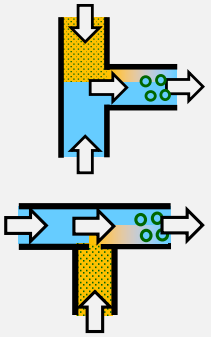
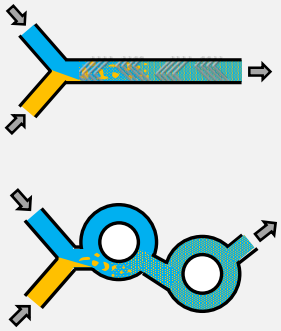
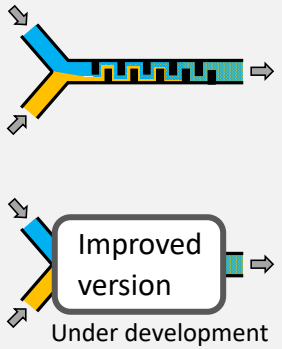
Recent research papers indicate that the biological activity of the nanoparticle depends on its size.



Ref.1: Journal of Controlled Release 327 (2020) 467–476
Ref.2: ACS Omega 2018, 3, 5044-5051
Ref.3: ACS Appl. Bio Mater. 2021, 4, 2, 1783–1793
Ref.4: J Pharm Sci. 2019 Aug;108(8):2668-2676

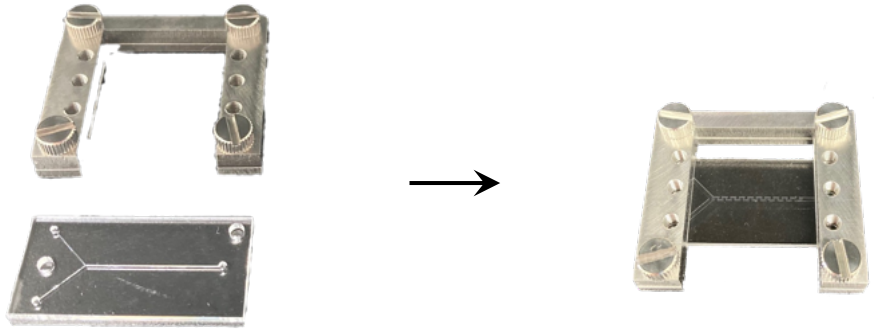
The iLiNP technology is easily adaptable to future particle size adjustment needs.

The iLiNP is Next Generation Technology for Manufacturing of Nanoparticles

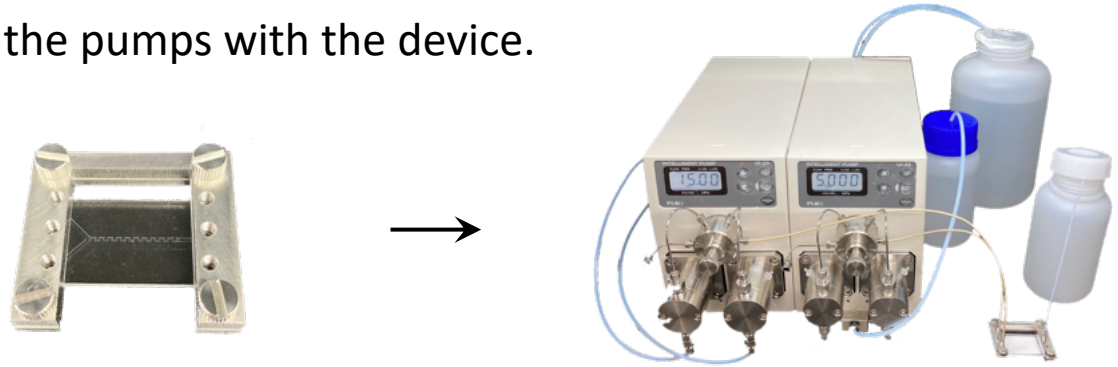
	1st Gen - 2010s	2nd Gen - 2021	3rd Gen Current	4th Gen Now and in the future
Type	Batch mixing & Extrusion 	In-line macro-mixing 	Rapid micro-mixing 	Controlled micro-mixing  Under development
Company /Product	Many	Polymun etc.	Precision Nano. /NanoAssemblr etc.	Lilac pharma /iLiNP
Scale-up	Difficult	Easy	Easy	Easy
Reproducibility	Difficult	Difficult	Reproducible	Reproducible
Particle size control by flow condition	Difficult	Difficult	Limited range	Wide range

How to Make Nanoparticles by Lilac's Microfluidic Products

1) Assemble the manufacturing device with the iLiNP microfluidic chip and its accessories.



2) Connect the pumps with the device.



3) Pump the ingredients solutions into the flow path to make nanoparticles.
4) Collect the nanoparticles suspension from the outlet of the device.
5) (Optional) Remove remaining organic solvent from the suspension by dialysis, etc.
6) (Optional) Concentrate the suspension by Ultrafiltration, etc.
7) Evaluate particle size distribution, surface charge (Zeta potential), etc. of the nanoparticles.

iLiNP1.0, iLiNP2.0

The most cost-saving models.

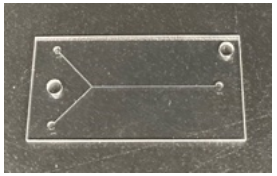
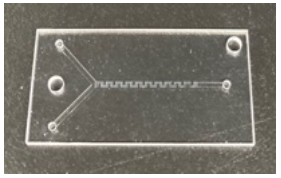
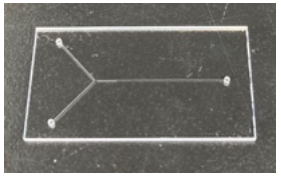
Pumps (Third party's product)

Nanoparticles manufacturing device
(iLiNP 1.0 or 2.0 chip + Accessories)

- This model consists of microfluidic chip and its accessories. Pumps are not included.
- You can choose base material (COP plastic resin or quartz glass) of the chip and channel width (narrow or wide), depending on the intended use.

iLiNP1.0, iLiNP2.0

The most cost-saving models.

Microfluidic chip	Accessories	Pump	Recommended for a person who want to
 **iLiNP1.0S (narrow channel)** Base material: COP plastic resin			- Save initial cost. - Save consumption of ingredients.
 **iLiNP1.0SW (wide channel)** Base material: COP plastic resin	 **Accessories set for iLiNPx.x**	Syringe pumps, Plunger pumps, etc. (Third party's product)	- Manufacture nanoparticles at a high flow rate condition. - Avoid clogging of flow path.
 **iLiNP2.0S (narrow channel)** Base Material: Quartz glass			- Use various types of organic solvents other than alcohol.

Specifications of the Microfluidic Chips iLiNP1.0 and 2.0

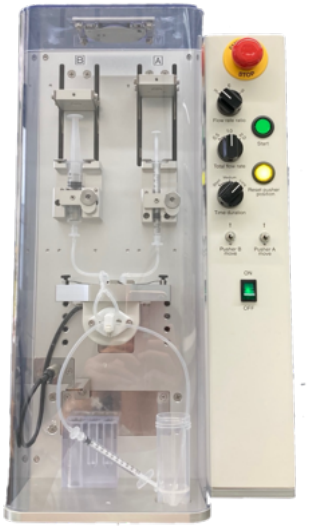
Product Name	iLiNP1.0		iLiNP2.0
Type	S	SW	S
Base material	COP (Cycloolefin polymer)		Quartz glass
Channel width	narrow	wide	narrow
Dead volume	Low	High	Low
Total flow rate in use (Recommended)	0.1 - 1.0 mL/min	0.3 - 6.0 mL/min	0.1 - 1.0 mL/min
Resistance to organic solvents other than Alcohol	Poor		Good
Resistance to hydraulic pressure	Poor		Good
Price	ask	ask	ask
Remarks			High-performance pumps may be required at a higher flow rate condition because of high back pressure.

LiNAS Series

Low-running cost models.

LM microfluidic chip

LiNAS-mini



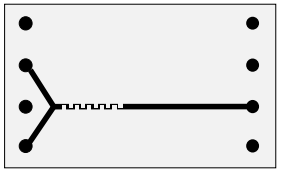
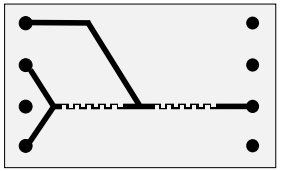
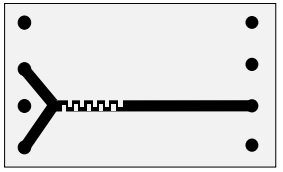
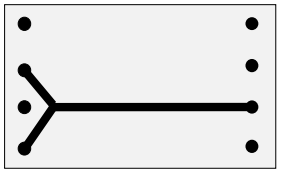
LiNAS-S platform

LiNAS-M platform

- This model adopts PDMS-made microfluidic chip (LM chip) that is low-cost but high quality.
- High-precision syringe pumps are integrated in the platforms, except for LiNAS-mini.
  (For LiNAS-mini, pumps are additionally required for the use.)

LM microfluidic chips

Low-running cost models.

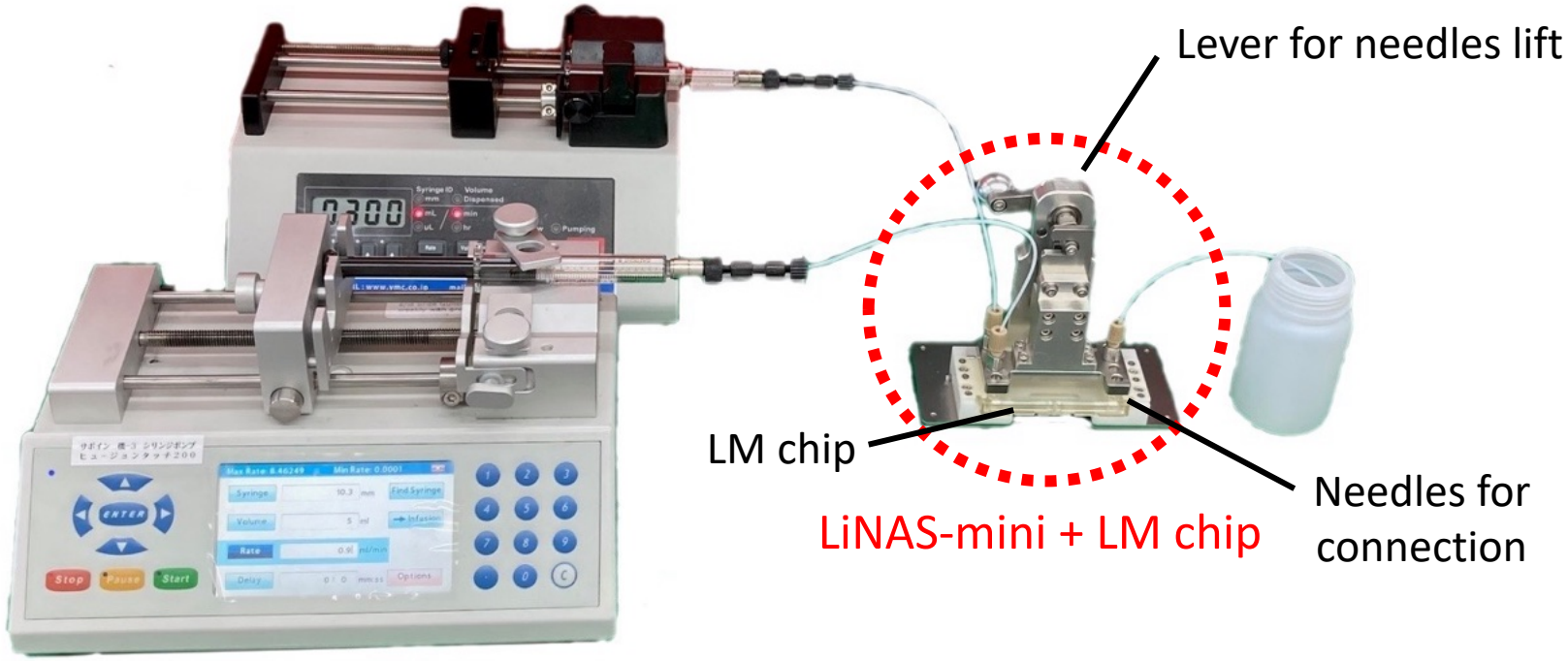
Microfluidic chip	Channel shape (Schematic)	Recommended for a person who want to
 **LM-iLiNP001 (narrow channel)** Base material: PDMS		- Save consumption of ingredients.
 **LM-iLiNP002 (3-inlets, 2x iLiNPl)** Base material: PDMS		- Suppress enlargement of particle size by rapid dilution. - Challenge on-device screening of lipid composition.
 **LM-iLiNP003 (wide channel)** Base Material: PDMS		- Manufacture nanoparticles at a high flow rate condition. - Avoid clogging of flow path.
 **LM-W001 (straight channel)** Base Material: PDMS		- Enlarge particle size in combination with low flow rate pumping.

LiNAS Series

Low-running cost models.

Microfluidic chip	Platform		Recommended for a person who want to
 **iLiNP chips** **LM-iLiNP001 (narrow channel)** **LM-iLiNP002 (3-inlets, 2xiLiNP)** **LM-iLiNP003 (wide channel)** **Normal chip** **LM-W001 (straight channel)**	 **LiNAS-mini**	+ Syringe pumps, Plunger pumps, etc. (Third party's product)	- Use a new microfluidic chip frequently. - Save initial cost.
	 **LiNAS-S platform**		- Use a new microfluidic chip frequently. - Manufacture nanoparticles by simple operations. - Manufacture nanoparticles under sterile condition.
	 **LiNAS-M platform**		- Use a new microfluidic chip frequently. - Challenge various flow conditions easily. - Manufacture tens of mL of nanoparticles.

LiNAS-mini

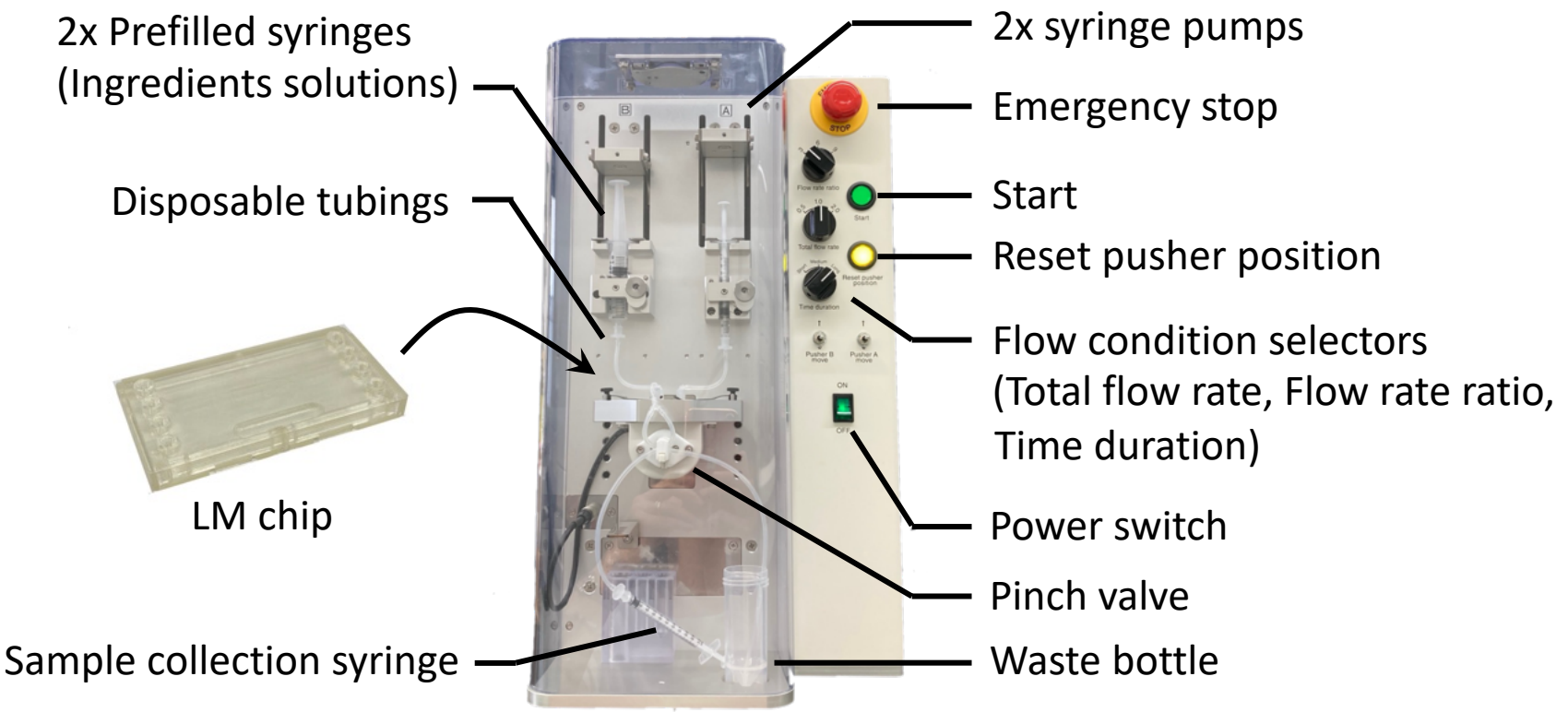


Pumps (Third party's product)

Features

1. This model consists of microfluidic chip and its accessories. Pumps are not contained.
2. Easy connection of the dedicated microfluidic chip with the pumps by original mechanism.

LiNAS-S: Simple Platform

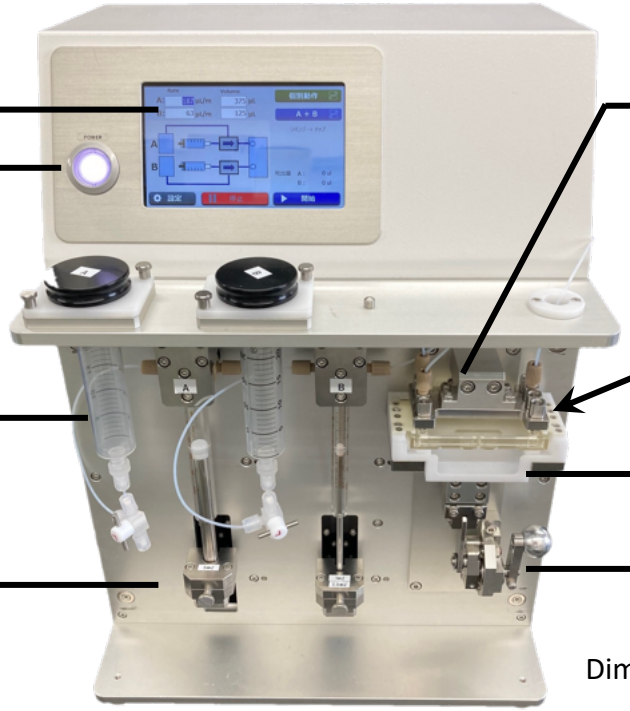


The photo is a prototype. The actual product may vary.

Features

1. Very easy operation; Select total flow rate (TFR), flow rate ratio (FRR) and time duration from preset typical conditions, then press start button. That's all.
2. Nanoparticles can be manufactured aseptically by using sterilized syringes, microfluidic chip and tubings even if you do not have any clean bench.

LiNAS-M: Multi Functional Platform



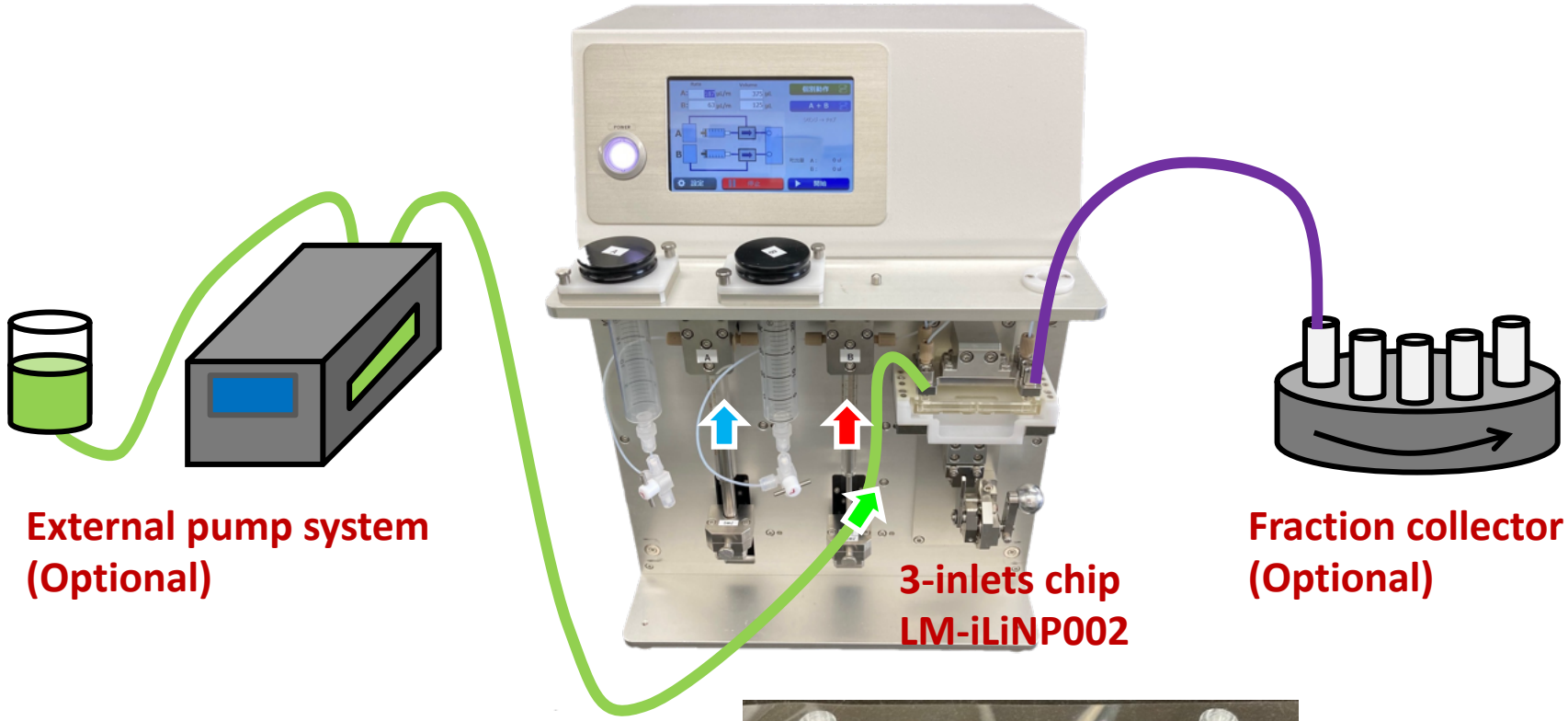
Dimension : 250(W)×300(D)×408(H)mm

New version (LM-003) will be on sale in October 2023.

Features

1. High reproducibility of nanoparticle production is achieved by using a high-precision pump.
2. Easy connection of the dedicated microfluidic chip with the pumps by original mechanism.
3. Easy to set flow conditions and operate pumps via touch panel.
4. Easy to try several pumping conditions using the same ingredients solutions and chip.
5. Easy to manufacture tens of mL of nanoparticles by repeated pumping in combination with using reservoir.
6. High flexibility of piping allows customers to easily customize the pumping route.

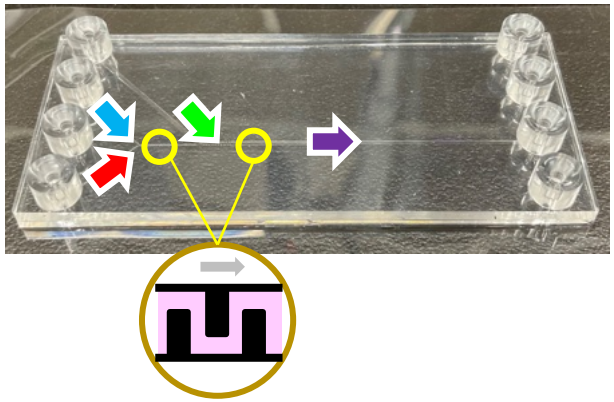
Expansion of the Platform for the use of 3-Inlet Type Microfluidic Chip



External pump system
(Optional)

3-inlets chip
LM-iLiNP002

Fraction collector
(Optional)

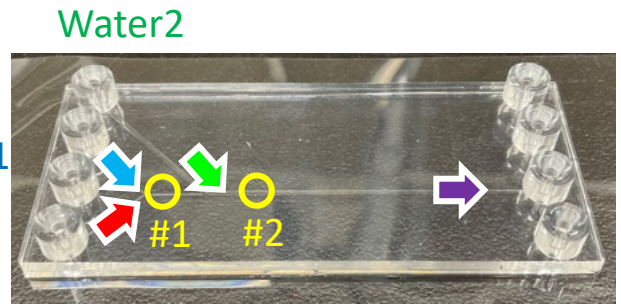


2x iLiNP

3-Inlets Type Microfluidic Chip: Applications

Application #1

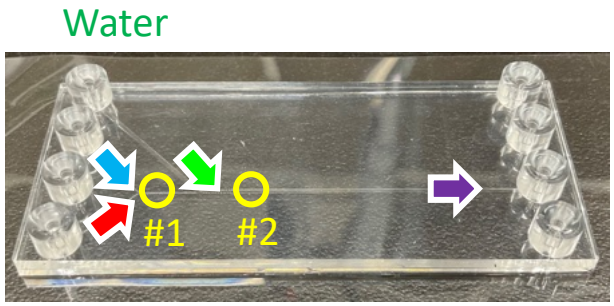
On-device rapid dilution for reduction of particle size.



The lipid nanoparticles suspension formed in the iLiNP #1 region is rapidly diluted with water (diluent) in the iLiNP #2 region. This Rapid dilution will prevent the undesirable aggregation or fusion of lipid nanoparticles even at the high-lipid-concentration condition. This contributes to reduction of the particle size and improvement of the polydispersity index (PDI). (ACS Appl. Mater. Interfaces 2020, 12, 34011−34020.)

Application #2

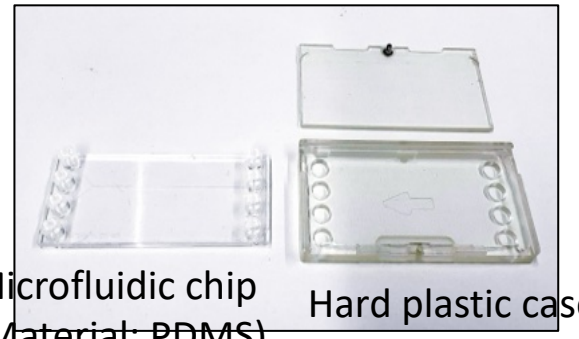
On-device screening of lipid composition.



Lipid mixture solution is prepared in the iLiNP #1 region and then mixed with water (or aqueous drug solution) in the iLiNP #2 region to form lipid nanoparticles. The lipid composition in the lipid mixture can be easily changed by changing the flow rate ratio of lipid solutions (Lipid1, Lipid2). This eliminates the need to exchange the syringe each time, which is necessary for other companies' products, and contributes to streamline the formulation study. (ACS Appl. Eng. Mater. in press. https://doi.org/10.1021/acsaenm.2c00062)

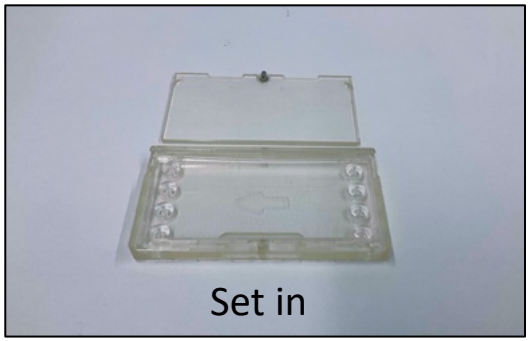
Microfluidic Device Assembly (LM chip) & Device Installation (LiNAS-M)

1. Assembly

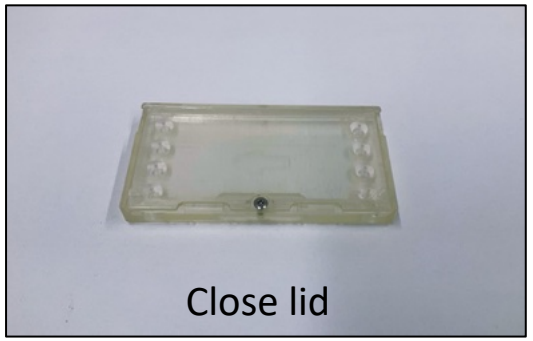


Microfluidic chip
(Material: PDMS)

Hard plastic case

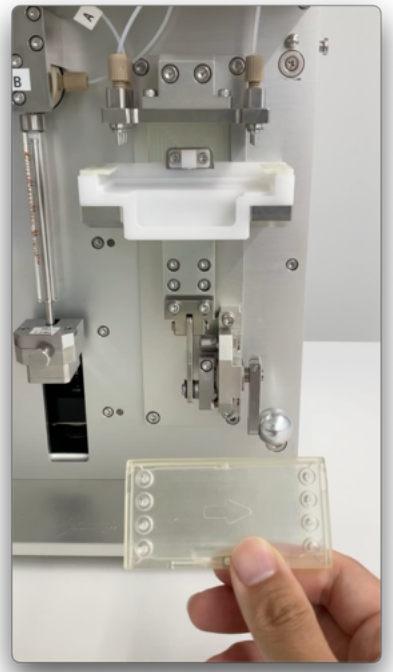


Set in



Close lid

2. Installation



Insert

Lift-up

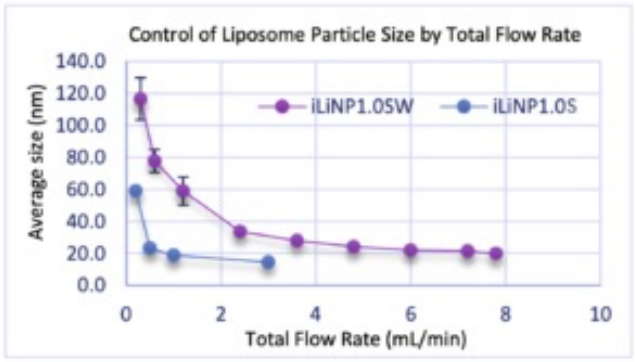
Specifications of the Microfluidic Chips LM-iLiNP001, 002, 003 and W001

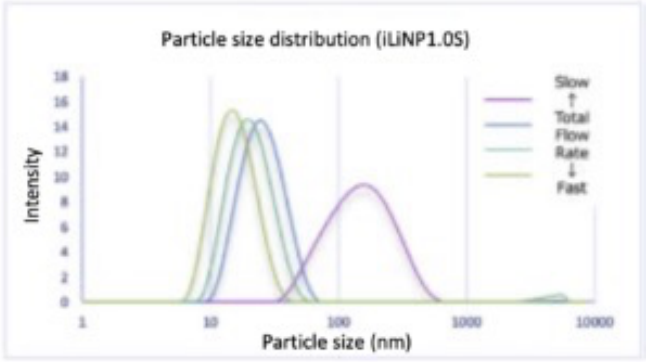
Product Name	LM-iLiNP			LM-W
Type	001	002	003	001
Base material	PDMS (Poly-dimethyl siloxane)			
Channel width	narrow	narrow	wide	semi-wide
Dead volume	Low	Low	High	High
Total flow rate in use (Recommended)	0.1 - 1.0 mL/min	0.1 - 1.0 mL/min	0.3 - 6.0 mL/min	0.1 - 5.0 mL/min
Resistance to organic solvents other than Alcohol	Poor			
Resistance to hydraulic pressure	Poor			
Price	ask	ask	ask	ask
Remarks		3-inlet model. 2x iLiNPs are sequentially placed. An additional pump is required for the use.		Straight channel.

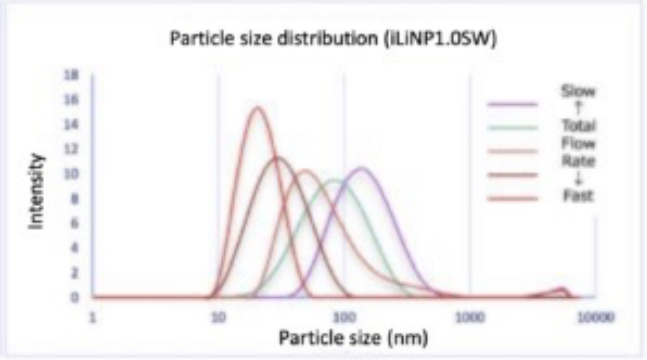
Applications

Case 1: Control of Liposome Particle Size by Total Flow Rate

- To investigate the particle size controllability characteristic of the iLiNP device, we measured the change in liposome average particle size as a function of flow rate variation.
  Lipid: POPC
  Device: iLiNP1.0S, iLiN1.0SW (wide channel)
  Flow conditions: TFR=0.1-7.8mL/min, FRR=5
 - After mixing POPC/ethanol solution and saline solution in the iLiNP device, the average particle size of liposomes generated was measured.



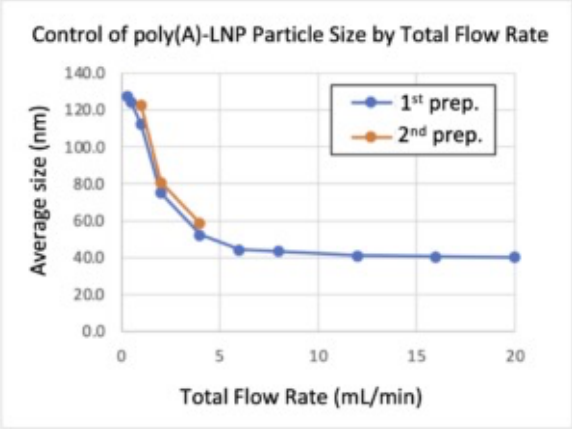




- Both devices were able to vary the average particle size significantly by changing the total flow rate.

Case 2: Control of poly(A)-LNP Particle Size by Total Flow Rate

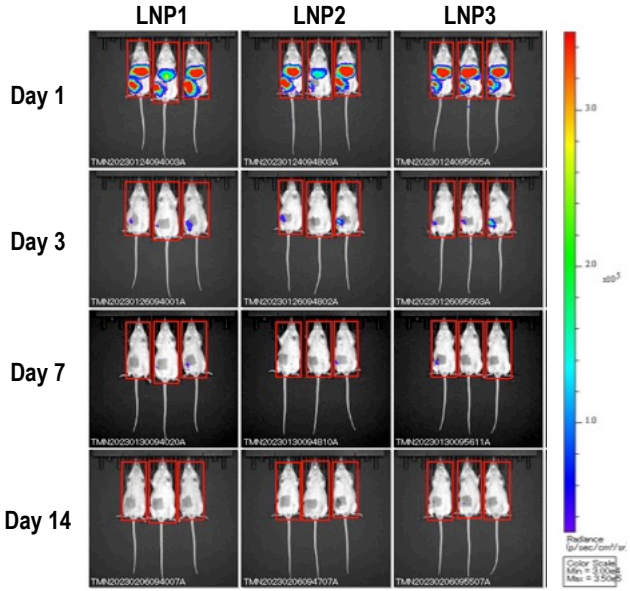
- To investigate the particle size controllability characteristic of the iLiNP device, we measured the change in poly(A)-LNP average particle size as a function of flow rate variation.
  Lipid: Ionizable lipid + helper lipids
  Device: iLiNP-RoA (new model)
  Flow conditions: TFR=0.3-20mL/min, FRR=3
- After mixing lipid/ethanol solution and poly(A)/acetate buffer solution in the iLiNP device, the average particle size of poly(A)-encapsulated lipid nanoparticles generated was measured.

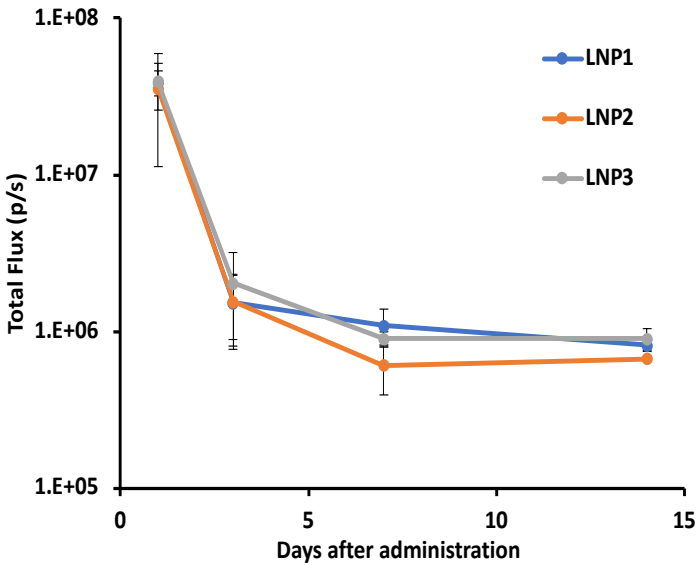


- Even in the case of nucleic acids-encapsulated LNPs, the average particle size could be varied significantly by changing the total flow rate.
- We have confirmed that there is no problem in the reproducibility of the production of LNPs with a particle diameter of 80-100 nm, which is a frequently requested range.
- It should be noted that the range of change in particle size varies greatly depending on the lipid used, the nucleic acid encapsulated, and the concentration of the lipid. For example, the particle size may not be smaller than expected in the case of payload with a large molecular weight.

Case 3: Manufacturing of mRNA-Encapsulated Lipid Nanoparticles and Evaluation of Effect of Remaining Ethanol on mRNA Delivery Activity

- Manufacturing of mRNA-encapsulated lipid nanoparticles by using the iLiNP device.
(Lipids: ALC-0315 + helper lipids. mRNA: Fluc mRNA (5moU). Device: LM-iLiNP001 equivalent (handmade device). Flow conditions: TFR=0.5mL/min, FRR=3. Post-treatment: dialysis against PBS buffer.)
- After the dialysis, Ethanol (final conc: 9% or 18%) is added to the LNP suspension.
- The LNP suspensions (0.01 mg/mouse as mRNA dose) was inoculated by a single intramuscular injection into the thigh of one hind paw of ICR mice, and 1 day, 3 days, 1 week and 2 weeks later, the expression level of luciferase was quantified as luminescence.



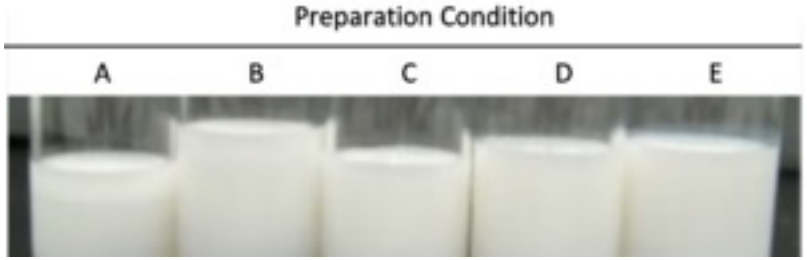


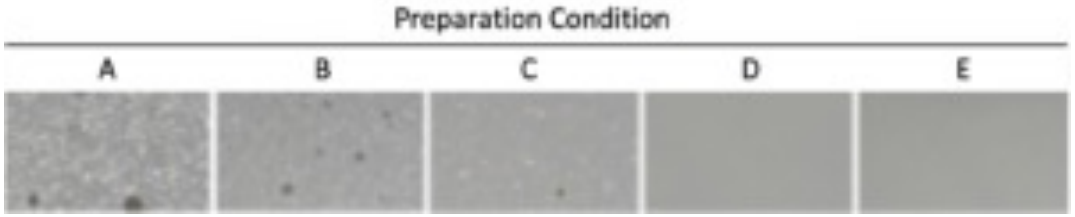
LNP1: mRNA LNP in PBS
LNP2: mRNA LNP in PBS + 9% EtOH
LNP3: mRNA LNP in PBS + 18% EtOH

- The mRNA LNPs manufactured by the iLiNP have enough activity to deliver the mRNAs into the cells.
- The mRNA LNPs manufactured by the iLiNP are tough enough and keep delivery activity even in the presence of high concentration of Ethanol.

Case 4: Preparation of O/W Type Emulsion

- The iLiNP device can also be used to produce particles (droplets) other than lipid nanoparticles. As an example, an O/W type emulsion (oil emulsion) was prepared using the iLiNP device, in which oil and water are vigorously mixed in a channel in the presence of a surfactant to form micro-droplets.





Left photo O/W emulsions of baby oil (liquid paraffin) made under different flow conditions. Synthetic surfactant used. Oil concentration is about 18%. Creaming occurs in the conditions A and B because large droplets were generated under these conditions. On the other hand, relatively small and uniform droplets were generated in the conditions C, D, and E, and thus no creaming is observed.
Right photo Stereomicroscopic images of the samples. In the conditions A and B, coexistence of large droplets is observed, but in the conditions C, D, and E, coexistence of large droplets is suppressed and the particle size is homogenized.

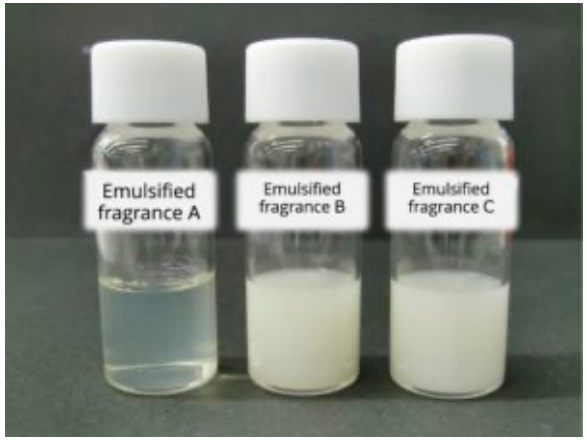
- The iLiNP has an excellent ability to control the mixing state by adjusting the flow rate, so it is possible to obtain droplets of the targeted particle size in a single step. As a result, "creaming," which tends to occur when large droplets coexist, is expected to be less likely to occur.
- The use of a palm-sized microfluidic device makes it possible to study emulsification conditions even with extremely small amounts of raw material liquid (~1mL). The small size of the device also makes on-site small-volume production of emulsions possible. If scaling up is required, the system can be flexibly adapted to meet demand by parallelizing and stacking the flow path chips.

Case 5: Preparation of PLGA Microspheres (Sustained Release Formulation)

- Micrometer-order particles can be prepared using the iLiNP device. As an example, microspheres made of PLGA (polylactic acid/glycolic acid copolymer), a biodegradable polymer, have been prepared. The microspheres can be used as a sustained release drug delivery system by encapsulating drugs.
- In the experiment, PLGA was dissolved in an organic solvent and mixed with an aqueous Camostat solution (model drug) to form a W/O emulsion, which was then suspended in a PVA solution to form a W/O/W emulsion. PLGA microspheres with an average particle size of approximately 0.5 µm and drug inclusion rate >99% were then obtained by removal of solvents.
- In each emulsification process, the iLiNP devices were used. Furthermore, by serializing iLiNP devices, W/O/W emulsions were successfully prepared continuously in one step from the raw material solution. By adjusting the pumping conditions, the particle size of microspheres can be further increased.

Case 6: Preparation of Emulsified Flavor

- As an example of application of emulsion preparation technology, we have made a prototype emulsified flavor.
- Emulsified flavor was obtained by mixing and emulsifying oil-soluble flavor (containing ethanol) and water in the presence of edible emulsifier, using the iLiNP (not-for-sale version) for emulsion production. Although the prepared sample contains about 25% ethanol, no increase in particle size or phase separation (creaming) was observed even after long-term storage at 4°C, and the particle size distribution remained unimodal. Samples in which ethanol is removed by dialysis also show similar stability.
- The high stability of the prototyped emulsified flavor is assumed to be due to the high particle size control characteristics of the iLiNP device.



Scientific Research Papers in which iLiNP was used (As of August 2023)

1. Development of the iLiNP Device: Fine Tuning the Lipid Nanoparticle Size within 10 nm for Drug Delivery.
ACS Omega 2018, 3, 5044−5051
2. The Use of a Microfluidic Device to Encapsulate a Poorly Water-Soluble Drug CoQ10 in Lipid Nanoparticles and an Attempt to Regulate Intracellular Trafficking to Reach Mitochondria.
J Pharm Sci. 2019 Aug;108(8):2668-2676
3. Development of a Microfluidic-Based Post-Treatment Process for Size-Controlled Lipid Nanoparticles and Application to siRNA Delivery.
ACS Appl. Mater. Interfaces. 2020 Jul 29;12(30):34011-34020
4. Lipid nanoparticles loaded with ribonucleoprotein–oligonucleotide complexes synthesized using a microfluidic device exhibit robust genome editing and hepatitis B virus inhibition.
J Control Release. 2021 Feb 10;330:61-71
5. One-Step Production Using a Microfluidic Device of Highly Biocompatible Size-Controlled Noncationic Exosome-like Nanoparticles for RNA Delivery.
ACS Appl. Bio Mater. 2021, 4, 1783−1793
6. Ultra-small lipid nanoparticles encapsulating sorafenib and midkine-siRNA selectively-eradicate sorafenib-resistant hepatocellular carcinoma in vivo.
J Control Release. 2021 Mar 10;331:335-349
7. Delivery of Oligonucleotides Using a Self-Degradable Lipid-Like Material.
Pharmaceutics. 2021 Apr 13;13(4):544.
8. Preparation of size-tunable sub-200 nm PLGA-based nanoparticles with a wide size range using a microfluidic platform.
PLoS One. 2022; 17(8): e0271050.
9. Microfluidic Device-Enabled Mass Production of Lipid-Based Nanoparticles for Applications in Nanomedicine and Cosmetics.
ACS Appl. Nano Mater. 2022, 5, 6, 7867–7876
10. Microfluidic Platform Enabling Efficient On-Device Preparation of Lipid Nanoparticles for Formulation Screening.
ACS Appl. Eng. Mater. 2023, 1, 1, 278–286
11. Fine-tuning the encapsulation of a photosensitizer in nanoparticles reveals the relationship between internal structure and phototherapeutic effects.
J Biophotonics. 2023 Mar;16(3):e202200119.
12. Self-homing nanocarriers for mRNA delivery to the activated hepatic stellate cells in liver fibrosis.
J Control Release. 2023 Jan;353:685-698.
13. Controlling lamellarity and physicochemical properties of liposomes prepared using a microfluidic device.
Biomater Sci. 2023 Feb 8. doi: 10.1039/d2bm01703b. Online ahead of print.
14. Mass production system for RNA-loaded lipid nanoparticles using piling up microfluidic devices.
Applied Materials Today 31 (2023) 101754
15. Lipid nanoparticle-based ribonucleoprotein delivery for in vivo genome editing.
J Control Release. 355 (2023) 406-416
16. A system that delivers an antioxidant to mitochondria for the treatment of drug-induced liver injury.
Scientific Reports volume 13, Article number: 6961 (2023)
17. Construction of the systemic anticancer immune environment in tumour-bearing humanized mouse by using liposome- encapsulated anti-programmed death ligand 1 antibody-conjugated progesterone.
Frontiers in Immunology (2023) 14:1173728.