Supporting Information

Vertical numbering-up microfluidic architecture for scalable and homogeneous lipid nanoparticle production

Zhaoyu Zhang‡ [a], Jaejeung Kim‡ [a, b], Jinwoo Hwang [a], Hyunjo Seo [a], Geonha Kim [a], Seoyeon Choi [a, b], Kyung-A Hyun [c] and Hyo-ll Jung* [a, b, d]

[a] School of Mechanical Engineering, Yonsei University, 50 Yonsei-ro, Seoul, South Korea

[b] The DABOM Inc., 50 Yonsei-ro, Seoul, South Korea

[c] School of Biopharmaceutical and Medical Sciences, Sungshin Women's University, 55 Dobong-ro, Seoul, South Korea

[d] Department of Integrated Medicine, Yonsei University, 50 Yonsei-ro, Seoul, South Korea

‡ These authors contributed equally to this manuscript.

* These authors are corresponding authors.

E-mail address: uridle7@yonsei.ac.kr (H. I. Jung)

Telephone number: +82-(0)2-2123-5814

Fax number: +82-(0)2-312-2159

2.3.1. Microfluidic chips surface hydrophilic treatment

To address the inherent hydrophobicity of polycarbonate (PC, water contact angle ≈ 70°), which can compromise device performance, a two-step surface modification was employed to create a stable hydrophilic interface. First, the chips were activated on each side for 5 min using an oxygen plasma cleaner (Femto Science Inc., Korea) at 100 W power, 4.10 Torr pressure, and a 30 sccm oxygen flow rate. Immediately following treatment, the microchannels were filled with a 20 w/v% PEG-2000 solution and incubated overnight at a 37°C with 30% humidity. Finally, the channels were rinsed with DI water and dried under a nitrogen stream to complete the hydrophilic modification.

Table S1. Summary of residence times in the mixing region for VeSMiC and single-layer devices at varying flow rates.

	Total flow rate (mL/min)	Flow rate per layer (Q, mL/min)	Residence time (t, ms)
VeSMiC	4.0	0.8	487.5
	8.0	1.6	243.8
	12.0	2.4	162.5
	16.0	3.2	121.9
Single layer	4.0	4.0	97.5

Fig. S1 Comparison of bubble formation in the re-Tesla channel under four different surface treatment methods: Untreated, Oxygen plasma treatment, PEG treatment, and combined Oxygen plasma- PEG treatment.

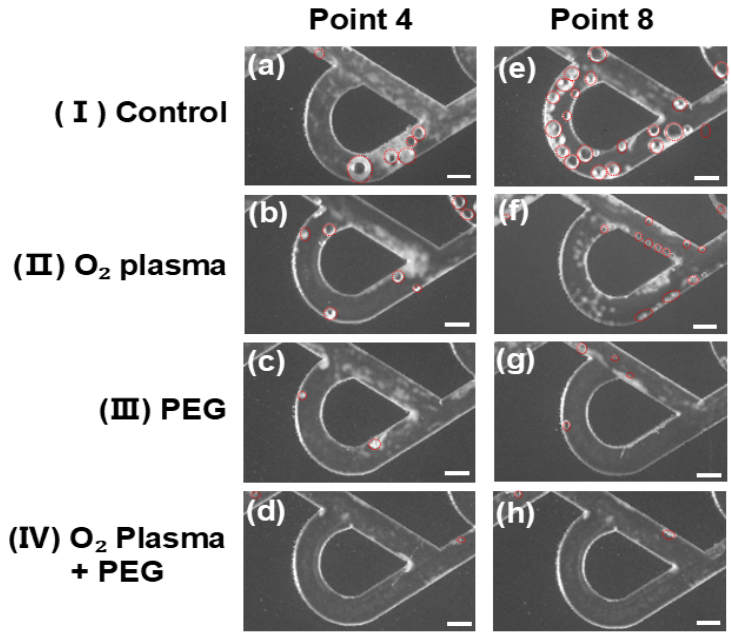

Fig. S2 Temporal stability assessment of the hydrophobic surface treatment. Comparative analysis of (A) average size and (B) PDI of LNPs synthesized using a freshly treated single-layer chip and a chip stored for 7 days. All experiments were conducted at a TFR of 4 mL/min.

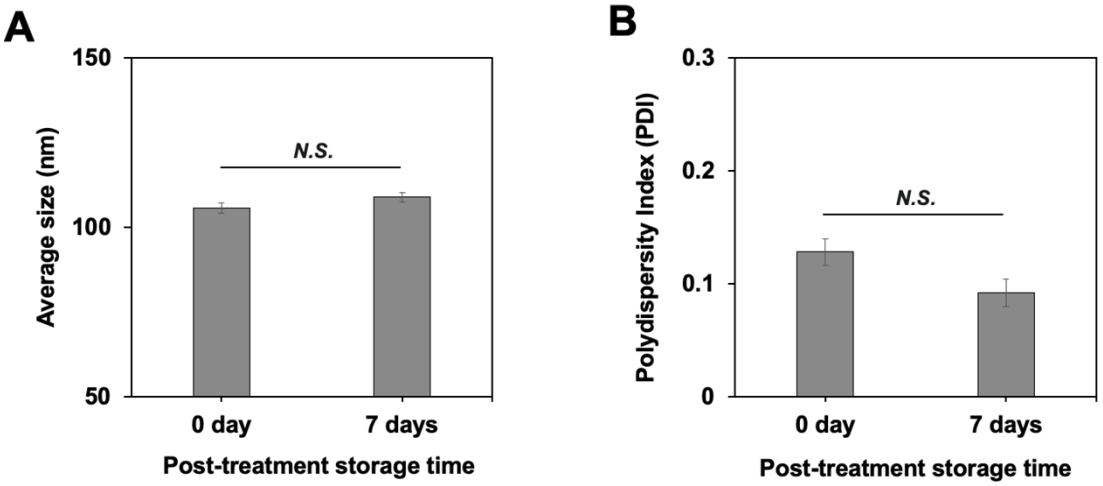

Fig. S3 Average particle size and PDI of LNPs produced by the 10-cycle re-Tesla chip (surface-treated) at TFR from 2-4mL/min.

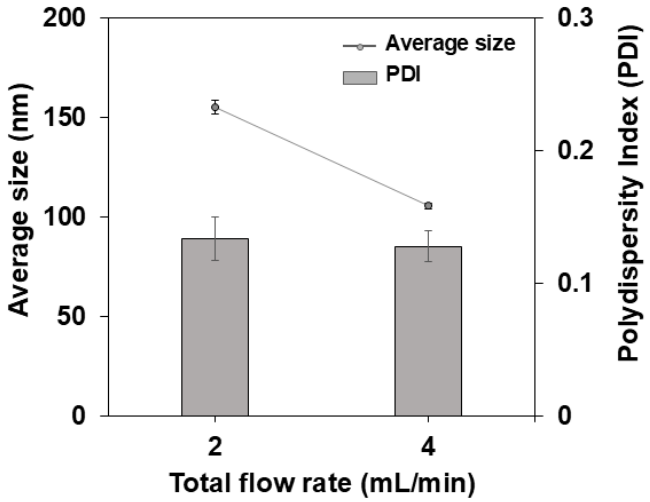

Fig. S4 Simulated pressure drop distribution across the five layers for straight, re-tapered, and tapered inlet structures at a TFR of **(A)** 12 mL/min; **(B)** 16 mL/min; **(A)** 20 mL/min.

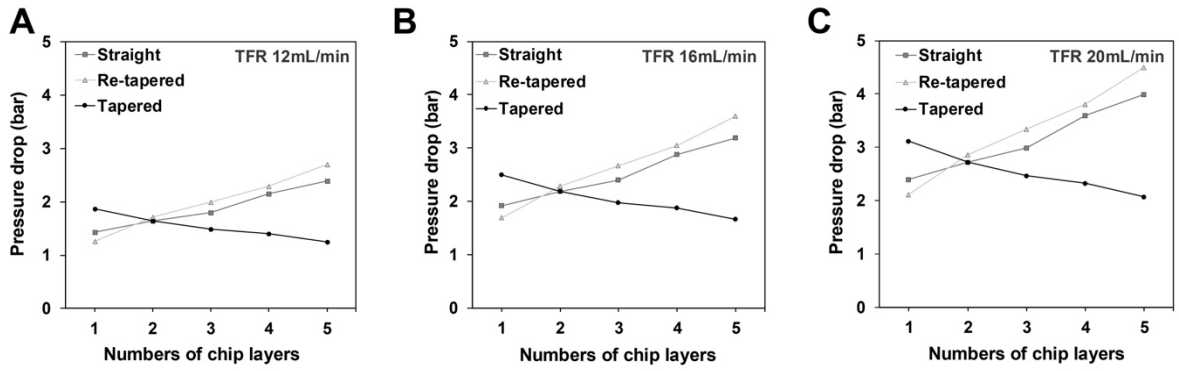

Fig. S5 (A) Epoxy resin adhesive: Common method for thermoplastics which prone to channel clogging. **(B)** Bolt fixation: Securing layers using only bolts, with alignment holes fabricated by CNC machining. **(C)** PDMS film lamination: Bonding Oxygen plasma-treated PDMS to a PC substrate to create an intermediate waterproofing lamination. **(D)** PSA tape interlayer: Using a substrate-matched film laminated with a PSA layer for interlayer bonding.