Supporting Information

Needle-Free Transdermal Delivery of mRNA Vaccine with Ionic Liquid Crystals and Effective Tumor Growth Inhibition

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1. [1]HNMR of ILs

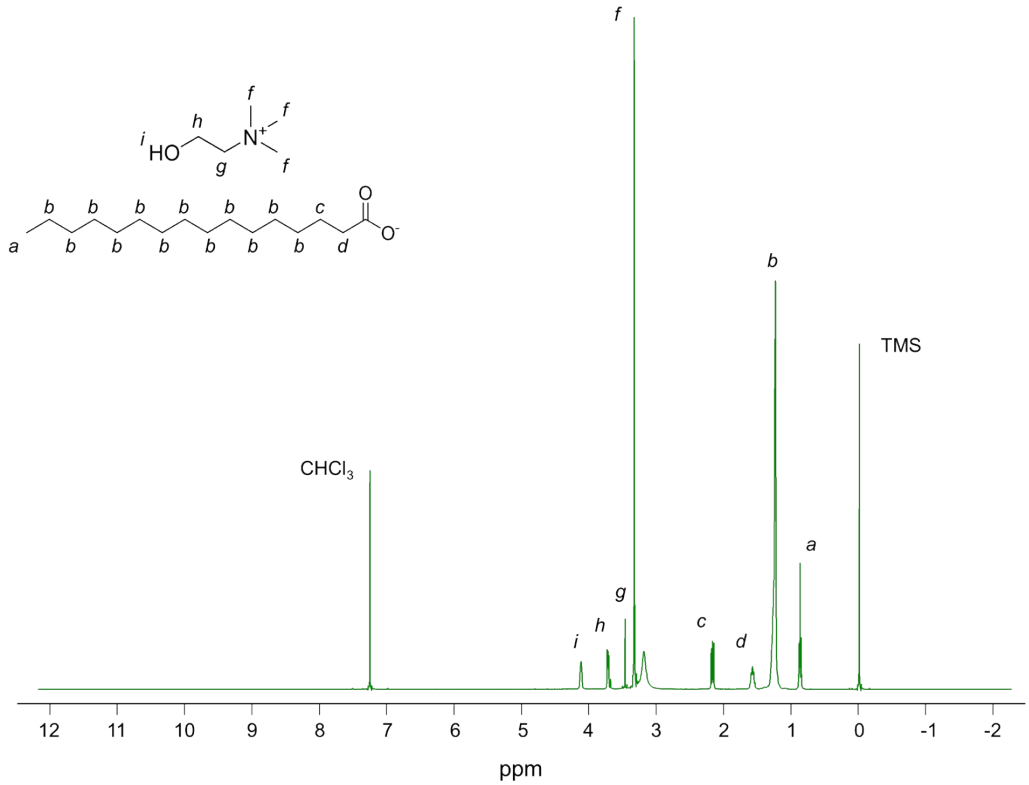

Fig. S1 [1]H NMR spectrum of [Cho][Pal] in $CDCl_3$.

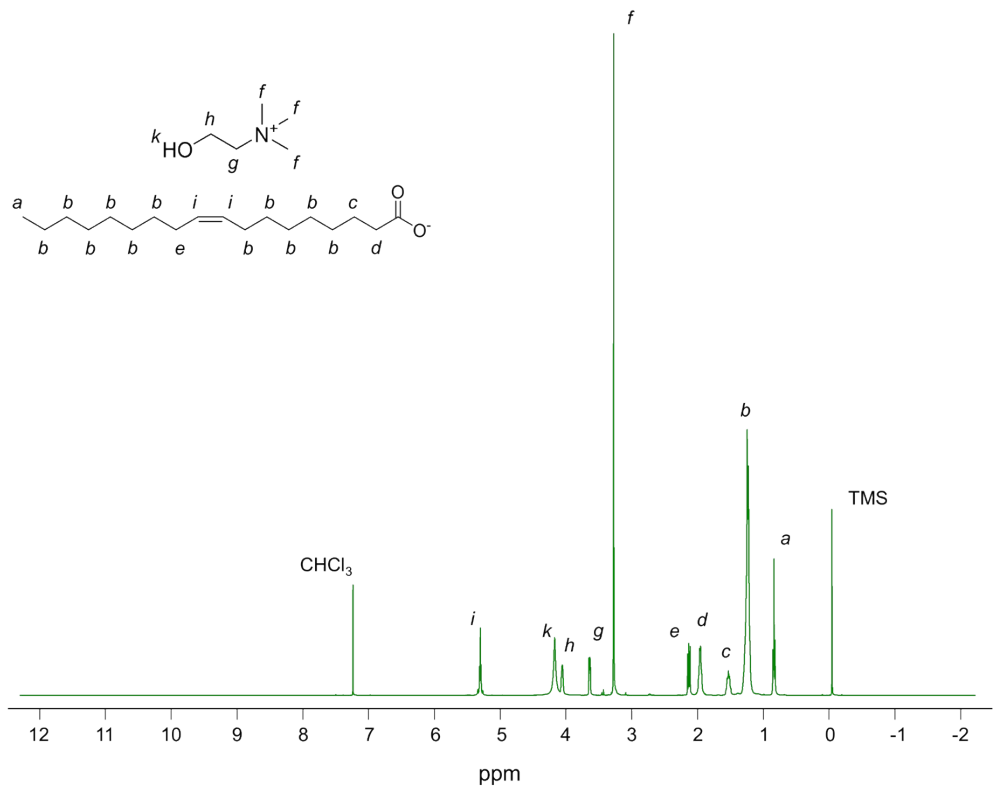

Fig. S2 ^{1}H NMR spectrum of [Cho][Ole] in $CDCl_3$.

2. SAXS measurement of mRNA-incorporated ILCs

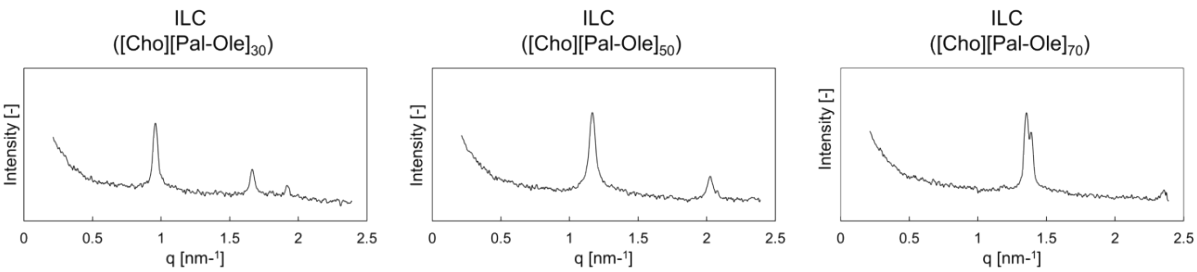

Fig. S3 SAXS diffraction pattern of ILCs encapsulating mRNA.

3. FRAP measurement of ILCs

Three types of ILC formulations ([Cho][Pal]$_{50}$, [Cho][Pal-Ole]$_{50}$, [Cho][Ole]$_{50}$) were prepared as microscope slides by placing them on glass slides. Fluorescence recovery was observed for 300 s after bleaching fluorescent molecules for 5 s using a confocal laser scanning microscope (CLSM). Normalization of fluorescence recovery data was performed according to Equations (1) and (2).

$$(Equation\ 1);\ I_{corrected} = \frac{I_{measured} * I_{unbleached}^{0}}{I_{unbleached}}$$

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Here, $I_{measured}$ represents the fluorescence intensity of the bleached region at each measurement time point, $I_{0unbleached}$ denotes the average fluorescence intensity of the unbleached region before photobleaching, $I_{unbleached}$ indicates the fluorescence intensity of the unbleached region at each measurement time point, and $I_{postbleach}$ refers to the fluorescence intensity of the bleached region immediately after photobleaching.

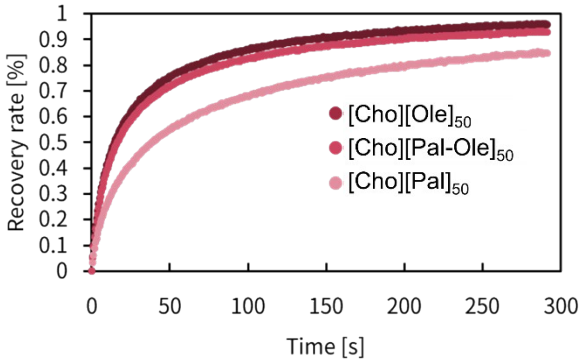

Fig. S4 Fluorescence recovery rates for various liquid crystal samples containing Nile Red.

4. Rheological properties of ILCs

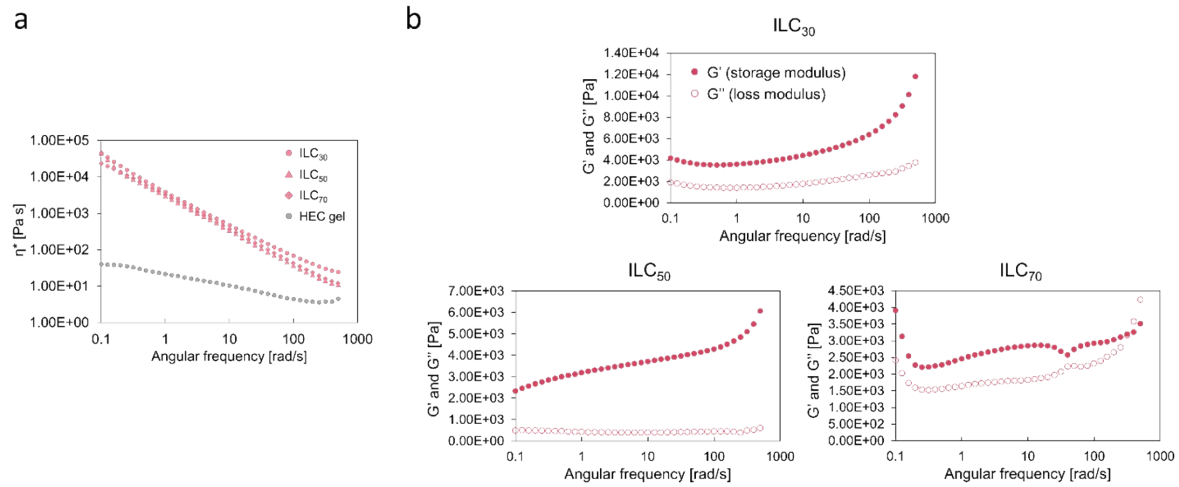

Fig. S5 Rheological properties of HEC gel and ILCs. (a) Complex viscosity and (b) Storage modulus (G') and loss modulus (G").

5. Stability of mRNA in ILCs

ILC formulations containing 10 μg of mRNA, as well as mRNA aqueous solutions, were prepared and incubated at 37 °C for 2 weeks. After incubation, the ILC samples were processed according to the procedure described in the section "Evaluation of RNase Resistance." Briefly, 800 μL of 99% ethanol was added to dissolve the ILC, and mRNA was subsequently recovered using a spin column purification method. The recovered mRNA was then subjected to reverse transcription followed by quantitative PCR (qPCR) to determine Ct values. For the aqueous mRNA samples, reverse transcription and qPCR were performed after dilution to match the mRNA concentration recovered from freshly prepared ILC samples, without column purification. In the aqueous solution, an increase in Ct value was observed after storage, suggesting partial degradation of mRNA under these conditions. In contrast, no significant change in Ct value was detected for mRNA recovered from the ILC formulation before and after incubation, indicating that the ILC system effectively preserved mRNA integrity during storage.

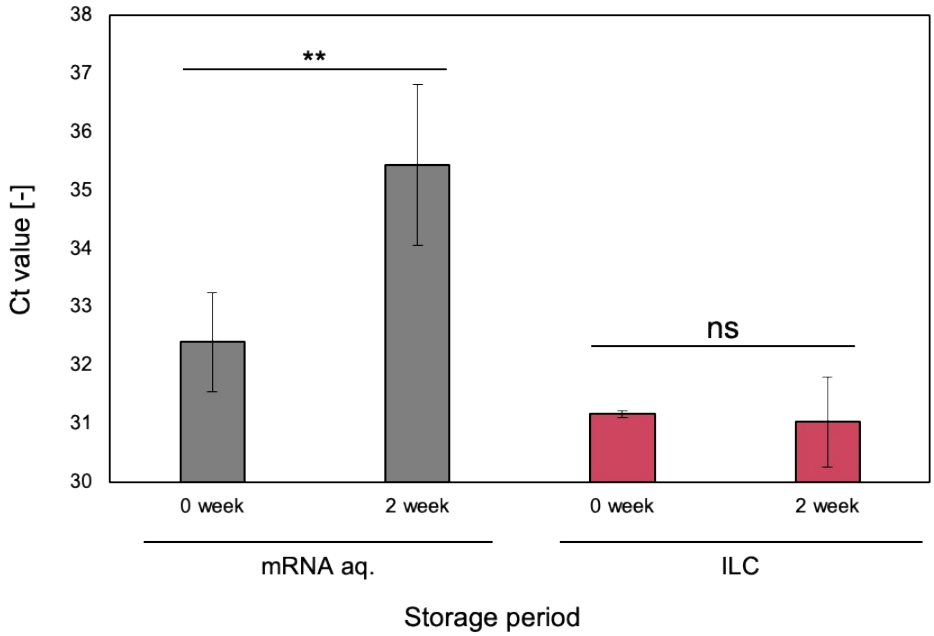

Fig.S6 Stability of mRNA under different storage conditions. The remaining mRNA amount was quantified after storage, and values are presented as mean ± SD (N = 3).

6. *Therapeutic anti-tumor effects*

Initially, E.G7-OVA was transplanted into C57BL/6N mice, and then mRNA was administered by injection or transdermally. The specific administration schedule is shown in Fig. S7a. Tumor volume and mouse weight changes are summarized in Fig. S7b, c. The results showed that tumor growth was inhibited in the ILC-treated group compared to the untreated group. The fact that no significant changes in body weight were observed suggests that the ILC formulation is not toxic to the whole body.

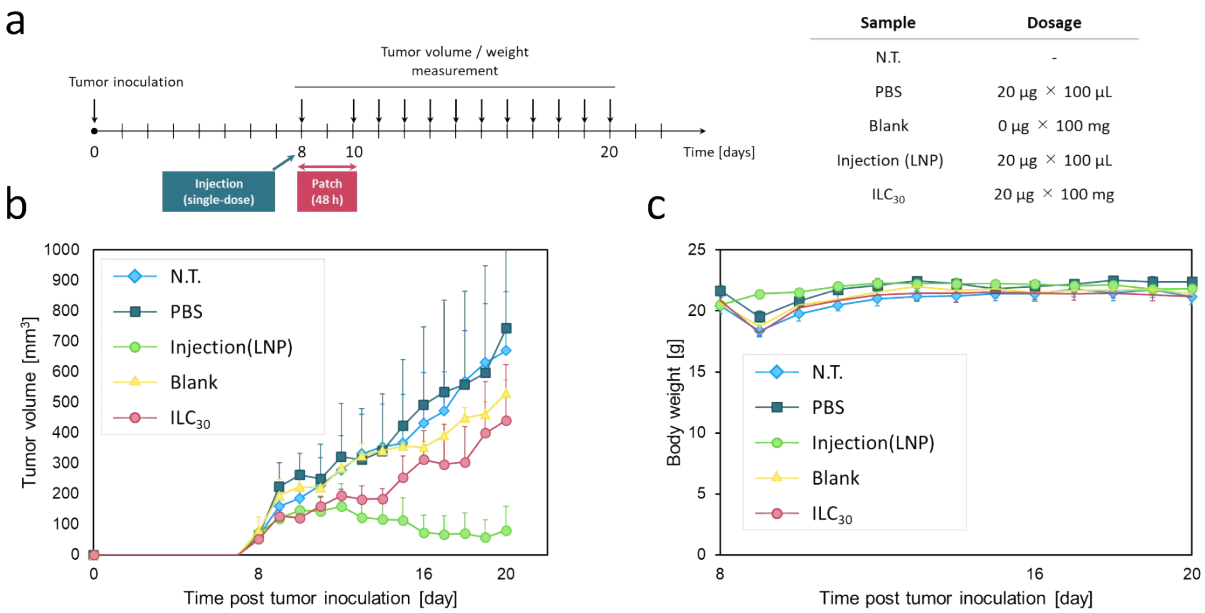

Fig. S7 Therapeutic antitumor effect on E.G7-OVA cells. Prior to tumor inoculation, mRNAOVA-epitope was administered to C57BL/6N mice by injection and transdermal patch. a) Time course of this experiment, (b) changes in tumor volume. (c) changes in body weight. $N = 3$.

7. *Biotoxicity assessment*

To further evaluate local skin safety, we monitored transepidermal water loss (TEWL) following topical administration of the ILC formulation as an indicator of skin barrier disruption. Immediately after administration, the ILC-treated group exhibited markedly higher TEWL values compared to the non-treated (N.T.) group, suggesting transient skin barrier disruption and increased irritation. However, the TEWL values gradually decreased over time and returned to baseline levels by day 12, indicating recovery of skin barrier function.

These results suggest that while the ILC formulation induces temporary skin barrier disruption, the effect is reversible and does not result in sustained barrier damage under the tested conditions.

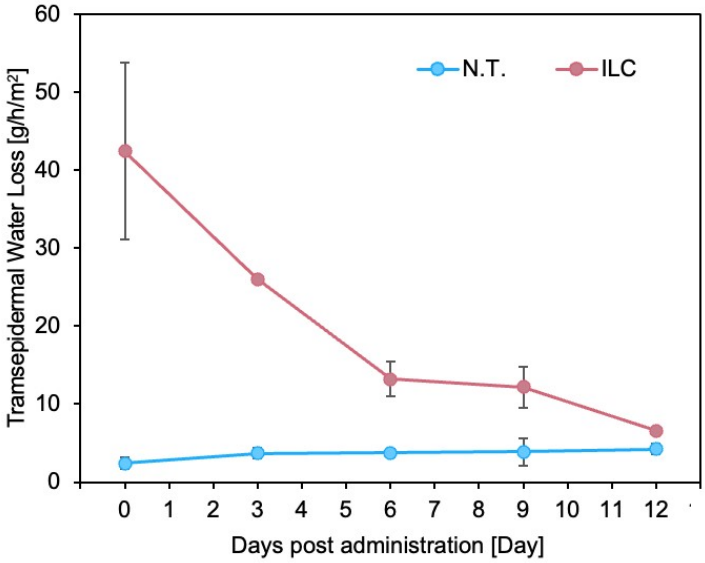

Fig.S8 Time course of TEWL after formulation application (N = 3, mean ± SD).

8. *Preparation of mRNA*

Plasmid DNA (pDNA) encoding NanoLuc was linearized according to the manufacturer's instructions for the Takara IVTpro™ mRNA Synthesis System. Briefly, template pDNA was mixed with 10× M Buffer, Nuclease-Free Water, and Hind III

under the conditions described in Table 1, followed by restriction enzyme digestion at 37 °C for 3 h. After digestion, one-tenth volume of 3 M sodium acetate (pH 5.2) and twice the volume of ethanol were added to the reaction mixture. The solution was mixed thoroughly and stored at –20 °C for at least 15 min to precipitate the DNA. The mixture was then centrifuged for 15 min, and the resulting supernatant was carefully discarded. The DNA pellet was washed with 1 mL of 70% ethanol, followed by another centrifugation under the same conditions. After removal of the supernatant, the pellet was air-dried and dissolved in Nuclease-Free Water to obtain linearized pDNA.

Table S1. Sample composition for linearized DNA

Component	Amount
Template pDNA	50 μg
10×M Buffer	20 μL
Nuclease-Free Water	X μL
HindIII (15 U/ μL)	10 μL
Total	200 μL

Subsequently, mRNA was synthesized from the linearized pDNA template following the Takara IVTpro™ mRNA Synthesis System protocol. The reagents listed in Table 2 were added sequentially to the reaction tube, mixed thoroughly, and incubated for 4 h at the recommended temperature. Following in vitro transcription (IVT), DNase treatment was performed by adding 4 μL of DNase I to the IVT reaction mixture, gently mixing, and incubating at 37 °C for 15 min to remove the DNA template. For LiCl precipitation, 30 μL of Nuclease-Free Water and 30 μL of Lithium Chloride Precipitation Solution were added to the DNase-treated IVT mixture. The reaction was mixed thoroughly and stored at –20 °C for 30 min to allow RNA precipitation. The RNA

was pelleted by centrifugation at 15,000 rpm for 15 min at 4 °C, the supernatant was discarded, and the pellet was washed with 100 μL of 70% ethanol. After a second centrifugation under the same conditions, the supernatant was removed, and the pellet was air-dried. The purified RNA was finally dissolved in 100 μL of Nuclease-Free Water.

Table S2. Sample composition for IVT

Component	Amount
Nuclease-Free Water	X μL
10×Transcription Buffer	2 μL
10×ATP	2 μL
10×CTP	2 μL
10×GTP	2 μL
10×UTP (10×m1ΨUTP *in vivo*)	2 μL
CleanCap Reagent AG	1.6 μL
Template linear DNA	1 μg
10×Enzyme Mix	2 μL
Total	20 μL

The following mRNA (NanoLuc) was used:

AUGGUUUUUACCCUGGAAGAUUUUGUGGGUGAUUGGCGUCAGACCGCAGGUU
AUAAUCUGGAUCAGGUUCUGGAACAGGGUGGUGUUAGCAGCCUGUUUCAGAA
UCUGGGUGUUAGCGUUACCCCGAUUCAGCGUAUUGUUCUGAGCGGUGAAAAU
GGCCUGAAAAUUGAUAUUCAUGUGAUCAUCCCGUAUGAAGGUCUGAGUGGUG
AUCAGAUGGGUCAGAUUGAAAAAAUCUUCAAAGUUGUGUAUCCGGUGGAUGA
CCACCAUUUUAAAGUGAUUCUGCAUUAUGGCACCCUGGUUAUUGAUGGUGUG
ACCCCGAAUAUGAUUGAUUAUUUCGGUCGUCCGUAUGAGGGUAUUGCAGUUU
UUGAUGGCAAAAAAAUCACCGUUACCGGCACCCUGUGGAAUGGUAACAAAAU
UAUCGAUGAACGCCUGAUUAAUCCGGAUGGUAGCCUGCUGUUUCGUGUUACA
AUUAAUGGUGUUACCGGUUGGCGUCUGUGUGAACGUAUUCUGGCAUGAUGAG
CUGGAGCCUCGGUGGCCUAGCUUCUUGCCCCUUGGGCCUCCCCCCAGCCCCUC
CUCCCCUUCCUGCACCCGUACCCCCGUGGUCUUUGAAUAAAGUCUGAGUGGGC
GGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAA

The following mRNA (OVA epitope) was used:

UAAUACGACUCACUAUAAGGAAUAAACUAGUAUUCUUCUGGUCCCCACAGACU
CAGAGAGAACCCGCCACCAUGAGCAUCAUCAACUUUGAAAAAUUGUGAGCUGG
AGCCUCGGUGGCCUAGCUUCUUGCCCCUUGGGCCUCCCCCCAGCCCCUCCUCCC
CUUCCUGCACCCGUACCCCCGUGGUCUUUGAAUAAAGUCUGAGUGGGCGGCAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAG
CUU