Supplementary Information

Needle-free transdermal patches for insulin delivery in diabetes treatment.

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1. Materials and methods:

1.1 Synthesis and characterization of characterization of biocompatible non-aqueous polar IL and surface-active IL.

Biocompatible [Cho][Pro] and [Cho][Ole] were synthesized by two-steps metathesis according to our previously published reports [1] [2] [3]. In brief, choline hydroxide/choline bicarbonate was mixed with propionic acid/oleic acid at stoichiometric ratio. To this mixture, Milli Q/methanol worked as solvent, and the solution was stirred at room temperature overnight. The solvent was then removed using a rotary evaporator, and the residue was freeze-dried for two days. (**Fig. S1**) The obtained

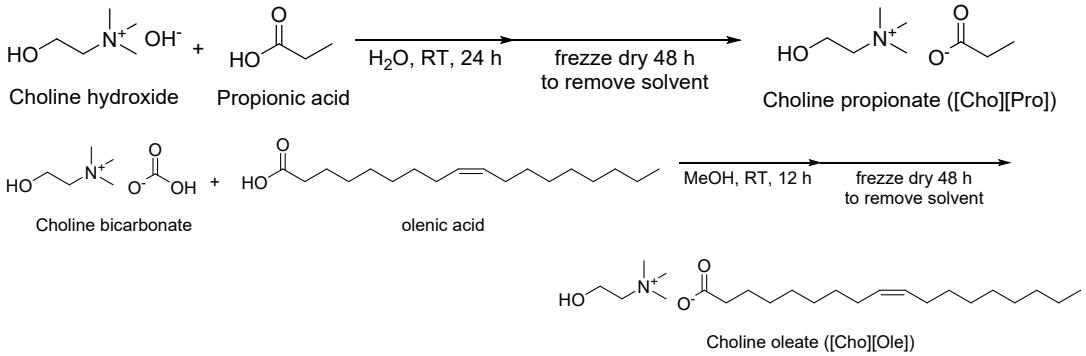


Figure S1. Scheme for IL synthesis.

product was analyzed by ^{1}H-NMR in $CDCl_3$/ DMSO-d6 to confirm its structure.

1.2 Particle size distribution of the IL/O ME formulations

Particle size distribution was analyzed using the Zetasizer Nano ZSP (Malvern Instruments Ltd., Malvern, Worcestershire, UK) via dynamic light scattering (DLS). The Z-average particle size and polydispersity index (PDI) were assessed to evaluate the storage stability of the IL/O ME formulation. The formulation process is depicted in **Fig.S2**. Additionally, TEM was employed to further confirm the nanoparticle characteristics.

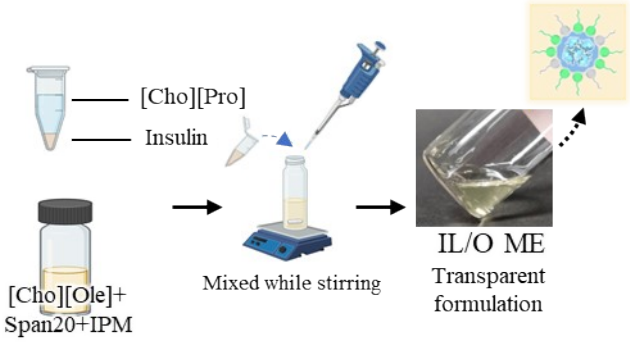


Figure S2. Protocol for IL/O ME modulation.

Table S1. Composition of IL/O ME.

	Polar Phase [Cho][Pro]	Surfactant [Cho][Ole]	Co-surfactant Span-20	Oil IPM
ME-[Cho][Ole]	2.5wt%	7.5wt%	5.0wt%	85.0wt%

1.3 Fluorescein isothiocyanate (FITC) labeling of insulin synthesis:

FITC labeling of Insulin was prepared following a previously reported protocol[4] with a slight modification. In brief, human insulin was dissolved in 0.01 M bicarbonate buffer (PH 9.3) to prepare a 15mg/ml solution, and FITC-I was dissolved in DMSO to obtain a 4 mg/ml FITC/DMSO solution. The two solutions were mixed at a 1:3 molar ratio and incubated at room temperature for 2.5 hours, protected from light with aluminum foil. During the reaction, the mixture was gently shaken every 30 minutes or continuously agitated using a shaker. The resulting conjugate was then purified using a PD-10 SephadexTM G-25 column and freeze-dried to obtain the final product.

2. Results and Discussion:

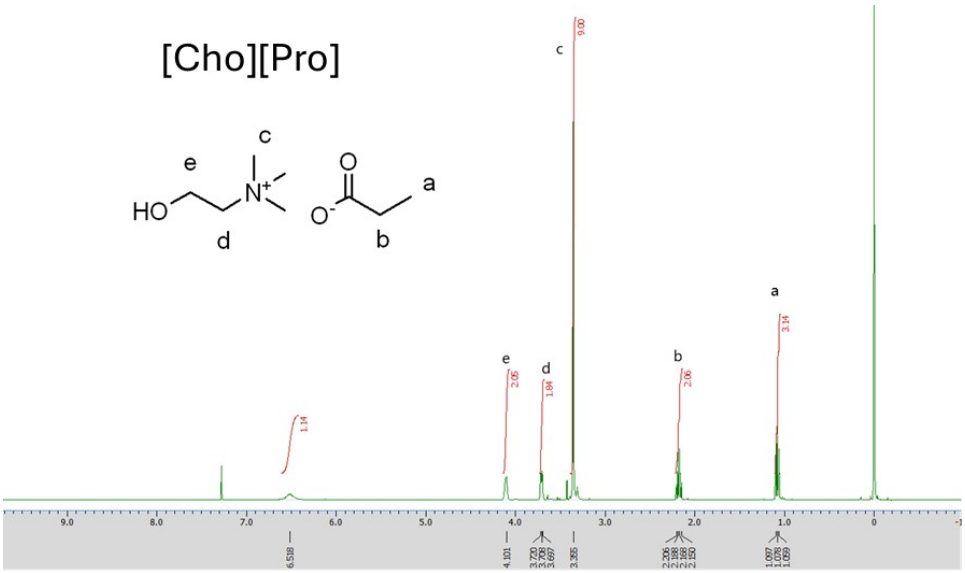
2.1 ^{1}H NMR-Based Structure Identification

[Cho][Pro]

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.07 (t, 3H), 2.16 (q, 2H), 3.35 (s, 9H), 3.71 (t, 2H), 4.10 (t, 2H).

[Cho][Ole]:

^{1}H NMR (DMSO-*d6*, 400 MHz): δ 0.85 (*t*, 3H), 1.24-1.28 (*m*, 20H), 1.40 (*quint*, 2H), 1.86 (*t*, 2H), 1.98 (*dt*, 4H), 3.14 (*s*, 9H). 3.44 (*t*, 2H), 3.85 (*t*, 2H), 5.30-50.32 (*m*, 2H).



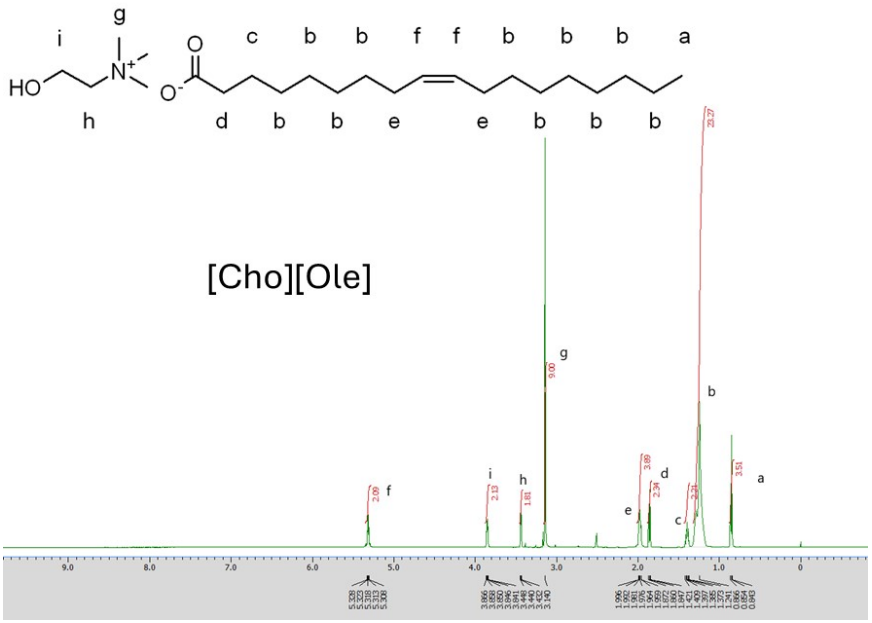


Figure S3. ^{1}H NMR spectroscopy of (a) [Cho][Pro], (b)[Cho][Ole].

2.2 Insulin solubility at varying water content:

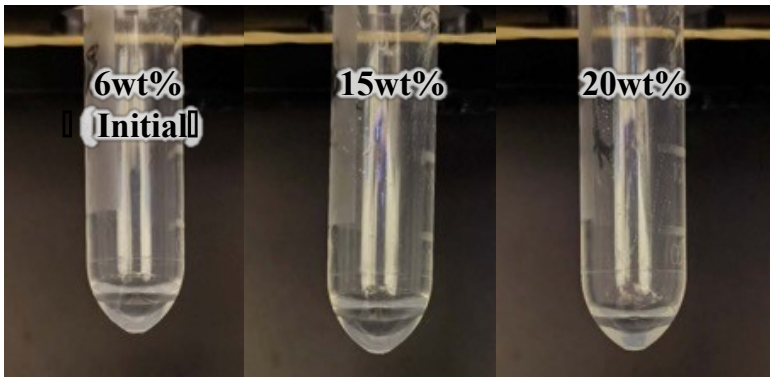


Figure S4. Insulin solubility in [Cho][Pro] at varying water content (wt%).

2.3 Insulin loaded IL/O ME formulation:

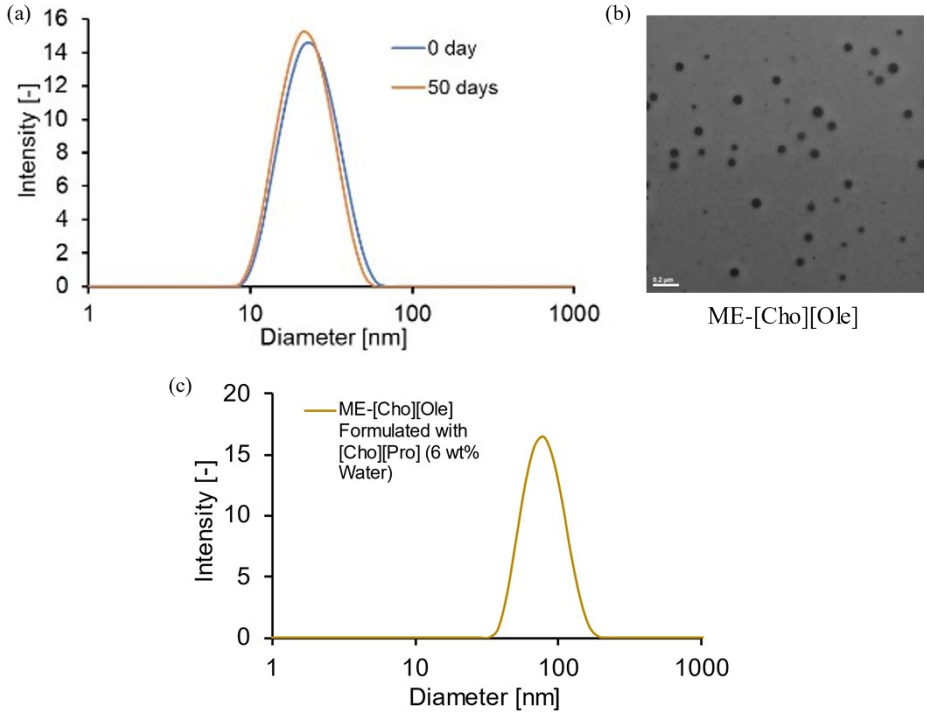


Figure S5. (a) Investigation of the physical stability of 3 mg/mL insulin-loaded ME-[Cho][Ole] (formulated with [Cho][Pro] containing 15wt% water) by monitoring particle size changes during storage at room temperature. (b) TEM analysis of the formulation. (c) ME-[Cho][Ole] formulated with [Cho][Pro] (6 wt% Water).

Table S2. Particle size and polydispersity index of ME-[Cho][Ole] (containing 15wt% water) after 50 days storage at 25 °c. (n = 3, mean ± SD).

ME-[Cho][Ole] (containing 15wt% water)	0 day	50 days
Diameter [nm]	21.17±0.2572	19.42±0.0755
PDI	0.167±0.012	0.156±0.003

Table S3. Particle size and polydispersity index of ME-[Cho][Ole] (containing 6wt% water) (n = 3, mean ± SD).

	ME-[Cho][Ole] (containing 6wt% water)
Diameter [nm]	94.96±5.591
PDI	0.381±0.005

2.4 *In vitro* permeation of different formulations.

Table S4: Characteristics of the PSA.

Name of PSA	Description	Vinyl acetate	Functional groups	Crosslinker	IL/O ME: PSA
DURO-TAK 87-4098	Acrylates copolymer	Yes	None	N/A	1:1

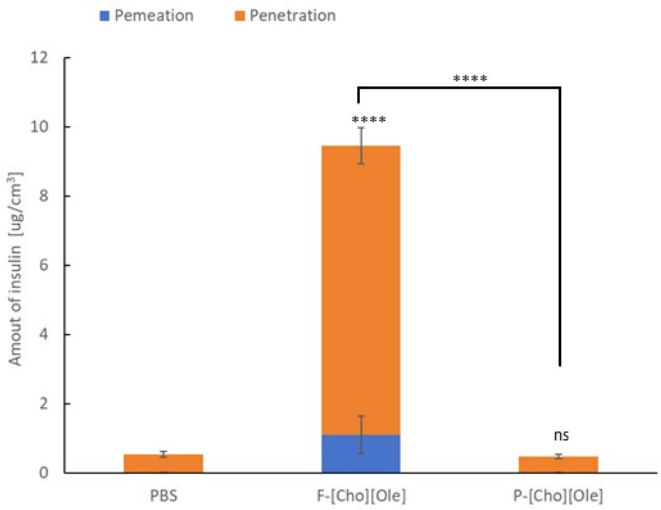


Figure S6. *In vitro* permeation study on YMP skin, total amount over 48 h.

Table S5. The administration amount of ME and patch

	PBS	**ME-[Cho][Ole]**	**P-[Cho][Ole]**
Administration amount (μg)	300	300	6.2±0.1

3. Reference:

[1] Islam MR, Uddin S, Chowdhury MR, Wakabayashi R, Moniruzzaman M, Goto M. *ACS Appl Mater Interfaces.* 2021, **13**(36):42461-42472.

[2] Ali MK, Moshikur RM, Wakabayashi R, Tahara Y, Moniruzzaman M, Kamiya N, Goto M. *J Colloid Interface Sci.* 2019, **551**:72-80.

[3] Tahara Y, Morita K, Wakabayashi R, Kamiya N, Goto M. *Mol Pharm.* 2020, **17**(10):3845-3856.

[4] Shah D, Guo Y, Ocando J, Shao J. *J Pharm Anal.* 2019, **9**(6):400-405.