Electronic supplementary information

Biocompatible Ionic Liquid-Based Formulations for Topical Delivery of ε-Poly-L-Lysine to Combat Subcutaneous Fungal Infections

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1. Abbreviations

Abbreviation	Name/description
PBS	Phosphate buffer saline
ILs	Ionic liquids
SAILs	Surface-active ionic liquids
MEFs	Microemulsion formulations
IL/O	Ionic liquid-in-oil formulations
SC	Stratum corneum
FAs	Fatty acids
C18:1	Oleic acid
EPL	ε-poly-l-lysine
Span-20	Sorbitan monolaurate
IPM	Isopropyl myristate
[Cho][Ole]	Choline oleate
[Cho][Pro]	Choline propionate
PDA	Potato dextrose agar
pI	Isoelectric point
Mw	Molecular weight
SDS	Sodium dodecyl sulfate
DLS	Dynamic light scattering
PDI	Polydispersity index
FDC	Franz diffusion cell
FTIR	Fourier-transform infrared

2. CD Spectra of EPL in IL

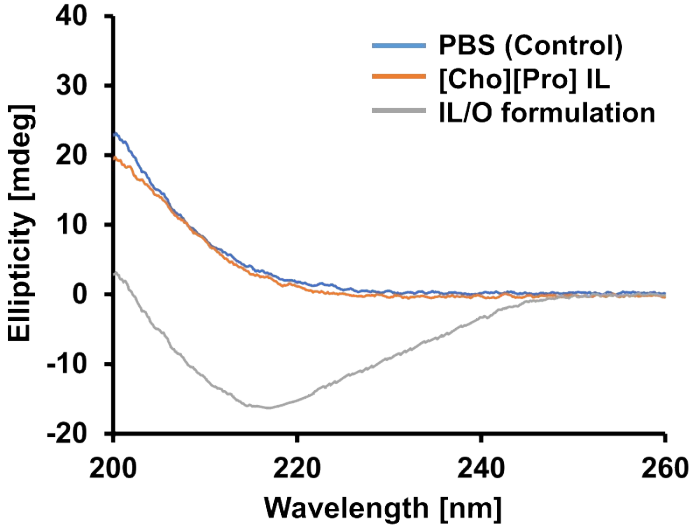

Figure S1. CD spectra of EPL in PBS or [Cho][Pro] and that of IL/O formulation at 25 °C.

3. MALDI-TOF-MS Analysis of unmodified and Fluorescein-labelled EPL

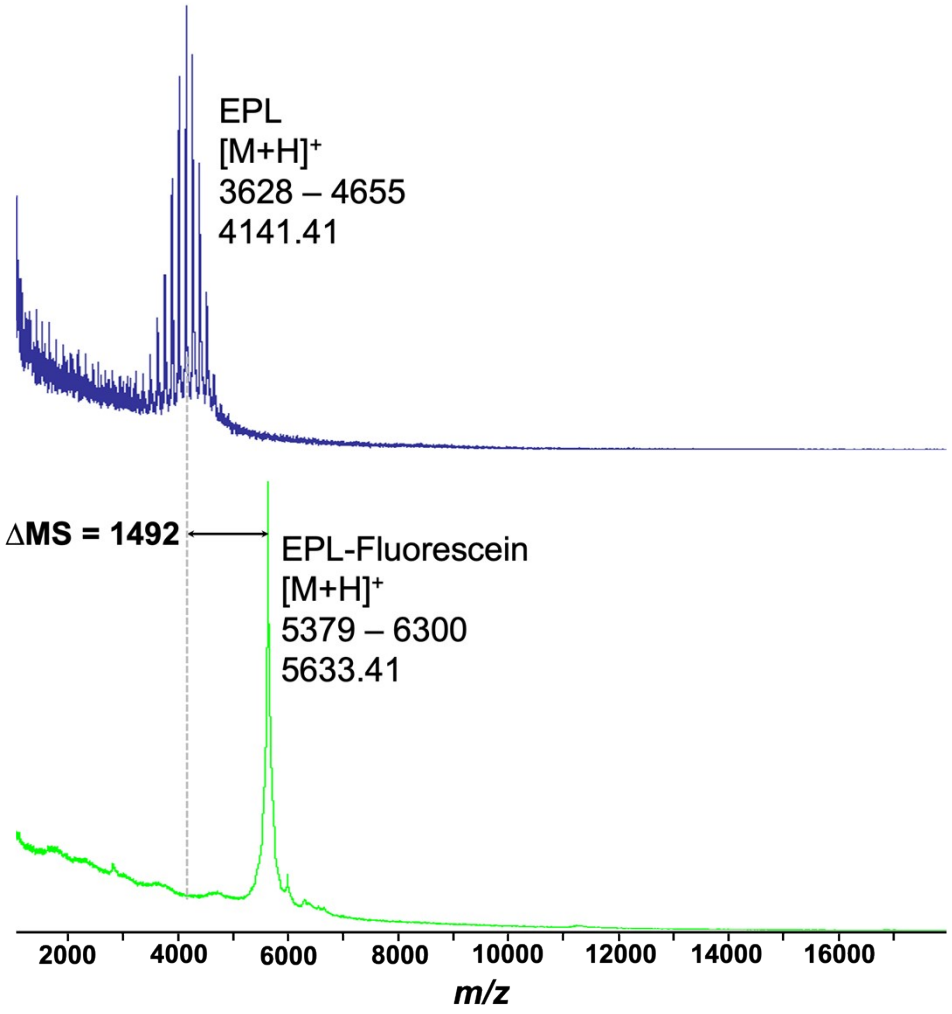

Figure S2. MALDI-TOF-MS spectra analysis of EPL and Fluorescein-labelled EPL. The theoretical ΔMS for modification with four fluorescein molecules is 1501 *m/z*.

4. FTIR spectrum of IL/O formulations-treated stratum corneum

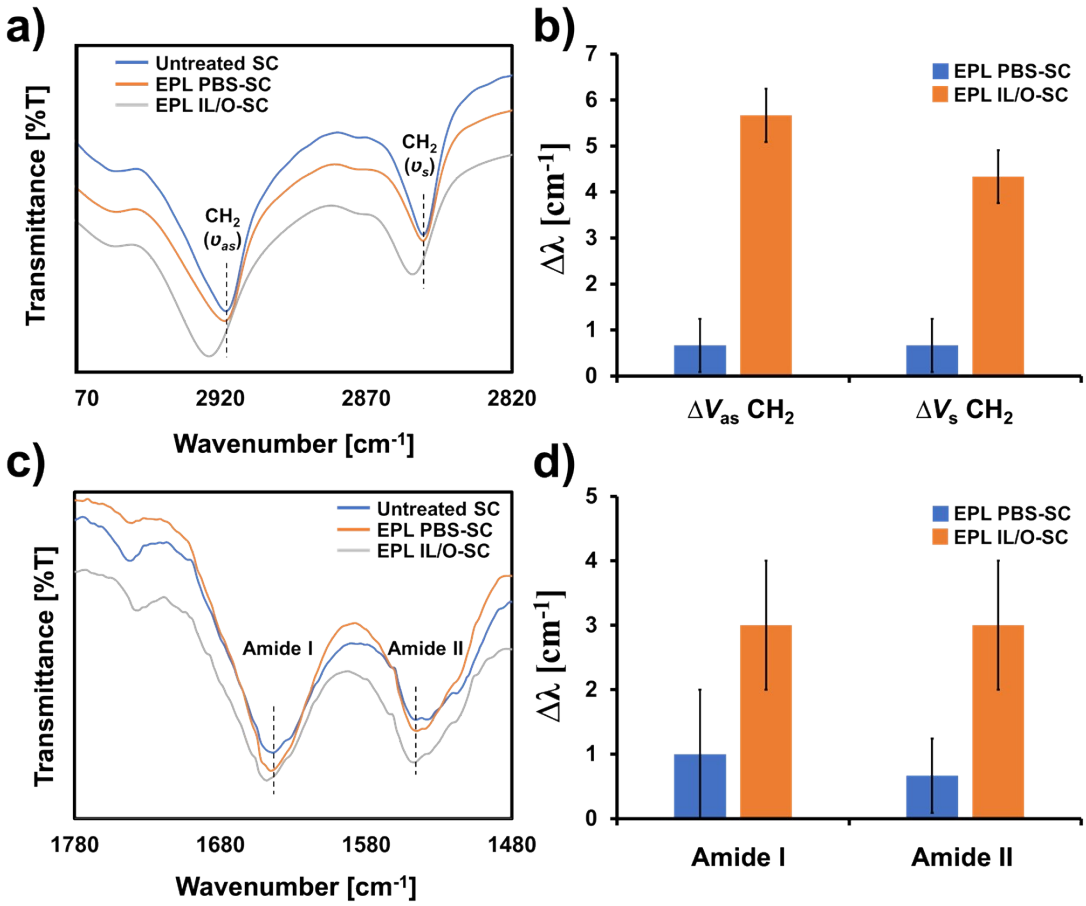

Figure S3. Analysis of the skin permeation mechanism. FTIR spectra of mice SC treatment with EPL-loaded IL/O formulations. a) Peaks near 2850 $cm^{-1}$and 2920 cm^{-1} correspond to symmetric (ΔV_s) and asymmetric (ΔV_{as}) CH_2 vibrations of the SC lipids, respectively. b) Shifts in symmetric and asymmetric CH_2 stretching peaks relative to untreated SC lipids. c) Peaks near 1630 cm^{-1} and 1545 cm^{-1} correspond to Amide I and II vibrational modes of the SC protein, respectively. d) Amide I and II vibrational peaks shift compared with nontreated SC protein. This experiment was conducted in triplicate (n = 3), mean ± SD.

5. Antifungal activity of controls

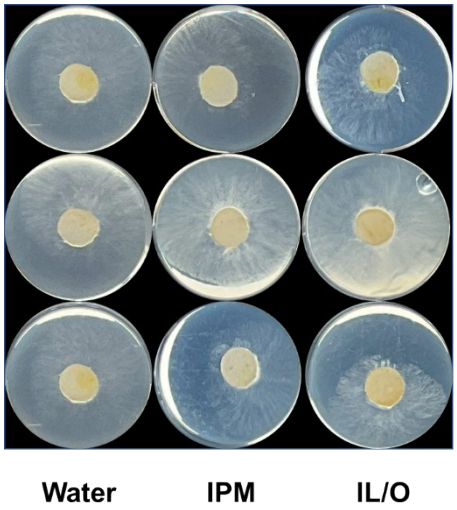

Figure S4. The picture of antifungal activity of controls, including water, IPM, and IL/O alone (without EPL) against a fungal strain growing beneath the mice skin, subsequently overlying the freshly prepared sample solutions to the SC containing *Trichoderma viride*. The experiments were conducted in triplicate (n = 3) using the skin with *T. viride* in 6-well different microplates.

6. Antifungal activity of freshly prepared EPL-loaded MEFs

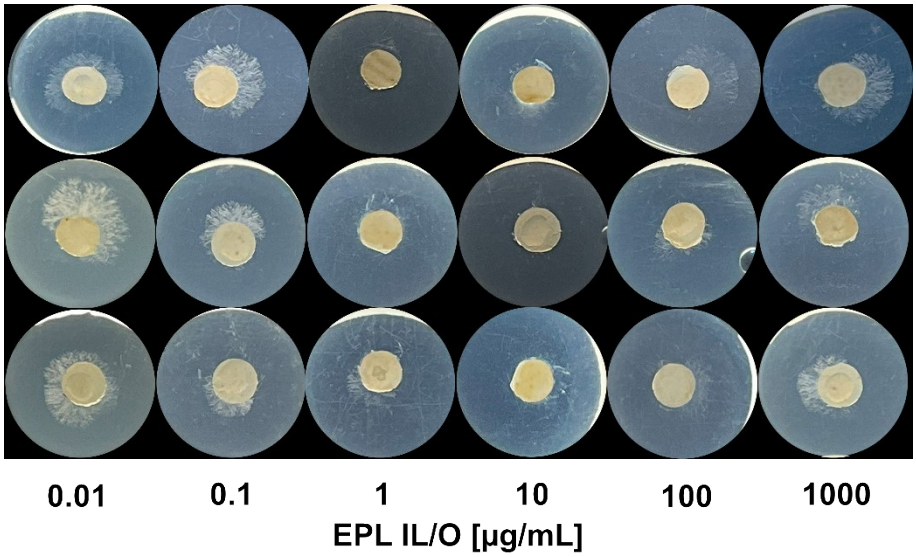

Figure S5. The picture of all antifungal activity assay results shown in Figure 4a,c. The antifungal activity of EPL-loaded IL/O formulation against a fungal strain actively growing beneath the skin, subsequently overlying the freshly prepared sample solutions to the SC containing *T. viride*. The experiments were conducted in triplicate ($n = 3$) using the skin containing *T. viride* in 6-well different microplates.

7. Antifungal activity of freshly prepared EPL in PBS

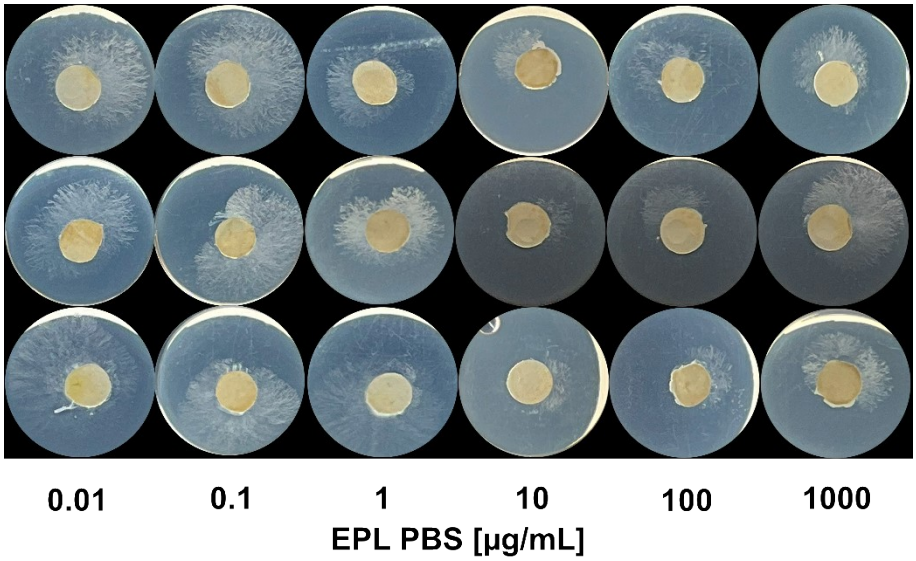

Figure S6. Topical antifungal activity results of EPL dissolved in PBS shown in Figure 4b,c. Potato dextrose agar (PDA) plate after subcutaneously growing *T. viride* treated with EPL dissolved in PBS at 25 ^{o}C for 12 h. The experiments were conducted in triplicate (n = 3) using the skin containing *T. viride* in 6-well different microplates.

8. Live/dead assay

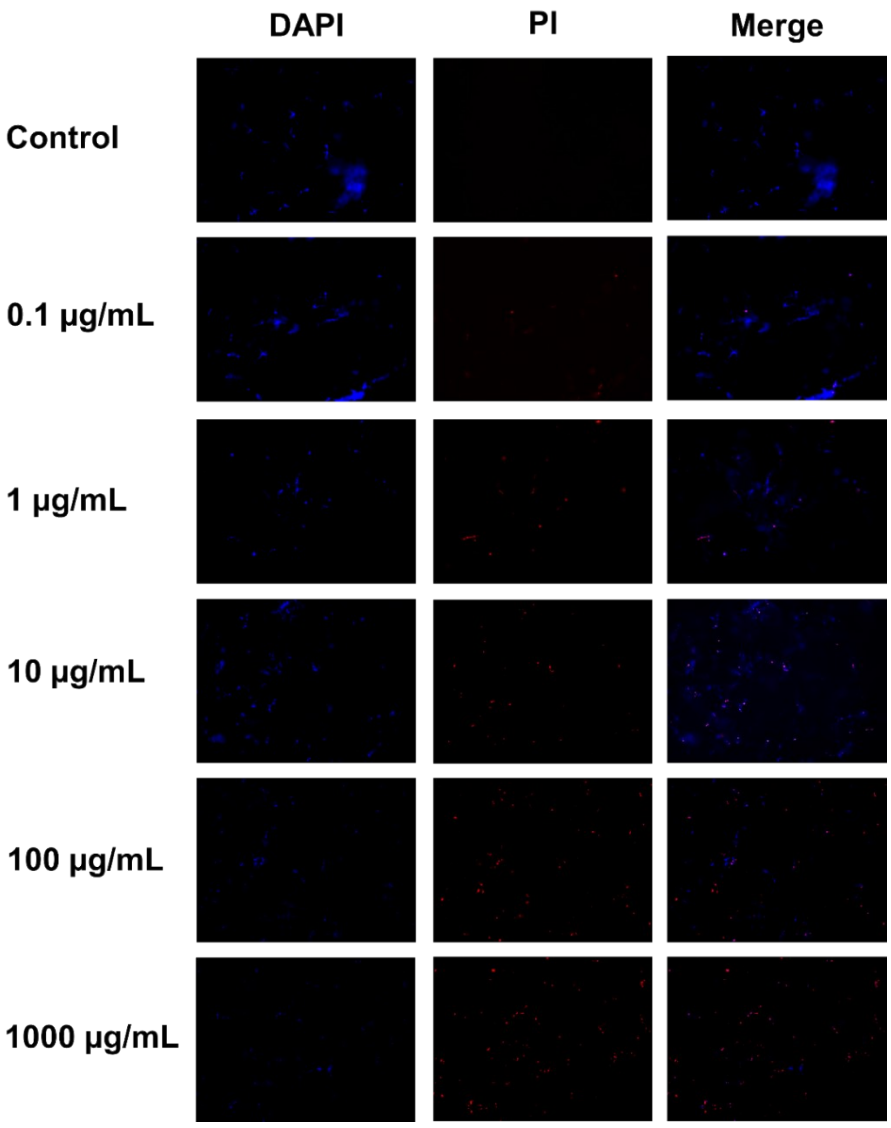

Figure S7. Fluorescence microscopy result of dead cell of *T. viride* treated with EPL shown in Figure 4d. Fluorescence microscopy of *T. viride* staining with PI (red, for dead cells) and DAPI (blue, for live cells) after treated with various EPL (0 – 1000 μg/mL) after 24 h.

9. The long-term therapeutic efficacy of EPL-loaded IL/O formulations

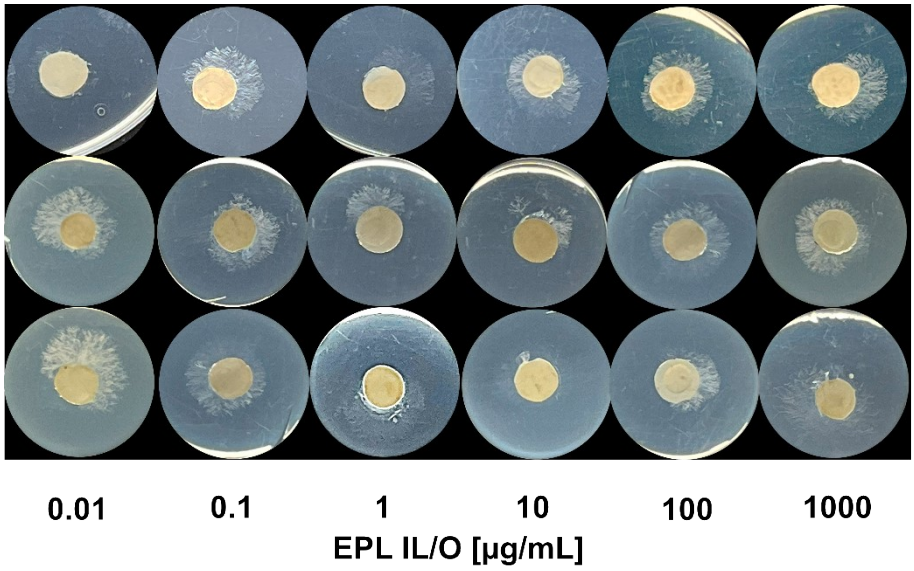

Figure S8. The picture of antifungal activity assay using *T. viride* in the topical application to assess the long-term therapeutic efficacy of EPL-loaded IL/O formulations over 28-day of storage at room temperature shown in Figure 6a. The experiments were conducted in triplicate (n = 3) in 6-well different microplates.

10. The long-term therapeutic efficacy of EPL in PBS

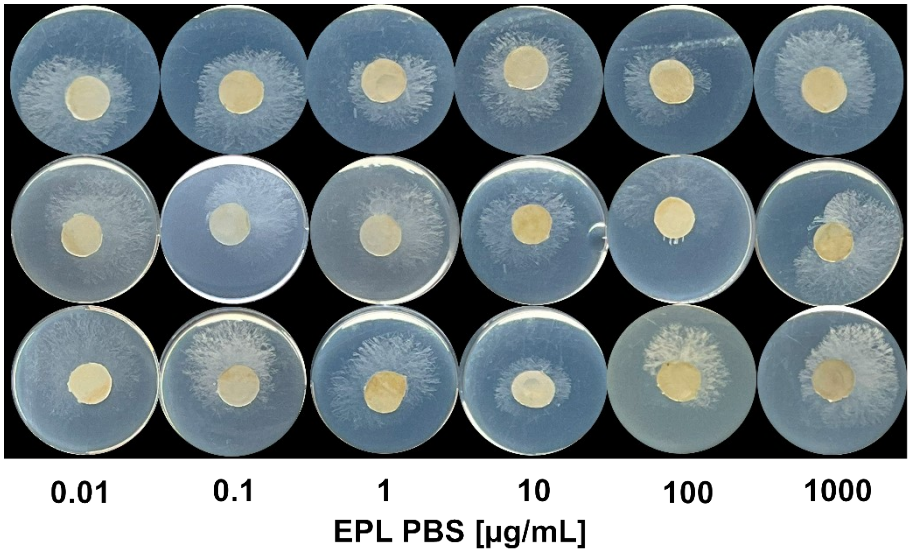

Figure S9. The picture of antifungal activity assay using *T. viride* in the topical application to assess the long-term therapeutic efficacy of EPL in PBS over 28-day of storage at room temperature shown in Figure 6b. The experiments were conducted in triplicate (n = 3) in 6-well different microplates.