Ethyl oleate-based *in situ* forming gel for long-term peptide delivery via lamellar phase transition

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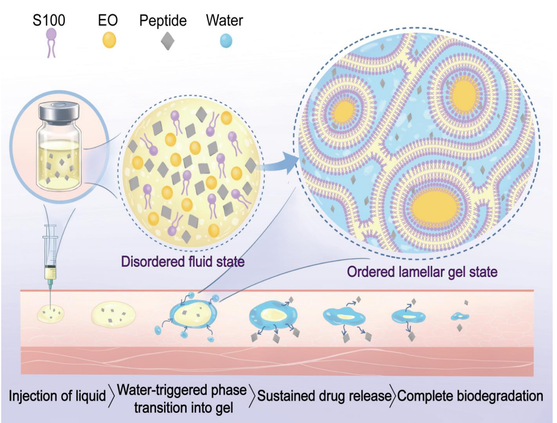
Abstract

Peptide therapeutics are increasingly attractive due to their high potency and specificity, yet their clinical application is often limited by short systemic half-life, enzymatic instability, and rapid clearance. Here, we report a minimally invasive injectable *in situ* forming gel (ISG) composed of phospholipids dissolved in ethyl oleate (EO), replacing conventional hydrophilic organic solvents, which rapidly forms a depot upon exposure to aqueous environments. By adjusting the phospholipid-to-EO ratio, we systematically characterized the ISG's microstructural evolution, rheological behavior, water absorption, and drug release profile, revealing a water-triggered phase transition mechanism toward a lamellar gel structure. Compared with conventional hydrophilic organic solvent-based formulations, the EO-based ISG exhibited superior biocompatibility and enhanced protection of encapsulated peptides. Sustained *in vivo* release of peptides with distinct physicochemical properties was maintained for over one month, and the liraglutide-loaded ISG demonstrated prolonged glucose-control efficacy in a diabetic mouse model. Collectively, this study establishes a simple, scalable ISG platform for long-term peptide delivery, offering a promising strategy to enhance peptide stability and therapeutic performance.



Platform Design & Characterization

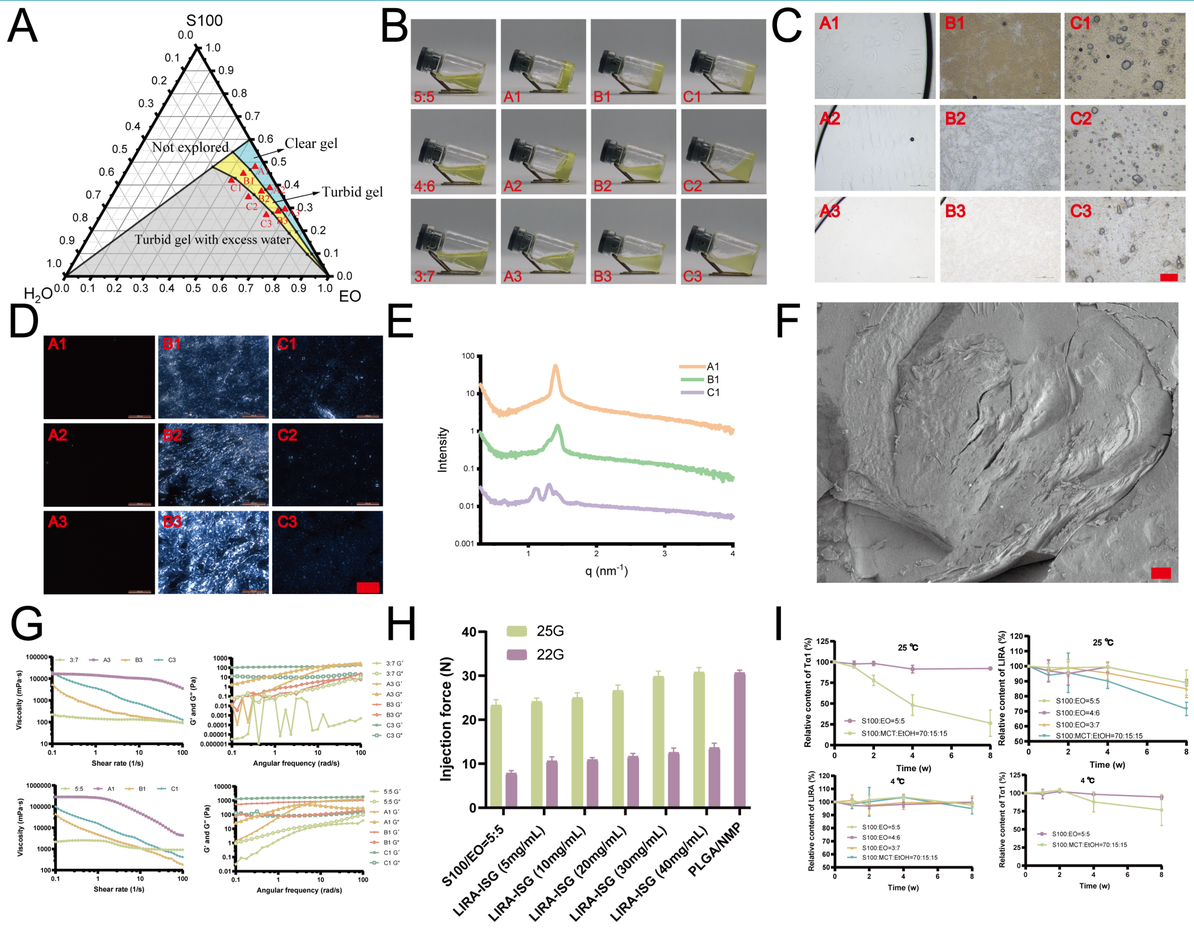


Figure 1. Phase behavior, microstructure, and physicochemical properties of the phospholipid/ethyl oleate ISG platform. (A) Ternary phase diagram of S100, EO, and water at 25 °C. **(B)** Macroscopic appearance of S100/EO formulations at weight ratios of 5:5, 4:6, and 3:7, corresponding to the points marked in (A). **(C)** Optical microscopy images of the samples from different regions (scale bar: 100 μm). **(D)** Polarized light microscopy (PLM) images showing the evolution of liquid crystalline textures upon water exposure (scale bar: 500 μm). **(E)** Small-angle X-ray scattering (SAXS) profiles of representative samples, revealing structural transitions and the emergence of lamellar ordering. **(F)** Cryo-SEM image of a hydrated formulation, revealing a multilamellar structure (scale bar: 40 μm). **(G, H)** Rheological properties demonstrating the gel strength and injectability. **(I)** Stability of the model peptide encapsulated in the ISG under different storage conditions.

Biocompatibility & Degradation

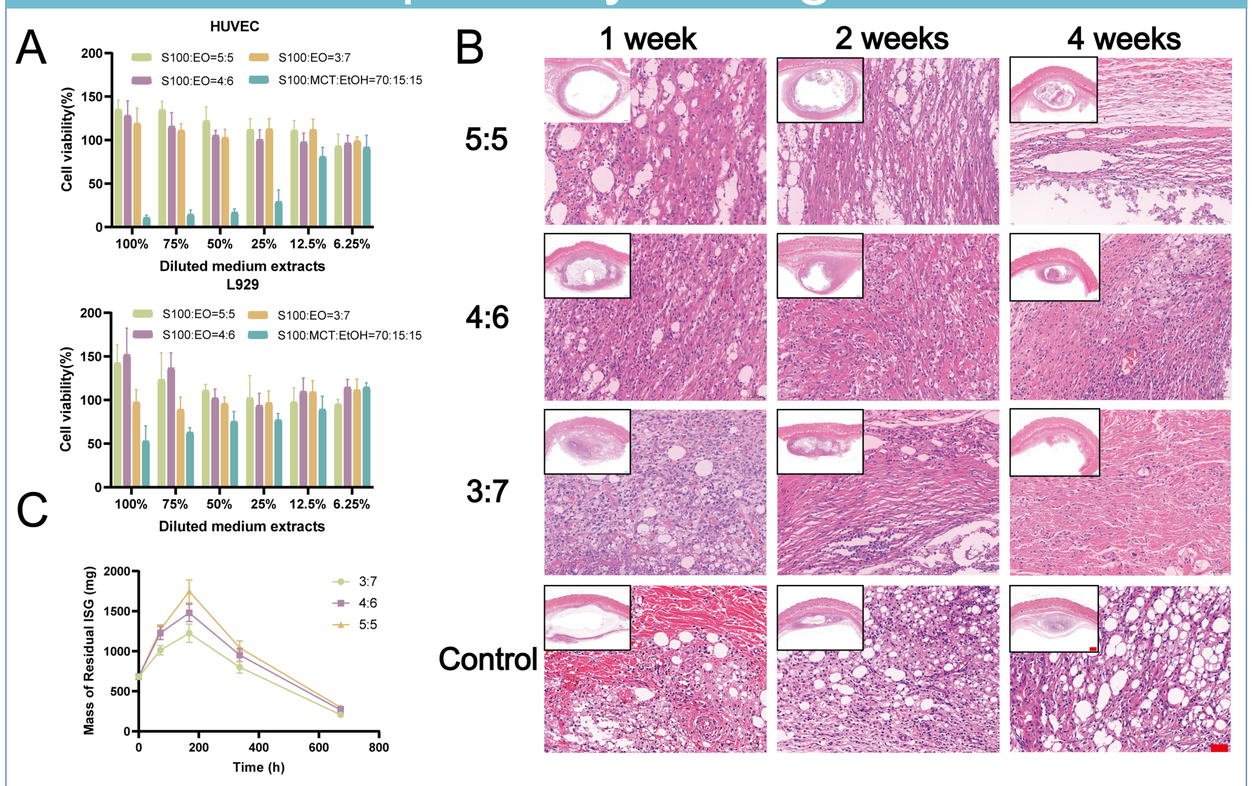
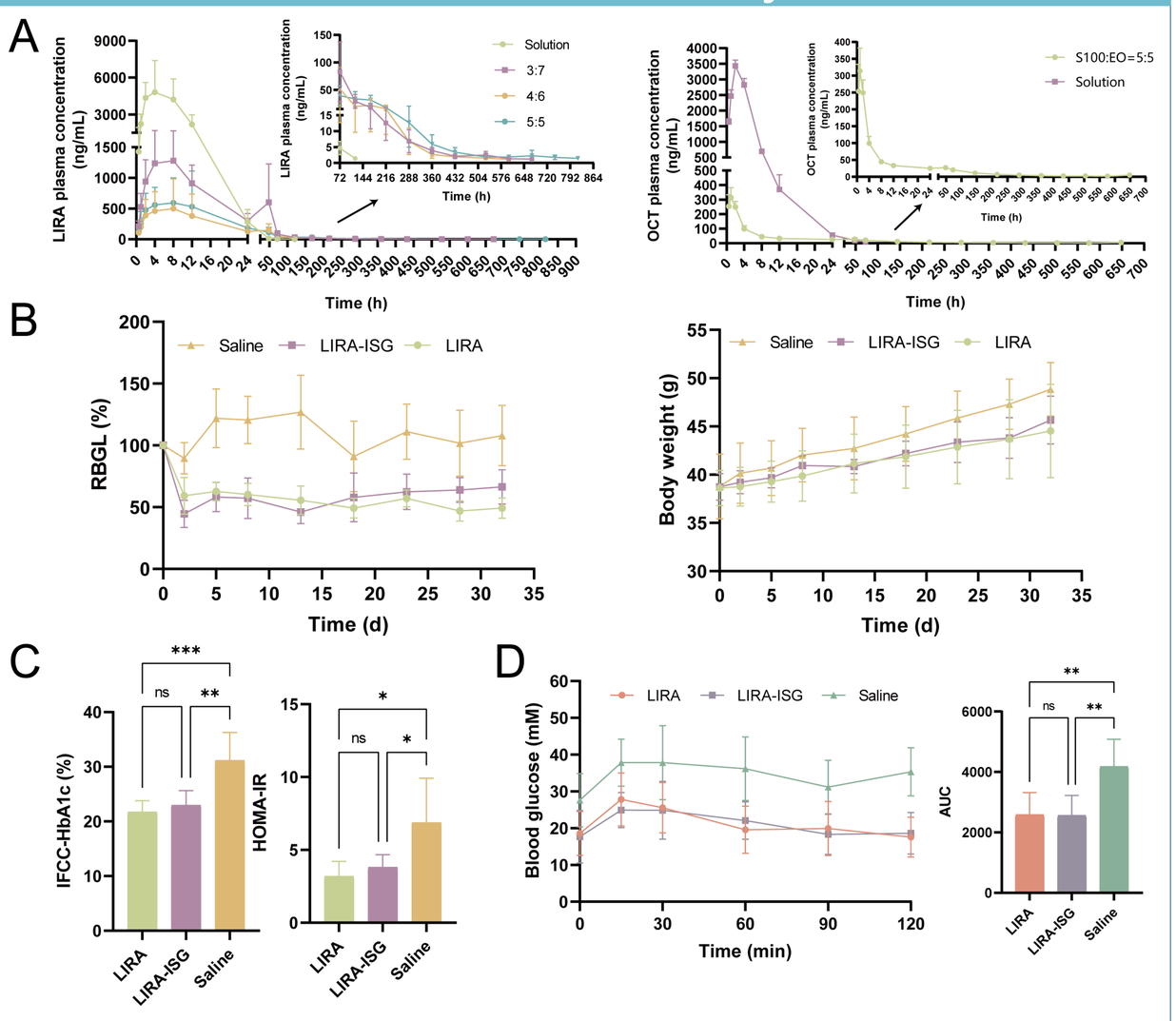


Figure 2. *In vitro* and *in vivo* biocompatibility of the EO-based ISG. (A) Cytotoxicity of gel extracts toward HUVECs and L929 cells. **(B)** Representative H&E-stained histological sections of subcutaneous tissue at the injection site, showing minimal local tissue response (scale bars: 1000 μm [overview] and 50 μm [magnified view]). **(C)** Quantitative analysis of residual ISG mass over time after subcutaneous administration, indicating *in vivo* degradation behavior.

In Vivo Efficacy



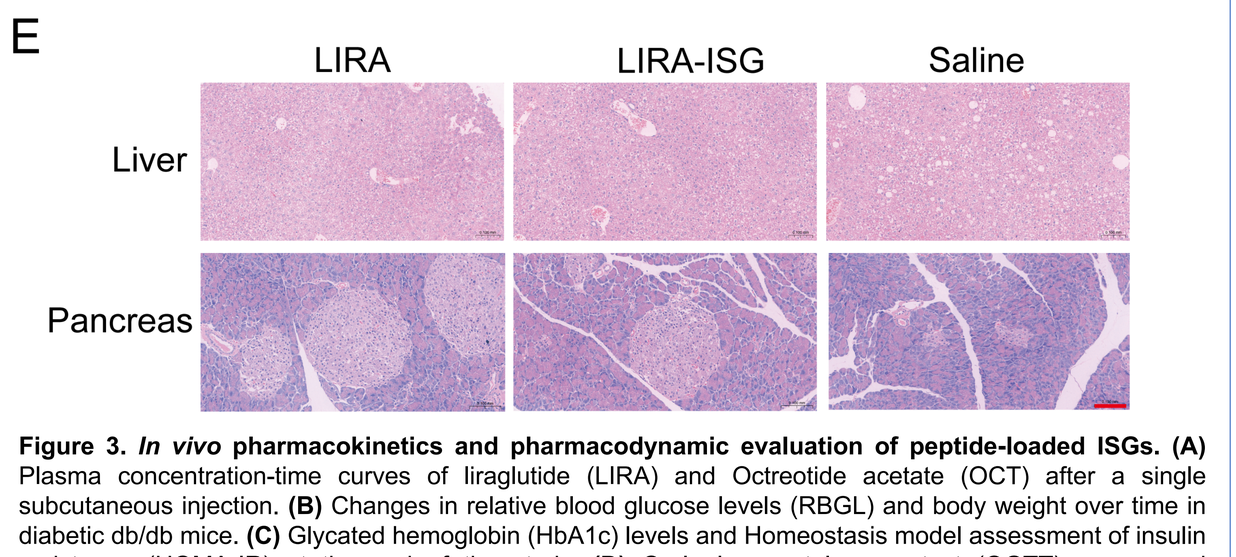


Figure 3. *In vivo* pharmacokinetics and pharmacodynamic evaluation of peptide-loaded ISGs. (A) Plasma concentration-time curves of liraglutide (LIRA) and Octreotide acetate (OCT) after a single subcutaneous injection. **(B)** Changes in relative blood glucose levels (RBGL) and body weight over time in diabetic db/db mice. **(C)** Glycated hemoglobin (HbA1c) levels and Homeostasis model assessment of insulin resistance (HOMA-IR) at the end of the study. **(D)** Oral glucose tolerance test (OGTT) curves and corresponding AUC. **(E)** Representative H&E staining images of liver and pancreatic tissues.

Conclusion

This work presents a simple, scalable, and mechanistically distinct ISG platform that integrates material design, phase-transition mechanism, and therapeutic validation. The formulation undergoes *in situ* solidification solely through inward diffusion of water, inducing phospholipid self-assembly. The absence of bidirectional solvent-water exchange represents a clear mechanistic departure from classical ISGs and underlies the improved biocompatibility and depot stability observed *in vivo*. This work offers a safer and more versatile strategy for long-acting delivery of peptides, proteins, and other biologics.