Hybrid Chondrocyte Biomembranes for Targeted and Efficient Delivery of SIRT6 Activators for Osteoarthritis

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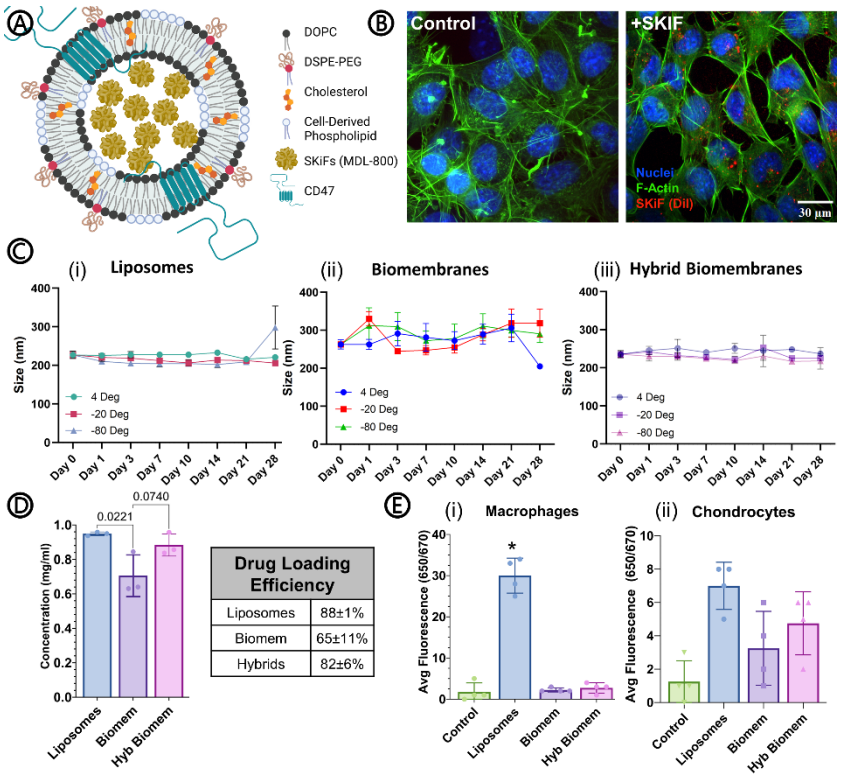
Introduction: Osteoarthritis (OA) is a chronic degenerative joint disease marked by progressive cartilage loss, inflammation, and impaired mobility, affecting ~7.7% of the global population. Current therapies largely alleviate symptoms but fail to modify disease progression. Sirtuin 6 (SIRT6) has recently emerged as an attractive therapeutic target owing to its regulatory roles in inflammation, oxidative stress, and chondrocyte senescence, key drivers of cartilage degeneration. To advance targeted OA therapy, we developed a hybrid chondrocyte biomembrane system (**Fig. 1A**) for the delivery of MDL-800, a potent SIRT6 activator. This platform integrates natural chondrocyte membranes with synthetic lipids to combine the biological specificity of cell-derived membranes with the stability and loading efficiency of liposomes. The resulting hybrid vesicles provide chondrocyte-specific targeting, enhanced colloidal stability, and prolonged intra-articular retention. By leveraging micellar nanocarriers (SKiF) to encapsulate the hydrophobic MDL-800, the system achieves improved solubility and bioavailability (**Fig. 1B**). Together, these design elements enable efficient, cell-directed delivery of SIRT6 activators for potential disease-modifying therapy in osteoarthritis.

Methods: Nanoparticle Preparation: Liposomes were formulated from DOPC, cholesterol, and DSPE-PEG$_2$K (2:1 v/v chloroform: methanol solution). A lipid film, formed by solvent evaporation, was rehydrated with 1 mL of PBS, PBS containing MDL-800, or PBS containing SKiF particles (40°C, 20 min), followed by vortexing (5 min) and extrusion (11 passes, 100 nm PC membranes). Cell-derived biomembranes were isolated from ATDC5 cultures. Cells were lysed in ice-cold 0.25× PBS with 30 mM Tris-HCl and 2 mM EDTA, and membranes were pelleted (15,000 × g, 20 min, 4°C). Pellets were rehydrated with 1 mL of PBS, PBS containing MDL-800, or PBS containing SKiF particles, then extruded (11 passes, 100 nm PC membranes). Hybrid biomembranes were prepared by rehydrating lipid films directly with the biomembrane suspension, followed by brief vortexing and extrusion (11 passes, 100 nm PC membranes). Characterization**:** Nanoparticle stability (size, polydispersity) was monitored via dynamic light scattering (DLS) at 4°C, −20°C, and −80°C. Drug loading efficiency was determined by high-performance liquid chromatography (HPLC). Cellular uptake and internalization were assessed in ATDC5 chondrocytes and IC-21 macrophages using DiI-labeled membranes.

Results: Liposomes with SKiFs **(Fig. 1Ci)** displayed average sizes of 210–230 nm with low PDI (~0.12–0.18), indicating uniformity and stability across all storage conditions. Biomembranes with SKiFs **(Fig. 1Cii)** showed larger, more variable sizes (240–330 nm, PDI ~0.25–0.35), reflecting greater heterogeneity due to membrane complexity. Hybrid biomembranes with SKiFs **(Fig. 1Ciii)** maintained intermediate sizes of 220–260 nm with moderate PDI (~0.18–0.25), suggesting stable, well-balanced assemblies combining liposomal and biomembrane features. HPLC quantification revealed that liposomes achieved the highest SKiF loading efficiency (88.01%), confirming their robust encapsulation capacity. Biomembranes exhibited lower loading efficiency (65.42%), consistent with their structural variability and reduced encapsulation potential (**Fig.1D**). Hybrid biomembranes, though slightly below liposomes, retained high efficiency (81.99%), validating their ability to merge liposomal stability with membrane-derived specificity. Cellular uptake studies in IC-21 macrophages **(Fig. 1Ei)** revealed the lowest internalization for hybrid biomembranes, indicating effective evasion of phagocytosis, likely mediated by CD47 surface protein expression. Uptake studies in chondrocytes (**Fig. 1Eii**) showed no significant differences among the formulations, suggesting comparable cellular interaction across all groups.

Discussion: This study reports the development of hybrid chondrocyte biomembranes incorporating SKiF nanocarriers for targeted delivery of the SIRT6 activator MDL-800 to osteoarthritic cartilage. The hybrid formulations achieved size uniformity (220–260 nm) and moderate polydispersity comparable to liposomes, while maintaining reduced reticuloendothelial uptake. Although HPLC analysis indicated lower than expected drug concentrations across all formulations, liposomes retained the highest encapsulation, and biomembranes the lowest. The hybrid configuration achieved a favorable balance between retention, integrity, and homogeneity. By integrating cell-derived components, these biomembranes exploit homotypic targeting for enhanced chondrocyte specificity and minimized systemic clearance, representing a promising strategy for sustained intra-articular drug delivery. Future studies will quantify intracellular MDL-800 accumulation in culture media and cells, assess cytotoxicity using Live/Dead, PrestoBlue, and PicoGreen assays, and evaluate senescence through β-galactosidase staining to confirm functional safety and efficacy.



Significance: Osteoarthritis therapies remain largely symptomatic, lacking disease-modifying efficacy. The hybrid biomembrane platform introduced here unites synthetic liposomal stability with biological membrane functionality, enabling enhanced cartilage targeting and prolonged therapeutic residence. Incorporating SKiF micellar carriers resolves hydrophobic drug-loading limitations while maintaining high encapsulation efficiency and structural stability. This system represents a significant advancement toward clinically viable, targeted SIRT6 activation for mitigating chondrocyte senescence, reducing inflammation, and slowing cartilage degeneration, potentially improving quality of life for millions with osteoarthritis.

Figure 1. *(**A**) Schematic of hybrid biomembrane composition. (**B**) Uptake of pure SKiF by chondrocytes showing no cytotoxicity. (**C**) Size and stability under storage at 4°C, −20°C, and −80°C (n = 6). (**D**) Comparison of MDL-800 loading efficiency. (**E**) Comparative cellular uptake among formulations.*

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