Surfactant-Stripped Micellar Delivery of a SIRT6 Activator Targets Senescence in Post-Traumatic Osteoarthritis

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Introduction: Osteoarthritis (OA) remains a predominant cause of pain and disability among older adults, yet no disease-modifying therapies are currently available; existing interventions are limited to symptomatic pain management. Addressing this critical therapeutic gap, we have developed surfactant-stripped, kinetically frozen (SKiF) nanocarriers engineered for the high-capacity encapsulation of hydrophobic agents in aqueous media. This platform is particularly suited for targeted intra-articular delivery, where injection volume is constrained. We encapsulated MDL-800, a potent Sirtuin-6 activator, within poloxamer-based SKiF nanocarriers to mitigate the senescence burden of chondrocytes, a key pathological driver in OA (**Fig. 1A**). These nanocarriers demonstrate robust MDL-800 loading, exceptional stability, efficient cellular uptake and demonstrate therapeutic potential, collectively positioning SKiF as a promising modality for OA intervention.

Methods: We synthesized SKiF nanocarriers through drug solubilization and a surfactant-stripping process[1]. Briefly, MDL-800 was dissolved in an organic solvent at 10 mg/mL and added dropwise to a 10 wt% poloxamer (F127 block copolymer) solution with stirring. The suspension was stirred for 3 hours to remove the organic solvent, followed by a critical micellar concentration (CMC) switching process and multiple washes to maximize MDL-800 loading without compromising nanocarrier stability. The solution was then diluted in cold PBS, and loosely bound poloxamer molecules were removed using a 100 kDa molecular weight cut-off filter at 4°C, followed by sterilization through a 0.22 µm nylon syringe filter.

The resulting SKiFs were characterized for size via dynamic light scattering (DLS, Brookhaven Instruments), morphology via transmission electron microscopy (JEOL JEM 2010), and drug and carrier concentration via spectrophotometry (Perkin Elmer). For functional assays, ATDC5 prechondrogenic cells were cultured at a density of 10^5 cells/cm² in RPMI supplemented with 10% FBS and 1% antibiotic-antimycotic solution. Senescence was induced with either 100 nM doxorubicin (LC Laboratories) for 4 days or 10 Gy irradiation. To assess SKiF efficacy, senescent cells were treated with SKiF at 40 µM and 200 µM for 4 days in growth media. Controls included a 10% poloxamer solution and MDL-800 in DMSO. Senescence was quantified using a Senescence-Associated β-galactosidase (SA-β-gal) assay kit (Cell Signaling).

In vivo efficacy was evaluated in a murine anterior cruciate ligament rupture (ACL-R) model of OA. 23 mice, 12 male and 11 female, were treated with either 10 µL of PBS (n = 12) or 10 µL of SKiF containing MDL-800 at a concentration of 13.15 mg/ml (n = 11) at weeks 0, 1, 3, and 6 post ACL-R. Pain tolerance was quantified at weeks 8, 10, and 12 using the Randall-Selitto method, and both injured and uninjured legs were collected at week 12. Legs were fixed and then micro-CT scanned in PBS (Voxel Size = 10 microns). Micro-CT scans were analyzed using Bruker's CT software. All animal studies were approved by the SUNY University at Buffalo IACUC.

Results: The SKiF nanocarriers measured 36.2 ± 2.6 nm in diameter, as determined by DLS. Our surfactant-stripping technique significantly enhances the MDL-800-to-poloxamer molar ratio, achieving a 19.2:1 ratio (mass ratio 1:1) in a streamlined process (**Fig. 1B**). This SKiF formulation demonstrated stability, with no observable precipitation or aggregation for at least one week at room temperature and for several weeks at -20°C and -80°C (**Fig. 1C**). Preliminary studies using the ATDC5 chondrocyte cell line indicate rapid SKiF internalization, as confirmed by fluorescent imaging, which reveals their accumulation in endosomal vesicles within hours (**Fig. 3D**). Additionally, SA-β-gal staining showed a 12.5% reduction in senescence in SKiF-treated cells compared to controls, suggesting a promising anti-senescence effect.

In vivo, SKiF-MDL-800 restored pain tolerance in ACL-R mice to levels comparable to uninjured controls by week 8 (n = 11, $p > 0.05$), whereas PBS-treated mice showed no recovery (**Fig. 1E**). Micro-CT revealed greater osteophyte formation and tibial subchondral bone loss in PBS-treated mice versus SKiF-treated animals (**Fig. 1F**). Quantitatively, Micro-CT analysis revealed significantly increased trabecular separation (n = 11, $p < 0.05$), in the proximal epiphyseal and metaphyseal tibia, in PBS-treated versus SKiF-treated mice suggesting that the bone is deteriorating in the PBS-treated mice while the SKiF-treated mice maintain a denser and healthier trabecular network (**Fig. 1G**).

Discussion: These findings establish SKiF nanocarriers as a promising disease-modifying platform for OA, targeting senescence-associated pathways central to disease progression. The surfactant-stripping approach enables high drug loading with minimal carrier, potentially reducing off-target effects. Efficient cellular uptake and significant reduction in senescence markers in vitro, together with functional and structural improvements in vivo, underscore the therapeutic potential of MDL-800–loaded SKiF. Future studies will focus on long-term efficacy, safety, and translation to clinical models.

Significance: Inflammatory cytokines, which comprise the senescence-associated secretory phenotype (SASP)[2], contribute significantly to the progression and symptom aggravation of OA. Current OA treatments focus primarily on pain relief, with no FDA-approved therapies that address the underlying mechanisms of disease development. MDL-800-loaded SKiF nanocarriers offer a novel, targeted intra-articular therapy by delivering therapeutics directly to senescent cells at clinically relevant concentrations. This approach addresses a critical unmet need in OA treatment, with the potential to modify disease progression and significantly improve patient outcomes.

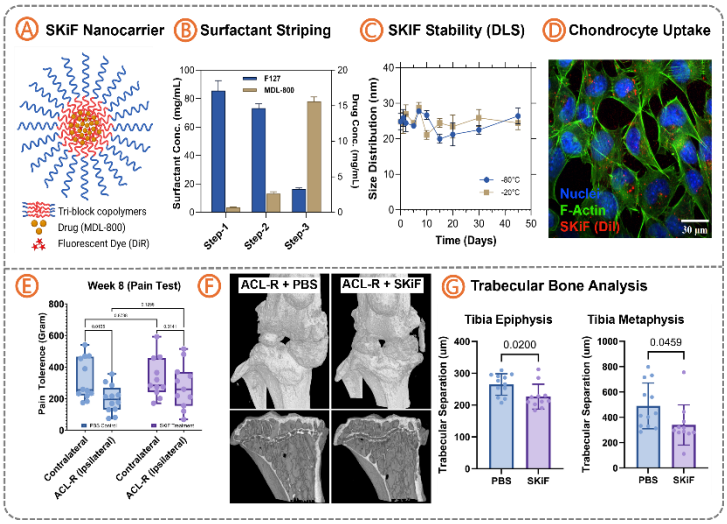

Figure 1: SKiF nanocarrier synthesis and characterization. *(**A**) Schematic of SKiF nanocarrier structure and composition. (**B**) High-concentration MDL-800 loading via surfactant-stripping. (**C**) DLS analysis showing SKiF stability at –20°C and –80°C over several weeks. (**D**) Rapid uptake of MDL-800–loaded SKiFs by chondrocytes in vitro. (**E**) In ACL-R mice, SKiF-MDL-800 restored pain tolerance by week 8 to levels comparable to uninjured knees (n = 11, $p > 0.05$), unlike PBS controls. (**F**) MicroCT images showing greater osteophyte formation and tibial subchondral bone loss in PBS group versus SKiF-treated mice. (**G**)MicroCT analysis showing significantly increased trabecular separation in PBS-treated versus SKiF-treated mice in both the proximal epiphyseal and metaphyseal tibia (n = 11, $p < 0.05$).*

References: 1. Zhang, Y. et al. Therapeutic surfactant-stripped frozen micelles. Nature Communications 7, 11649 (2016). https://doi.org:10.1038/ncomms11649, 2. Lozano-Torres, B. et al. The chemistry of senescence. Nature Reviews Chemistry 3, 426-441 (2019). https://doi.org:10.1038/s41570-019-0108-0.

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