Injectable Thermogel for Prolonged Joint Retention of Nanocarriers Loaded with Sirt6 Activators in Post-Traumatic Osteoarthritis

Zachary Varrenti[1], Yiting Song[1], Jacqueline Shine[2], Mingqian He[2], Jayishnu Roy[1], Brian O. Diekman[2], Jonathan F. Lovell[1], Ramkumar T. Annamalai[1]

[1]University at Buffalo, Buffalo, NY, [2]University of North Carolina, Chapel Hill, NC

Statement of Purpose: Osteoarthritis (OA) is a leading cause of pain and disability in older adults, yet no disease-modifying therapies currently exist. To address this gap, we developed surfactant-stripped, kinetically frozen (SKiF) nanocarriers capable of high-capacity encapsulation of hydrophobic agents in aqueous media. MDL-800, a potent Sirtuin-6 activator, was loaded into poloxamer-based SKiFs to reduce chondrocyte senescence, a key driver of OA pathology (**Fig. 1A**). SKiFs exhibit high drug loading, stability, and efficient cellular uptake, demonstrating strong therapeutic potential. However, *in vivo* imaging revealed rapid clearance from the knee joint within 3–6 hours, underscoring the need to improve intra-articular retention. To overcome this limitation, SKiFs were incorporated into a F127 matrix exhibits sol-gel transition at body temperature, forming an injectable depot for prolonged SKiF retention and sustained MDL-800 release.

Methods: SKiF nanocarriers were synthesized via drug solubilization and surfactant-stripping. MDL-800 (10 mg/mL in organic solvent) was added dropwise to a 10 wt% poloxamer F127 under stirring. After solvent removal and critical micellar concentration (CMC) switching, excess surfactant was eliminated by 100 kDa filtration (4°C) and sterilized (0.22 µm filter). SKiFs were characterized by dynamic light scattering (DLS, Brookhaven Instruments) for size, transmission electron microscopy (TEM) for morphology, and spectrophotometry for drug loading. ATDC5 cells were cultured in RPMI + 10% FBS, and senescence was induced by 100 nM doxorubicin (LC Laboratories) or 10 Gy irradiation. Senescent cells were treated with SKiFs (40 µM or 200 µM) for 4 days; controls included 10% poloxamer and MDL-800 in DMSO. Senescence was quantified using a SA-β-gal assay (Cell Signaling). In vivo efficacy was tested in a murine ACL rupture model (23 mice; PBS, n=12; SKiF-MDL-800, n=11; 13.15 mg/mL; 10 µL doses at weeks 0, 1, 3, and 6). Pain tolerance was assessed at weeks 8, 10, and 12 (Randall-Selitto), followed by leg collection, fixation, and micro-CT (10 µm voxel, Bruker software). All animal studies were approved by the SUNY University at Buffalo IACUC. Thermogelling properties of F127 (15-35 wt%) ± SKiF was analyzed by rheological temperature sweep (0–40°C, 1°C/min, 10 s^{-1} shear rate, 1500 µm gap, 20mm sandblasted geometry) (TA Instruments).

Results: SKiF nanocarriers measured 36.2 ± 2.6 nm and achieved a high MDL-800-to-poloxamer molar ratio of 19.2:1 (1:1 mass ratio) (**Fig. 1B**). The formulation remained stable for at least one week at room temperature and several weeks at −20°C to −80°C (**Fig. 1C**). ATDC5 chondrocytes rapidly internalize SKiFs and accumulate in endosomal vesicles within hours, as confirmed by fluorescent imaging (**Fig. 1D**). 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 near-baseline by week 8 (n = 11, p > 0.05), while PBS-treated mice showed no recovery (**Fig. 1E**). Micro-CT revealed greater osteophyte formation and subchondral bone loss in PBS versus SKiF-treated groups (**Fig. 1F**). DiR-labeled SKiFs showed strong local retention immediately post-injection with rapid clearance in 3–6 h. Rheology showed all F127 matrices ≥20 wt% transitioned from liquid to gel at 37°C, with higher F127 or MDL-800 content lowering gelation temperature (**Fig. 1G**).

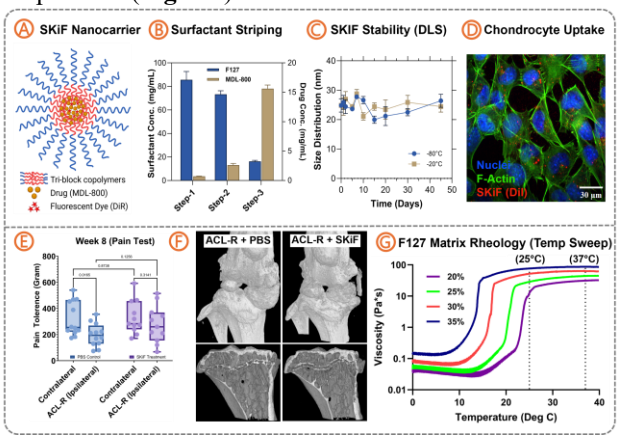

Figure 1: SKiF nanocarrier synthesis/retention and F127 matrix thermogelling characterization.
*(**A**) SKiF nanocarrier structure and composition. (**B**) High-concentration MDL-800 loading via surfactant-stripping. (**C**) DLS analysis showing SKiF stability at –20°C and –80°C over several weeks. (**D**) 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 show greater osteophyte formation and tibial subchondral bone loss in PBS group versus SKiF-treated. (**G**) Temperature sweep revealing the thermogelling properties of F127 matrices (wt% + 1 mg/mL MDL-800).*

Conclusions: These findings establish SKiF nanocarriers as a promising platform for disease modification in OA through targeting senescence-associated pathways. While the surfactant-stripping method enables high drug loading with minimal carrier effect, rapid clearance limits intra-articular efficacy. Incorporating SKiFs into thermogelling F127 matrices has the potential to enhance local retention and enable sustained release. Rheological data confirms gelation at physiological temperature, supporting this injectable, slow-release system for future optimization and evaluation in post-traumatic OA models.