

## Localisation of Fluorescently Labelled Drug-Free Phospholipid Vesicles to the Cartilage Surface of Rat Synovial Joints

**Authors :** Sam Yurdakul, Nick Baverstock, Jim Mills

**Abstract :** TDT 064 (FLEXISEQ®) is a drug-free gel used to treat osteoarthritis (OA)-associated pain and joint stiffness. It contains ultra-deformable phospholipid Sequeosome™ vesicles, which can pass through the skin barrier intact. In six randomized OA studies, topical TDT 064 was well tolerated and improved joint pain, physical function and stiffness. In the largest study, these TDT 064-mediated effects were statistically significantly greater than oral placebo and equivalent to celecoxib. To understand the therapeutic effects of TDT 064, we investigated the localisation of the drug-free vesicles within rat synovial joints. TDT 064 containing DiO-labelled Sequeosome™ vesicles was applied to the knees of four 6-week-old CD® hairless rats (10 mg/kg/ joint), 2-3 times/day, for 3 days (representing the recommended clinical dose). Eighteen hours later, the animals and one untreated control were sacrificed, and the knee joints isolated, flash frozen and embedded in Acrytol Mounting Media™. Approximately 15 sections (10 µm) from each joint were analysed by fluorescence microscopy. To investigate whether the localisation of DiO fluorescence was associated with intact vesicles, an anti-PEG monoclonal antibody (mAb) was used to detect Tween, a constituent of Sequeosome™ vesicles. Sections were visualized at 484 nm (DiO) and 647 nm (anti-PEG mAb) and analysed using inForm 1.4 (Perkin Elmer, Inc.). Significant fluorescence was observed at 484 nm in sections from TDT 064-treated animals. No non-specific fluorescence was observed in control sections. Fluorescence was detected as discrete vesicles on the cartilage surfaces, inside the cartilaginous matrix and within the synovial space. The number of DiO-labelled vesicles in multiple fields of view was consistent and >100 in sections from four different treated knees. DiO and anti-PEG mAb co-localised within the collagenous tissues in four different joint sections. Under higher magnification (40x), vesicles were seen in the intercellular spaces of the synovial joint tissue, but no fluorescence was seen inside cells. These data suggest that the phospholipid vesicles in TDT 064 localize at the surface of the joint cartilage; these vesicles may therefore be supplementing the phospholipid deficiency reported in OA and acting as a biolubricant within the synovial joint.

**Keywords :** joint pain, osteoarthritis, phospholipid vesicles, TDT 064

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