INTRODUCTION: Osteoarthritis (OA) is amenable to local drug delivery due to its involvement of a single or few joints. The major challenge to local drug delivery in OA is the short residence time of small molecules and large polymers alike. The residence time of non-steroidal anti-inflammatory drugs (NSAIDs) in synovial fluid following intra-articular injection is 1-5 hours (1) and the half-life of intra-articular hyaluronic acid preparations is in the order of hours (2). Micelles and liposomes are two popular nanocarrier platforms that are widely used to control target tissue drug accumulation and temporal release (3,4). The aim of this project is to evaluate time-dependent cartilage surface association, penetration and chondrocyte uptake of micelles and liposomes using an in-vitro bovine cartilage plug model.

METHODS: In-vitro cartilage model: Full-thickness articular cartilage plugs (12 mm diameter) were harvested from skeletally-mature bovine femoral condyles. The plugs were immobilized with 1% agarose in 12-well plates to ensure a level surface and prevent exposure of the sides of the plugs to test preparations. Viability of the plugs was maintained by incubation at 37°C in 95% humidity and 5% CO₂ in DMEM media containing 1% penicillin/streptomycin. Cartilage plugs were incubated with fluorescently-labeled micelles, fluorescently-labeled liposomes at a final concentration of 1 µg/ml for 1, 2, and 4 hours (n=3 in each group at each time point). Non-treated cartilage plugs served as controls (n=3 at each time point).

Liposomes and micelles containing equivalent amounts of total lipid (5 mg/ml) and 1,2-dioleoyl-sn-glycerol-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) ammonium salt (Rh-PE) for fluorescent labeling were prepared as previously described (5,6). All preparations were subjected to measurement of average particle size and assayed for rhodamine fluorescence to ensure the homogeneity of preparations across treatment time points. A predefined cartilage region of interest (ROI) and a penetration depth measured as the depth in microns at which the fluorescence intensity was 30% of the fluorescence intensity in the superficial layer of the cartilage. Statistical analysis: Analysis of Variance (ANOVA) was used to analyze differences among normalized fluorescence intensity in the micelle and liposome groups at different time periods. The ratio of normalized fluorescence was reported as mean ± 95% confidence interval (95% CI) at different time points. Cartilage penetration depths were reported as mean ± standard error of the mean (SEM).

RESULTS: At 1, 2 and 4 hours, both micelles and liposomes associated with articular cartilage as observed in histological specimens (Fig 1A). Across different time points, the fluorescence intensity of micelle-treatment was significantly higher (p<0.001) than the corresponding liposome treatment as determined by a ratio of micelle to liposome fluorescence (mean (95% CI) of 11.33 (6.83-15.78), 10.41 (3.97-16.84) and 7.83 (4.50-11.17) at 1, 2, and 4 hours, respectively (Fig 1C). The fluorescence intensity of the 4-hr micelle treatment was significantly higher (p<0.05) than the 1 and 2 hr micelle treatment and the 1, 2, and 4 hr liposome treatment. The fluorescence intensity of the 2-hr micelle treatment was significantly higher (p<0.05) than the fluorescence intensity of the 1, 2, and 4 hr liposome treatment. Comparing the histological slides of the different time points, micelles were shown to be uptaken by chondrocytes in the middle and deep zones of cartilage at the 2 and 4 hour time points (Fig 1B). In contrast, the 2 and 4hr liposome treatment did not exhibit chondrocyte uptake in the middle and deep cartilage zones. The depth of cartilage penetration in the 2-hr micelle treatment was 305±190 micron and was not significantly different (p=0.43) from the cartilage penetration depth in the 2-hr liposome treatment (118±69 microns).

DISCUSSION: Micellar nanoparticles exhibited a time-dependent increase in cartilage diffusion and chondrocyte uptake. Overall, micelles showed significantly better association and accumulation in articular cartilage compared to liposomes. Our preliminary data strongly support our general hypothesis that nanocarrier platforms differ in their association with and disposition within articular tissue. A mechanistic basis for this differential association is yet to be determined but the difference in average particle size of micelles and liposomes could have some influence. Additionally the presence of an abundance of proteoglycan component of cartilage. Micelles showed appreciable penetration and association with chondrocytes warranting further investigation of this platform in the delivery of OA disease-modifying drugs.