INTRODUCTION: Osteoarthritis, as a debilitating epidemic, affects 3.8% of the worldwide population and an even higher percentage of the United States population. Despite the high prevalence of the disease little is known about the pathophysiology at the molecular level. Exosomes are small membrane bound vesicles that carry miRNA and are thought to play an important role in cell-cell communication. The primary purpose of this study was to characterize the differences between exosomes isolated from synovial fluid of patients with and without OA. We also investigated the impact of these extracellular vesicles on chondrocytes and the role of synoviocytes in secreting exosomes carrying OA-related miRNAs. We propose that the exosomal miRNA’s transferred between chondrocytes and synoviocytes could play a significant role in the pathogenesis of osteoarthritis.

METHODS: Synovial fluid was isolated from patients between the ages of 40-60 without OA (n=12) and with OA (n=7). The size and concentration of exosomes within the synovial fluid were quantified using Zetaview with nanoparticle tracking analysis (n=6). In addition, CD-63, an exosome specific cell surface marker, was labeled using an anti-CD63 antibody to confirm the specificity of these isolated particles. Exosomes were labeled with the membrane dye PKH67, and chondrocytes were then treated with these labeled exosomes to explore cellular uptake kinetics and endocytosis using confocal microscopy.

RESULTS: Nanoparticle tracking analyses showed that neither the concentration (OA 1.18E10 particles/mL n=6, Non-OA 1.59E10 particles/mL n=6) nor size (OA 0.128 μm n=6, Non-OA 0.127 μm n=6) of exosomes differed between the groups. The anti-CD63 labeling antibody bound strongly to the vesicles isolated from the synovial fluid, consistent with the morphology of exosomes. Confocal imaging demonstrated that chondrocytes readily endocytosed PKH67-labeled exosomes (Fig. 1). MicroRNA (Fig. 2) arrays revealed that the expression of several miRNAs was altered in exosomes from OA patients compared to healthy subjects: miR-99a-5p (Fold change=-2.07, +/-1.51 p=.71), miR-28-5p (Fold change=1.95, +/-1.8 p=.19, miR-200C-3p (Fold change=2.50 +/- 1.73, p=.04), miR-23b, miR-155-5p (Fold change=1.95, +/-1.8 p=.19), miR-7-5p (Fold change 1.98, +/-1.11 p=.03), and miR-150 (Fold change=-2.52, +/-3.5 p=.36). miR-200C-3p, miR-7-5p, and miR-155-5p have previously been suggested to play a role in TNF-α signaling, and so we examined the effects of TNF-α treatment on the expression of these miRNAs in synoviocyte-derived exosomes. Results showed that 24-hr TNF-α treatment increased the abundance of miR-155-5p by 2.5 fold in synoviocyte-derived exosomes, while miR-7-5p, and miR-200c-3p expression were not significantly changed.

DISCUSSION: The secretion of exosomes by synoviocytes and their uptake by chondrocytes may represent a clinically important communication system. Since TNF-α is known to be upregulated by chondrocytes in patients with OA, the upregulation of miR-155-5p could stimulate a potential positive feedback loop with the chondrocytes as miR-155-5p has been demonstrated to mediate inflammation caused by TNF-α. Although TNF-α did not lead to the upregulation of miR-7-5p, and miR-200c-3p, this upregulation in OA patients could be due to either other chemical mediators in the synovial fluid or the secretion of these exosomes from multiple types of cells including synoviocytes.

SIGNIFICANCE: These differences in exosome profiles between patients with and without OA could have important clinical applications, either as a potential biomarker for the progression of OA or as a potential therapeutic target for OA.

Figure 1: The top row (A,B,C) shows chondrocytes treated with DAPI and unlabeled exosomes. The bottom row (D,E,F) shows chondrocytes treated with DAPI and PKH67 labeled exosomes. The left column shows only DAPI, the middle column shows only PKH67 labeling and the right column shows a combination of DAPI and PKH67.