Introduction: Posttraumatic osteoarthritis (PTOA) is a debilitating degenerative disease that follows trauma to the cartilage, bone, and/or soft tissues in the joint. PTOA of the hip, knee, and ankle affects approximately 5.6 million Americans. However, because PTOA develops following trauma, there is an opportunity for early intervention to delay progression of disease. Mesenchymal stem cells (MSCs) and other progenitor lines have been targeted as a potential cellular therapy for the treatment of osteoarthritis. Studies have focused both on their regenerative capabilities and also their immunomodulatory functions in the joint. Because MSCs are native to the joint, in particular at the synovium, articular cartilage, and subchondral bone, it is possible that they could be a source of reparative cells.

Cartilage impaction or injurious compression has been shown to lead to chondrocyte death in many in-vitro models. In-vivo models using this cyclical compression mechanism have been developed to successfully create and study an osteoarthritic phenotype in the knee. The ankle also experiences compressive axial loading with this mechanism. We observed an intra-articular cellular response in the ankle to this loading, with cells appearing on the surface of the talar cartilage. We hypothesize that these cells have progenitor characteristics, and may potentially be a population of MSCs involved in the response of cartilage to injury and PTOA.

Methods: In this study, 3-month old FVB, male mice (Jackson Laboratories; Bar Harbor, Maine) were injured. All animals were handled using protocols approved by an institutional animal care veterinarian (IACUC # 11-037).

Axial compression was performed using a Bose Electroforce® 3200 (Framingham, Massachusetts) biomechanical testing device. Various loading protocols were used to test for a dose dependent response to injury. The first protocol called for loading 10N of force at 5mm/second for 10, 80, 240 cycles to test for dose dependent changes by number of impactions. The second protocol, testing for differences in phenotype following high-energy loading and ACL rupture, loaded 12N at 5mm/second for 240 cycles or until the ACL ruptured. Control mice were anesthetized and injected with buprenorphine, but were not subjected to any compressive injury. In total, seventy-six (change this number to what’s appropriate) joints were analyzed in this study. Following either loading protocol, mice were allowed ad-lib activity and euthanized at 1-week or 8-weeks after injury.

Ankles were dissected, with the majority of the soft tissues removed and fixed in zinc buffered formalin (Anantech LTD; Battle Creek, MI) at 4°C overnight. Ankles were decalcified in a 0.45M ethylenediamine...
tetraacetic acid solution (EDTA) solution for one week. Following decalcification, knees were dehydrated and embedded in paraffin (Richard-Allan Scientific; Kalamazoo, MO) for sectioning. Seven micron-thick sagittal sections were taken using a Microm® HM 325 Microtome. Sections between samples were standardized to location based upon the depth from the start of the lateral joint surface. The talar-tibial joint was of interest in this study. Both tibia and talus were divided into posterior, middle, and anterior thirds along the articular surface. Sections were stained using Safranin-O (Sigma; St. Louis, MO)/Fast Green (Sigma; St. Louis, MO) to evaluate proteoglycan content (PG) and cartilage morphology. Immunohistochemistry for Lubricin/Proteoglycan 4 (PRG4), CD44, CD90/Thy-1, Sca-1/Ly6A/E, and integrin beta-1 (CD-29) was performed. All stains were counter-stained using a 1:5 solution of hematoxylin. Histologic scoring was performed by adapting the OARSI mouse specific scoring scale.

**Results:** Grossly, injured ankles developed a cellular layer on the surface of the talar cartilage. There does not appear to be a progression of disease from one to eight weeks, based upon histologic scoring for osteoarthritis. The cell layer above the ankles remains at eight weeks and does not appear to change phenotypically. There were no statistically significant differences in the osteoarthritis scores at the tibia or talus between the various groups at both one and eight weeks. These results are summarized in micrographs in Figure 1. In control ankles, Lubricin is expressed by chondrocytes, particularly at the superficial layer. In areas of injury, where there is proteoglycan staining loss, chondrocytes stain negatively for Lubricin/PRG4. The cellular layer above these lesions, however, stain strongly for Lubricin/PRG4. Figure 2 shows representative images highlighting the differences in lubricin/PRG4 expression between injured and control ankles. The cellular layer stained positive for CD44 and integrin β1 (CD29), while staining negative for CD90/Thy-1 and Sca-1/Ly6A/E. Figure 3 shows representative micrographs of slides stained with the panel of antibodies, highlighting positive and negative staining at the cellular layer.

**Discussion:** The initial goal of this study was to create model that can be used to study PTOA following chondral injury in the ankle. Although we did not see a progression of degenerative cartilage disease between one and eight weeks, we did observe the presence of the cellular layer above lesion areas. Thus, our primary interest turned to characterizing the cells present above the articular cartilage lesions. In this study, we have found that these cells are phenotypically positive for some markers that would make suggest that they are some kind of progenitor cell line. Furthermore, the high positive lubricin staining exhibited by these cells leads us to believe that these cells are acting to repair the injured cartilage. Thus, it is our belief that these cells could represent a potential autologous source for engineering cartilage. What this study could not elucidate was the source of these cells and what role they play in the injured ankles. Future studies should focus on understanding the origin of these cells and their role in articular cartilage injury in the talar-tibial joint.

**Significance:** This model demonstrates that upon injury to the ankle, a population of cells forms above the area of articular cartilage lesion. The cells observed and characterized in this study could represent an ideal population of cells that could be used for autologous tissue implantation into the lesions in the articular cartilage.
Figure 1: (A) Representative histological samples, Safranin-O stained and pictures at 10x magnification of control and injured (10, 60, 120 and 240 cycles loads, as well as ACL ruptured). (B) Micrograph at 20x magnification showing cellular layer over cartilage lesion characterized by loss of staining and chondrocyte absence.

Figure 2: Representative micrographs of anti-Lubrinic/PRG4 stained ankles at 10x, 20x, and 63x magnification for control and injury treated ankles. Note strong chondrocyte staining (highlighted by red arrows) in the control and the lack of staining (highlighted by blue arrows) in injured chondrocytes. In injured ankles, the cellular layer is expressed Lubrinic/PRG4 with a high signal.
Figure 3: Representative micrographs of anti-CD44, CD90/Thy-1, CD29, and Sca-1 stained ankles at 10x, 20x, and 63x magnification injured ankles. The cells in the cellular layer above the articular cartilage lesions is positive for CD44 and CD29 and negative for CD-90/Thy-1 and Sca-1/Ly6A/E.

ORS 2015 Annual Meeting
Poster No: 1317