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<b>College *</b>	College of Medicine
<b>Department *</b>	Orthopaedics
<b>Title of Research *</b>	Characterization of a Stem Cell-Like Population in Cartilage
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### Introduction & Purpose \*

Articular cartilage is an avascular and aneural tissue that is believed to be incapable of healing after an injury. Acute chondrocyte necrosis and apoptosis induced by mechanical injury to articular cartilage is thought to contribute to the pathogenesis of post-traumatic osteoarthritis (PTOA). While routinely studying the effects of injury on cartilage explants, confocal microscopy revealed a cell population with fibroblast-like morphology in and around injury sites where extensive chondrocyte death had occurred. Since our model is an isolated osteochondral explant system, it is believed that these cells are derived from the cartilage itself. The morphology and migratory activity of these cells is atypical for chondrocytes, which are normally spheroid in shape and non-motile. Our work supports the idea that these fibroblastic cells are derived from a sub-population of chondrocytes activated by injury. We have been studying the characteristics and functions of these endogenous stem cells through multiple techniques in order to better understand their protective and reparative capabilities. We hypothesize that an upregulation or introduction of a population of these cells in cartilage, could have profound implications for the treatment of articular injuries.

### Experimental Design \*

Separation of the superficial one-third of cartilage from the deep two-thirds: A custom measurement device was fabricated to allow cartilage to be consistently cut. This apparatus allowed attachment of a micrometer (with precision of 2µm) and a blade; permitting an accurate separation of these areas. A six millimeter biopsy punch was used to obtain the specimens from bovine cartilage.

Clonogenic Assay: After separation, cartilage was digested overnight (pronase and collagenase). Using a serial dilution, two hundred cells from each group were grown for ten days in normal culture conditions. Richardson's stain was utilized to stain the fixed colonies.

Trypsinization of chondrocytes from superficial surface: Bovine osteochondral explants were injured using a blunt impact model two days post-harvest. Trypsin was used to detach cells from the superficial surface five to seven days post-injury, depending on the experiment.

Flow Cytometry: Primary antibodies to stem cell markers CD105, CD166, Stro-1, and NOTCH-1 were conjugated to four different fluorophores (absorbance: 390, 494, 550, and 605nm; emission: 479, 521, 575, and 667nm). A concentration of 4µg/ml of antibody was used for each. Cells were analyzed 2-5 days post-harvest.

Chondrocyte Transfer: Cells were harvested (trypsin method) five days post-blunt impact and were cultured for one day before a lentivirus expressing Green Fluorescent Protein (GFP) was introduced (six days post-injury). These cells were placed, superficially and locally, on newly injured explants (100,000 cells introduced to each injury). One week

post-injury, the explants were counterstained with Calcein Red and imaged using a confocal microscope.

## Results \*

The ability of the superficial third of cartilage to grow clones is substantially amplified compared to the deep two-thirds. The total number of clones, the total area covered, and the area per colony are all vastly increased compared to the lower two-thirds. Preliminary data comparing trypsinized cells show similar results; more assays are being performed to further analyze the difference between the trypsinized cells and superficial chondrocytes.

Flow Cytometry: Preliminary data revealed the presence of all four stem cell markers on the surface of cells post-trypsin and culture. The cells were analyzed for double expression of these antigens; all possible combinations of double positive cells were present. CD105/NOTCH-1, Stro-1/CD1-5, and Stro-1/NOTCH-1 showed the highest number of cells expressing both proteins. The surface expression of these markers is being determined for the chondrocytes within the cartilage matrix; superficial third and deep two-thirds being analyzed separately.

Chondrocyte Transfer: Cells expressing GFP were present at the same level within the tissue that the endogenous chondrocytes inhabited. Figure 3 illustrates the intermingling of these cells from two separate bovine explants.

## Conclusions \*

Ongoing work is focused on comparing the stem cell markers present within cartilage to the markers present on the population of cells that propagate on the surface. Immunohistochemistry will be performed on the lentivirus/GFP labeled samples to determine if these stem cell markers can be identified on cells residing within the cartilage. These specimens will also be analyzed for proteoglycan (PG) content to determine if the introduction of these cells at the time of an injury reduces PG depletion. The lentivirus-infected cells were also cultured for clonogenic activity; these will be compared to controls containing no virus. In previous work, we have shown that this population of cells has multipotent potential, an increased ability to migrate, and an increased growth rate.

By combining previous and present work, we can conclude that these cells are an endogenous multipotent stem cell population that propagates under certain conditions. If these cells are shown to reduce PG depletion or reverse cartilage degeneration, a local application of such cells could be used as a clinical therapy for osteoarthritis.

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