**ABSTRACT INTRODUCTION:**

Fresh osteochondral allografts in joint reconstruction are utilized to treat a broad spectrum of articular cartilage pathology. One of the major factors limiting the widespread use of fresh osteochondral allografts is the limited period of time available prior to the onset of irreversible chondrocyte necrosis. The currently available literature has consistently shown a decline in chondrocyte viability beyond fourteen days.1 It has been hypothesized that hibernation is initiated by the interaction of opioid receptors with a “hibernation induction trigger” (HIT) ultimately slowing cell death.2,3 An osteopetrotic phenotype may result in osteoarthritic disease in high affect cartilage metabolism.4

**METHODS:**

Fresh bovine distal femur allografts were used to obtain full thickness cartilage plugs with a diameter of 4 mm. Plugs were taken from the femoral trochlear groove under aseptic conditions. A total of fifty-two osteochondral plugs were harvested from thirteen femurs within twenty-four hours of death. The specimens were prospectively randomized to and stored in a culture medium that consisted of one of three treatment groups:

1. DMEM + 1% Penicillin/Streptomycin + 1% Amphotericin B (Antimicrobial/Antifungal, AB/AF) + 10% Fetal Bovine Serum (FBS) (DMEM)
2. DMEM + AB/AF + FBS + DADLE 2.7 μM (Sigma) (DADLE)
3. Lactated Ringer’s Solution (LR)

Articular cartilage was analyzed at 7, 14, 21, and 28 days of storage. At each time point, full-thickness cartilage specimens were cut into three ~0.5 mm thick axial disks. Each disk was submerged in 5 μM propidium iodide (PI, Sigma) and in 10.75 μM 5-chloromethylfluorescein diacetate (CMFDA, Invitrogen). PI is a membrane impermeable dye, which binds to the DNA of nonviable cells and has a red fluorescence. CMFDA diffuses through membranes of live cells, and reacts with intracellular components to produce cells that have a green fluorescence. Stained specimens were viewed using a laser confocal microscope (Zeiss LSM 510). Each disk was imaged at three random locations producing a total of nine images per cartilage plug, for a total of 36 measurements per treatment group at each time point. Viable and nonviable cells were tabulated with the use of an automated cell counting program, ImageJ (U.S. National Institutes of Health). The number of total cells was determined [total cell number = live cell number + dead cell number] and used to calculate the percentage viability of cells [% viability = (live cell number/total cell number) x 100]. Two-way analysis of variance with post hoc comparisons with Bonferroni correction was used to compare the effects of storage time on cell viability. Statistical significance of mean differences was set at P = 0.05.

**RESULTS:**

Chondrocyte percentage viability at Day 7, 14, 21, 28 of storage is shown in Figure 1. At the time of harvest, chondrocyte viability was 88.7% ± 3.1% (standard error). At Day 7, chondrocyte viability measured by confocal microscopy was significantly less (ANOVA, p < 0.05) in the LR treatment group compared with the DADLE and DMEM groups. At Day 14, chondrocyte viability was significantly greater (ANOVA, p < 0.05) in DADLE than in DMEM and LR. At Day 21, DADLE had a significantly greater viability (ANOVA, p < 0.05) than DMEM and LR. At Day 28, DADLE had a greater viability (ANOVA, p < 0.05) when compared to DMEM and LR. DMEM had a significantly greater viability (ANOVA, p < 0.05) compared to LR at 21 and 28 days of storage.

**DISCUSSION:**

The findings of this study support the hypothesis that the addition of DADLE to storage media improves chondrocyte viability over a period of up to 28 days. The percentage viability of DADLE was significantly greater than the standard media, DMEM, at fourteen to twenty-eight days of storage, and significantly greater than lactated ringer’s solution from seven to twenty-eight days. DMEM had greater viability than LR from seven to twenty-eight days of storage as well, which concurs with previous reports.1 We also found that DADLE maintained its cell viability from 7 to 28 days of storage. This is the first report on the use of an opioid agonist for osteochondral allograft preservation. Future studies are warranted to elucidate the response of human osteochondral allografts to storage with the addition of opioid agonists, as well as to uncover the molecular basis for which opioid agonists affect cartilage metabolism.

**REFERENCES:**