CHONDROCYTE APOPTOSIS IN HUMAN ARTICULAR CARTILAGE STORED AT 4°C IN TISSUE CULTURE MEDIUM FOR AN EXTENDED PERIOD

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Introduction
The use of osteochondral allografts for repair of articular cartilage defects is increasing, but is limited by storage constraints. Harvested allografts are typically used for transplantation within 72 hours of the donor’s death because of decreased chondrocyte viability, but little research has been conducted to assess the nature or extent of the tissue viability (Convery, 1991). The few studies that have examined the time course of articular cartilage viability have not been conclusive because of differences in study design, the use of different species, varied storage periods, and different analytical techniques (Schachar, 1994; Amiel, 1989). Regardless of these differences, it is apparent that viability decreases in relation to storage time. Likewise, few studies have attempted to determine the cause of the decreased viability. Apoptosis in chondrocytes has been shown in vivo and in vitro (Adams and Horton, 1998). Studies have demonstrated that apoptosis increases with age (Adams and Horton, 1998), is involved in osteoarthritis (Blanco, 1998), endochondral ossification (Gibson, 1998), and in damaged cartilage (Tew et al, 2000; Colwell et al. 2001). The current study was undertaken to characterize the extent of apoptosis in human articular tissue stored in tissue culture medium at 4°C for up to 21 days.

Methods
For the current study, osteochondral tissue was collected from spared areas of four femoral condyles at the time of total knee arthroplasty. On the day of harvest, a total of 66 osteochondral plugs, six-millimeter in diameter, was removed with a sterile coring system. The plugs were stored at 4°C in sterile wells containing RPMI 1640 supplemented with 10% fetal bovine serum, antibiotics, MEM non-essential amino acids, and α-tocopherol. The media was exchanged every 48 hours. Three plugs were removed daily for 21 days and frozen in OCT media at -120°C. Using a cryotome, 6-μm thick sections were cut from each plug, and in situ detection of apoptotic nuclei was performed by using the TUNEL method (Promega Corporation, Madison, WI). Day 0 sections served as the negative tissue control. Tissue stored under the same conditions for 12 months served as the positive control. The degree of apoptosis was calculated by dividing the number of positively staining cells by the total number of cells.

Essential Results
Results indicated that apoptosis increased linearly in relation to storage time of tissue. The percentage of apoptotic cells after one day of storage was 2%. After storage for 9 days, the percentage of apoptotic cells increased to 46%, and by 21 days of storage, the percentage had increased to 80%.

Discussion
Clinical survival of osteochondral transplants may be improved by increasing cell viability, and apoptosis rates can serve as an indicator of cell viability. Results from this study demonstrate a time-dependent increase in apoptosis when human osteochondral sections are stored in refrigerated tissue culture media. This data suggests that after eight days in culture, 50% of the cells are apoptotic, thus limiting the clinical utility of grafts stored for this length of time. Future studies will determine the degree to which chondrocyte apoptosis is affected by temperature, contents of the media, and other variables. Prolonging survival of chondrocytes in tissue culture will maximize the supply of available graft tissue, and will additionally allow adequate time for size matching of graft and defect, as well as testing for transmittable diseases.

References