New Insight into Allograft Cartilage: Lessons to be considered

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ABSTRACT INTRODUCTION:
More than 900,000 Americans suffer articular cartilage injuries each year. Patients under 40 years of age with full-thickness defects account for up to 5% of arthroscopies1,2. Treatment options include autologous chondrocyte implantation, osteochondral (autograft and allograft) grafting, microfracture, and arthroplasty. Osteochondral allografts are frequently used, either after being frozen or while relatively fresh following storage at 4°C in tissue culture conditions, in the management of large (greater than 2 cm) osteochondral defects. Recently, a prolonged-fresh preservation process has been developed (14-28 day storage) to extend the availability and viability of fresh allografts; but limited data exists outside of cell viability staining3,4. Furthermore, there are a number of questions that must be answered in order to optimize the technique and increase the use of osteochondral grafting as a procedure to improve cartilage repair and delay the development of post-traumatic osteoarthritis.

The purpose of this study was to evaluate the effect of pro-inflammatory cytokines on the metabolism and survival of chondrocytes obtained from prolonged-fresh osteochondral allografts as compared to fresh chondrocytes obtained from age-matched human organ donors. Our hypotheses were: 1) the metabolism of refrigerated prolonged-fresh osteochondral allografts will be lower than that of the fresh cartilage obtained from organ and tissue donors and 2) cell survival within the graft is compromised by exposure to acute inflammatory mediators (modeling the biology present at the time of surgery) potentially impairing metabolic activity of graft chondrocytes.

METHODS:
Nine prolonged-fresh osteochondral allograft specimens previously refrigerated for 14-28 days were collected at the time of osteochondral allograft surgery and six fresh hemicordylyes were obtained from normal donors within 24 hours of death through the Gift of Hope Organ and Tissue Donor Network. Cartilage was shaved off the bone and 4mm tissue explants were removed by a standard 4mm biopsy punch. Samples were then cultured in media containing 10% fetal bovine serum and divided into the following treatment groups: 1) Control culture (serum only), 2) IL-1β (0.1ng/ml), 3) IL-6 (3ng/ml), 4) IL-1β (0.1ng/ml) + IL-6 (3ng/ml), 5) IL-1β (10ng/ml) + IL-6 (3ng/ml), 6) IL-1β (10ng/ml) + IL-6 (5ng/ml) soluble receptor (5ng/ml) was added to all cultures containing IL-6. Doses of cytokines were determined based on their levels in synovial fluid at the time of surgery of patients undergoing allograft transplantation. Treatment was administered every other day. Prior to initiation of treatment, all explants were kept in culture media for 5 days to allow cells to adapt to the culture and reach a steady state. Tissue and media samples were collected on days 0, 2, 7, and 14. Cell viability (live/dead assay), apoptosis (Tunel assay), histological appearance with Safranin O staining and modified Mankin score, proteoglycan (PG) synthesis and content (normalized to wet weight) were used to analyze cartilage survival and metabolism. Allograft and fresh samples were compared using a paired t-test. Results were considered significant if the p value was less than 0.05.

RESULTS:
At day zero, the viability of prolonged-fresh allograft chondrocytes was 24% lower and they contained 29% more apoptotic cells than fresh chondrocytes (p<0.05) (Fig. 1). Treatment with cytokines did not further increase significant cell death or apoptosis in prolonged-fresh allograft cartilage. However, in fresh cartilage, treatment with high dose IL-1 (10ng/ml) alone or in combination with IL-6 showed a significant decrease in chondrocyte viability by day 14 (decreases of 34% and 43% respectively, p<0.05 and p=0.05) as well as a significant increase in the percentage of apoptotic cells (increases of 38% and 43% respectively, p<0.05 and p=0.05), when compared to day 0 control. (Fig. 2)

Fresh chondrocytes showed 2.5 times greater PG synthesis (p<0.05) and only half as much release of PGs into the media (p<0.01) when compared to prolonged-fresh allograft chondrocytes at day 0. However, fresh chondrocytes were more susceptible to cytokine treatments: by day 14 high dose IL-1 alone or combined with IL-6 inhibited PG synthesis by more than 8-fold (p<0.02) vs 4-fold cytokines in allograft cells (p<0.02) and induced higher PG release, which resulted in higher Mankin score for fresh cartilage (1.3 vs 3.0, p<0.04). Treatment with lower doses of cytokines did not significantly alter cartilage metabolism in either the fresh or allograft tissue.

DISCUSSION:
The primary purpose of this study was to determine the effects of pro-inflammatory cytokines on the viability and metabolism of chondrocytes obtained from prolonged-fresh osteochondral allografts as compared to fresh chondrocytes. Two-week cultures of prolonged-fresh allograft cartilage in the presence of 10% serum did not enhance cell death, apoptosis, or matrix depletion. It also did not inhibit PG synthesis suggesting that allograft tissue may survive additional storage time without further significant impairment of its viability, structural integrity, and metabolic activity. Treatment with IL-6 or low dose IL-1, mimicking a mild inflammatory environment did not cause additional stress to prolonged-fresh allograft tissue and did not induce cell death, apoptosis, matrix depletion, or inhibition of PG synthesis. Only high levels of IL-1 alone or in combination with IL-6 induced cell death, apoptosis, matrix degradation, enhanced depletion of PGs, and strongly inhibited PG synthesis. However, these changes were less dramatic than in fresh cartilage suggesting that the prolonged-fresh osteochondral allograft process may provide some degree of protection against the catabolic effects of the inflammatory process.

The main limitations of our studies are the variability amongst human cartilage samples and an in vitro approach which may not directly mirror in vivo animal studies with longer-term follow-up. In conclusion, osteochondral allografts remain the best treatment option for young patients with larger cartilage defects (greater than 2 cm). Higher concentrations of pro-inflammatory cytokines have less impact on prolonged-fresh osteochondral allograft tissue compared to fresh cartilage tissue in terms of cartilage metabolism and survival. This supports the ongoing use of prolonged-fresh osteochondral allograft tissue despite initially lower amounts of cell viability compared to fresh allograft tissue.

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REFERENCES: