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Etanercept Enhances Preservation of Osteochondral Allograft Viability

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Background: Osteochondral allografting is an increasingly popular treatment method for articular cartilage lesions. With demand for osteochondral allografts in orthopaedics steadily increasing, supply of suitable graft tissue, rather than restrictions in indications, has become the limiting factor in their clinical application. Thus, one challenge to scientists and clinicians has been to optimize the efficacy and utilization of the available donor pool, and in turn maximize the number of patients who can benefit from this unique treatment. While improvements in allograft storage media composition have conveyed clear longevity benefits over traditional Lactated Ringer's solution, prolonged cold storage at 4° C nonetheless predictably leads to deteriorating graft health as a function of storage time despite continued attempts at optimizing storage parameters. Storage of articular cartilage allografts beyond 14 days has been associated with progressive decreases in chondrocyte viability at the time of graft implantation. Prior studies have demonstrated that apoptotic genes and pro-inflammatory cytokines including TNF are up-regulated during storage and may contribute to the loss of cell viability seen.¹ TNF itself has been shown to be a key signal in the apoptotic pathway. The use of a TNF inhibitor or anti-TNF agent will likely help to modulate the apoptotic response of stored tissue and increase the viability of stored articular cartilage allografts and may improve clinical outcomes post-implantation.

Purpose: The aim of this study is to limit the effects of pro-apoptotic proteins and inflammatory cytokines generated during storage via a common TNF inhibitor (Etanercept) and thereby maintain chondrocyte viability up until and beyond the time of graft implantation typically 21 to 28 days.

Methods: Osteochondral allografts were harvested from eight whole mature Boer goat distal femurs and placed into allograft storage media supplemented with 10 µg/mL of Etanercept and stored at 4° C. Cell viability was assessed at the 4 and 8 week mark by removing full-thickness cartilage from the subchondral bone and sectioning it into approximately 0.5-mm-thick coronal slices. These slices were imaged using laser confocal microscopy and a live/dead stain technique to determine cell viability and density. ² Cores were analyzed at each time point and compared to time zero, with and without treatment, to determine the progression of cell loss. Data was analyzed via a two way ANOVA with Bonferroni/Dunn correction.

Results: At both 4 and 8 weeks, Etanercept was found to significantly maintain the viability of the superficial zone above that of the untreated group. Superficial zone viability in the treated group was 69.3% ± 9.4 and 58.3% ± 24.3 at 4 and 8 weeks respectively, compared to 47.8% ± 19.1 and 21.9% ± 21.3 in the untreated group. Both differences were significant with p=0.0125 at 4 weeks and p=0.0065 at 8 weeks. It should be noted that despite the increase of cell viability at 4 and 8 weeks of the treated allograft when compared to the untreated, the levels of cell viability were much lower than at time zero in all cases. At 8 weeks, the full thickness cartilage of the treated group showed a trend towards increased viability vs the untreated group (p=0.099).

Discussion and Conclusion: Etanercept appears to successfully maintain cell viability and density during long term storage of these allografts significantly better than the current storage paradigm. In particular, maintaining the viability of the superficial zone will benefit outcomes by facilitating joint articulation via improved lubrication. Adding Etanercept to the storage media will likely help to modulate the apoptotic response of stored tissue and increase the viability of stored articular cartilage allografts and may improve clinical outcomes post-implantation. In addition, maintaining the cellular viability for increased periods of time will allow a greater window of time in which a suitable recipient may be found.

(continued)

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1 Robertson CM et al. Upregulation of apoptotic and matrix-related gene expression during fresh osteochondral allograft storage. *Clinical Orthopaedics and Related Research*. 2005; 442: 260-66.

2 Mossberg K, Ericsson M. Detection of doubly stained fluorescent specimens using confocal microscopy. *Journal of Microscopy*. 1990;158:215-224.