CHONDROCYTE VIABILITY IN PRESS-FIT CRYOPRESERVED OSTEOCHONDRAL ALLOGRAFTS

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INTRODUCTION: Lesions in articular cartilage have limited capacity of healing due to the avascular nature of the tissue and the lack of mesenchymal stem cells. Available treatments include abrasion chondroplasty, subchondral drilling, and microfracture technique which introduce blood supply and mesenchymal cells that differentiate into chondrocyte-like cells. The fibrocartilage formed as a result of these techniques has inferior weight-bearing properties when compared to articular cartilage. Osteochondral and osteoarticular allografts have produced good-to-excellent results; however, these techniques are associated with donor-site morbidity and increased length of surgery. Fresh osteochondral allograft transplantation allows for the use of autologous grafts which may result in similar weight-bearing properties but requires a lower degree of demarcation between graft and surrounding tissue.cryopreservation of chondrocytes retains some of the metabolic function and chondrocyte viability, while reducing the immune response. Cryopreservation with glycerol may be superior to DMSO. This study investigates the viability of press-fit glycerol-preserved allografts one year after transplantation into a weight-bearing area.

METHODS: Freshly weight-bearing osteochondral plugs (OCPs), 5 mm in diameter, were separated from subchondral bone and incubated in a 3% S-containing medium by slow-freezing. They were stored at -70°C up to 72 hours prior to transplantation. At 5 and 12 months following surgery, sheep were sacrificed and articular cartilage was sampled from the graft-host junction using OATS tools.

Macroscopic Assessment: Articular cartilage was graded at three sites - the graft, the host, and the graft-host junction - using modified Outerbridge criteria. Normal intact articular cartilage was assigned a grade of 0. Cartilage samples were grouped into WB or NWB, autografts or allografts, and then Outerbridge grade averages were calculated per group. A tonometer pen, Tonopen-XL, was used to quantify cartilage firmness (mmHg) and was applied to the cartilage surface at the center of each graft and to surrounding articular cartilage. Histology: Samples of cartilage with underlying bone were prepared for sectioning and stained with H&E, trichrome and Safranin-O. Samples were evaluated based on a modification of Mankin’s histological-chemical grading scale.

Sulfate-uptake: Cartilage samples from grafts and surrounding tissue were separated from subchondral bone and incubated in a 35S-containing media to determine the level of proteoglycan metabolism. Following incubation, cartilage was solubilized to allow for liquid scintillation media to determine the level of proteoglycan metabolism. Following Safranin-O staining when compared to WB allografts and autografts.

Confocal light microscopy: Reduced chondrocyte viability was found in allografts, 56±29% when compared to autografts, 78±15% and surrounding cartilage, 83±49%. However, WB allografts demonstrated viability 77±17% which is comparable to fresh autografts. In contrast, allografts at NWB sites displayed 25±2% viability. WB or NWB placement did not affect autograft viability to a logarithmic degree. WB allografts showed viable chondrocytes evenly distributed in intact lacunae. NWB allografts demonstrated an increased population of non-viable cells, cloning of cells within lacunae and areas of non-cellular matrix.

DISCUSSION: After one year of transplantation, articular cartilage of WB allografts had similar appearance and firmness to the surrounding cartilage and to control autografts. Gross appearance of allografts appeared similar to a previous study. Cryopreservation protocols do not affect mechanical behavior of articular cartilage as was seen by similar cartilage firmness measured in this study.