INTRODUCTION: Successful repair of full thickness defects to articular cartilage remains a clinical and scientific challenge. The use of fresh osteochondral allografts to replace damaged articular cartilage with healthy tissues harvested from human cadavers is a viable treatment option. Allografts are stored at 4°C prior to transplantation to await final culture and maintain sterility, allografts are stored in sealed packages prepared in ambient room air conditions that may lack sufficient CO2 with attendant hypotheses that (1) cold storage and pH changes increase chondrocyte necrosis and apoptosis, and that (2) optimizing CO2 normalizes pH and enhances chondrocyte viability when stored at 4°C.

OPTIMIZING CO2 ENHANCES CHONDROCYTE VIABILITY DURING COLD STORAGE

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RESULTS: After 5 days, flow cytometry revealed that chondrocytes cultured at 37°C/ambient CO2 had greater viability (Group C: 81.8% viable) than chondrocytes subjected to 4°C/ambient CO2, (Group D: 47% viable) (p<0.001) (Figure 2). Chondrocyte viability appeared related to pH. After obtaining a baseline pH of 7.71 prior to temperature/CO2 level segregation, Group D (Cold/Ambient CO2) had a pH of 8.02 by day 1 and increased to a pH of 8.25 by day 5. In contrast, Group B (Warm/ambient CO2) had a pH of 7.77 at day 1 and a pH of 7.36 by day 5 (Figure 2).

DISCUSSION: These results show that equilibration of media at 5% CO2 normalized pH and improved chondrocyte viability when stored in sealed containers at 4°C. Chondrocytes stored in sealed containers at 37°C had similar viability and pH irrespective of CO2 levels because an increase in pH due to low CO2 may have been prevented by the greater overall metabolic activity of chondrocytes at physiologic temperatures. The data also provides a partial explanation for the observed decrease in chondrocyte viability following cold storage in containers sealed at ambient CO2. Although apoptosis rates did not vary among temperature/CO2 level groups in this short term study, the five day period was sufficient for elucidating the differential effects of CO2 levels on chondrocyte viability and pH during cold storage at 4°C. While the effects of CO2 and pH on chondrocytes within intact articular cartilage may differ from our 3-D model, our data suggests that it may be important to store fresh osteochondral allografts in media equilibrated with 5% CO2, or in media supplemented with a CO2 independent buffering system to maintain chondrocyte viability at 4°C.


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METHODS: Three Dimensional (3-D) Cell Culture: Chondrocytes harvested from articular cartilage aseptically removed from freshly slaughtered bovine knees were prepared into alginate beads. The beads were stored in DMEM supplemented with 10% fetal bovine serum at 37°C/5% CO2 for a period of 3 days. Chondrocyte growth media was changed once prior to segregation into experimental groups. Following segregation, containers were sealed and the media was not changed in order to model the storage conditions of fresh osteochondral allografts.

Experimental Groups: After 3 days of incubation, beads were placed in media, equilibrated with either ambient CO2 or with 5% CO2, and assigned to 4 groups: A) Warm (37°C)/5% CO2; B) Cold (4°C)/5% CO2; C) Warm (37°C/Ambient CO2); D) Cold (4°C)/Ambient CO2.

Flow Cytometry: At days 1, 3, and 5 after temperature and CO2 level segregation, the pH for each group was recorded. Chondrocytes were recovered by dissolving the beads in 55mM sodium citrate. The cells were then labeled with Annexin V and Propidium Iodide according to the manufacturer’s protocol (Invitrogen) and analyzed by flow cytometry (Figure 1). Groups were compared with a Chi Square analysis.