The Effect of Warming on Release of Marrow Elements from Osteochondral Cores

Suzanne M. Tabbaa1,2, Camille Glazer1, C. Wayne McIlwraith1, David D. Frisbie1, William D. Bugbee1, Robert L. Sah1,2
1Colorado State University, Fort Collins, CO, 2University of California-San Diego, La Jolla, CA, 3Scripps Clinic, La Jolla, CA

DISCLOSURES: None

INTRODUCTION: The availability of fresh osteochondral allografts (OCA) for treatment of articular defects is limited in part by procurement and storage. Currently, the accepted shelf-life for OCA is limited to 28 days due to the decline in chondrocyte viability during prolonged cold storage (4°C).1,2 The mechanism for storage-associated chondrocyte death is unknown, but associated with inflammatory mediators3 and countered by anti-inflammatory agents.4 Few studies5 have investigated factors released from subchondral bone during storage that may adversely affect cartilage health6. Bone marrow is comprised primarily of adipose bone marrow, which contains over 98% triglycerides, a neutral lipid component7. During the OCA transplantation and typical in vitro analyses procedures, an OCA is removed from 4°C, exposed to room temperature for 10-20 minutes during graft handling, and then incubated, or implanted in the recipient site, at 37°C.8 We hypothesized the warmer temperatures during preparation (21°C) and usage (37°C) accelerates the release of lipid components resulting in cell death of chondrocytes and surrounding host tissue through mechanisms of lipotoxicity. The specific aims of this work were to determine if exposure of OCA to warm temperatures of 24°C and 37°C; (1) affects the release of lipid components and (2) may mediate chondrocyte lipotoxicity through the release of nitric oxide.

METHODS: A total of eight osteochondral cores (diameter = 8mm, bone thickness ~10mm) were harvested from the left and right medial trochlear ridge of two recently-deceased adult horses within 24 hours post-mortem with IACUC approval, and stored in culture medium consisting of Dulbecco’s Modified Eagle Medium with additives (ascorbic acid, L-glutamine, antibiotics, and 10% fetal bovine serum) for 28 days at 4°C. Samples were analyzed following (i) cold storage, (ii) warming in the same storage medium at room temperature (~21°C) for 20 minutes, followed by (iii) warming to 37°C in a humidified 5% CO2 incubator for 4 days in fresh storage medium. Chondrocyte viability at the surface (en face section) and through the depth (vertical section) were analyzed by applying LIVE/DEAD reagents. Total released lipid components in the media were quantified as the mass of lipid extracted with 2:1 chloroform:methanol. Residual lipid remaining in osteochondral cores was determined similarly after pulverizing the bone and normalizing the extracted lipid weight to the weight of the bone. Nitric oxide production. Nitric oxide production was measured in conditioned medium using the Griess reagent. Statistical analysis. Data is presented as mean±SEM. The effect of temperature on amount of lipid release, nitric oxide production, and chondrocyte viability was assessed using one-way ANOVA with post-hoc Tukey test.

RESULTS: Lipid release in media increased with warming conditions. Gross images (Fig. 1A) of extracted lipids from the media showed lipid (bottom yellow layer) with warming at 37°C (n=2) compared to conditions with no warming at 4°C (n=2), and warming at 24°C (n=2). Warming at 21°C and 37°C led to sequential increases in lipid release into the medium (p<0.05) compared to the 4°C samples (Fig. 1B). Gross images of extracted lipids from pulverized bone also demonstrated lipid-like residue (Fig. 1C) that decreased with warming conditions (Fig. 1D). Warming conditions reduced chondrocyte viability both in the en face views (Fig. 1E) and vertical sections (Fig. 1F) compared to cores tested after 28 days of cold storage. Warming conditions showed an effect in all regions of the cartilage. Nitric oxide production (Fig. 2G) increased with warming conditions. Warming the OCCs at 37°C showed higher (p<0.05) in nitric oxide production compared to OCCs from cold storage and after warming to 21°C.

DISCUSSION: This work suggests lipid components trapped within the trabecular bone of OCCs are affected by warming temperatures and may affect chondrocyte viability through mechanisms of lipotoxicity. Warming temperatures relevant to the clinical situation accelerate the release of lipids from the subchondral bone, which may affect chondrocyte and host cell viability and function. The warming conditions experienced by the graft after implant will be additionally affected by the microenvironment of the recipient site, as well as local biomechanics and biotransport not analyzed in this study. An important technical result of the present study is also that the evaluation of chondrocyte viability after storage can be affected by the conditions of incubation with LIVE/DEAD dye. Further understanding of the effects of released lipid marrow elements on chondrocytes and surrounding host tissue may provide an opportunity to enhance osteochondral repair using OCA.

SIGNIFICANCE: Warming of 4°C stored osteochondral grafts accelerates release of lipid components, which may lead to lipotoxic effects on chondrocytes and surrounding reparative host cells. Technically, evaluation of chondrocyte viability can be affected by post-storage sample manipulation.


Figure 1: Released lipid gross images (A) and weights (B) from treatments with warming (Ai-iii) and without warming conditions (Ai). Gross images (C) and weight measurements (D) of remaining lipid extracted from OCCs. Visualization of chondrocyte viability in en face view (E) from OCCs without warming (Ei-iii) and with warming conditions (Eiv-vi). Chondrocyte viability in surface, middle, and deep regions of vertical view (F) from OCCs without warming (Fi-iii) and with warming conditions (Fiv-vi). (G) Nitric oxide production following warming conditions (* indicates p<0.05)