Use of Mitochondrial Transfer to Increase Rabbit Articular Cartilage Explant Viability

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INTRODUCTION: Osteochondral Allograft Transplantation is commonly used to restore articular joint surface when large defects (>3cm^2) are present. The success of this procedure is closely linked to allograft health which decreases over time when stored at 4°C as chondrocyte viability, cell density, and tissue metabolism decrease while the extracellular matrix and bone change minimally. Efforts to improve the storage of these fresh-preserved allografts to increase chondrocyte viability after the standard 14-day testing period have been documented. It has been shown that media formula and presence of growth factors significantly impacts chondrocyte viability in cold storage. Storage at 37°C has been shown to significantly increase chondrocyte viability but has not been established as the standard storage temperature for fresh preserved allograft due to risk for infection. This study was a proof of concept for using freshly isolated mitochondria as either media supplementation or as a treatment meant for uptake. We hypothesized that presence of mitochondria isolated from rabbit cardiac muscle would boost chondrocyte viability of rabbit articular explants stored at 4°C.

METHODS: Mitochondria was isolated from one gram of rabbit cardiac tissue. The tissue was manually minced and underwent two rounds of homogenization and centrifugation using a Teflon pestle and glass homogenization tube with resuspension in mitochondrial isolation buffer containing BSA (MESH + BSA Buffer). Differential centrifugation was used to isolate mitochondrial fraction from the homogenate. The final mitochondria pellet was washed with a MESH buffer without BSA. A BCA protein assay was performed to measure mitochondria concentration. Mitochondria were stained with Mitotracker CMXROS Red. The isolation and staining were conducted on ice and centrifuge cycles were run at 4°C to preserve mitochondria respiration. Rabbit articular cartilage morsels up to 2mm in width and .5mm in depth were harvested by scalpel from rabbit knee allografts following dissection. Cartilage explants were randomly sorted into 9 wells of a 24 well plate and divided into 3 groups: control, in solution, seeded. The control group received 0.1µL MESH vehicle and the solution group wells received a 100µg dose of mitochondria into wells already containing 2mL of low glucose DMEM/F12 supplemented with 10% FBS and 1%AA. Both the control and in solution groups were stirred for three seconds for even treatment. The cartilage morsels in the seeded group were directly treated with a 100µg dose of mitochondria in 0.1µL MESH which was left for 30 minutes before supplementation with media. At 6 days, 13 days, and 20 days, all cartilage morsels were removed from one well for each group and stained with calcein AM and ethidium homodimer to analyze cell viability and mitochondria count via fluorescence microscopy. Images underwent pixel classification using Trainable Weka Segmentation on ImageJ followed by particle analysis. Imaging results were analyzed using a sample size of 10-14 per group per time point. The percent of live cells out of the total cell count and the mitochondria count was graphed for each treatment group at each time point. Both data sets were analyzed using a mixed-effects model with initial values based on GLM. A Tukey's multiple comparisons test was used for the percent live cells data and a Šídák's multiple comparisons test was used for the mitochondria count data.

RESULTS SECTION: At Day 6, no significant difference was found between the groups for viability or mitochondria count. At Day 13, the in-solution group performed significantly worse than both the control and seeded treatment group. At 20 days, both treatment groups were found to be significantly more viable than the control. At Day 13 and Day 20, the seeded cartilage was found to have significantly more stained mitochondria in the tissue than the in-solution group. A linear regression conducted the fluorescent data pooled from all time points and both treatment groups showed a weak correlation between mitochondria count and percent of live cells. This weak correlation was found to be significant with a two-tailed p value of 0.0044.

DISCUSSION: This proof of concept study demonstrated that freshly isolated mitochondria can increase cartilage viability at 4°C. Our finding suggests that mitochondria uptake is a weak predictor of the increase in viability observed in these explants. However, we speculate that the effect of mitochondria on cartilage viability may be multifactorial. For future direction, more studies should be conducted on explants of uniform size and with varying concentrations of mitochondrial treatment. One limitation of this study is the lack of mitochondrial respiration measurement prior to treatment. This might have provided more insights on the viability of the mitochondria and the treatment effect.

SIGNIFICANCE/CLINICAL RELEVANCE: Use of mitochondrial transfer to increase chondrocyte viability indicates future use of mitochondria to optimize osteochondral allograft storage at 4°C and resultant patient outcomes.

IMAGES AND TABLES:

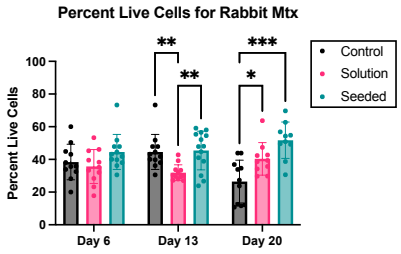

Figure 1 Live cell percentage for 3 treatment groups at 3 time points.
* p < 0.0332,
** p < 0.0021,
*** p < 0.0002,
**** p <0.0001

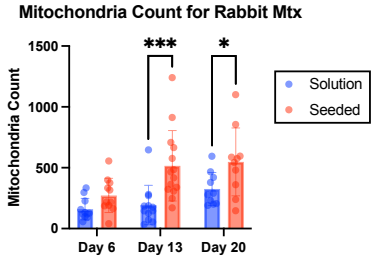

Figure 2
Mitochondria Count for 3 treatment groups at 3 time points.
* p < 0.0332,
** p < 0.0021,
*** p < 0.0002,
**** p <0.0001

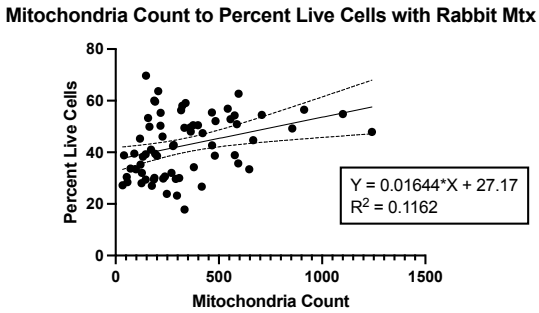

Figure 3 Mitochondria count to percent live cell for all in solution and seeded treated explants at all time points. Linear regression results are displayed on the graph with 95% confidence band. Pearson correlation resulted in p = 0.0044.