Rabbit Versus Rat Mitochondria Transfer to Increase Rabbit Articular Cartilage Explant Viability

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INTRODUCTION: Osteochondral Allograft Transplantation success rate is closely linked to chondrocyte viability, cell density, and tissue metabolism which decrease over time when cartilage is stored at 4°C while the extracellular matrix and bone change minimally. Storing cartilage at 37°C significantly increases chondrocyte viability but has not been widely accepted due to risk for infection. There are many studies conducted to improve the storage of these fresh-preserved allografts. Mitochondria treatment has been shown to preserve cartilage integrity in OA models by restoring metabolic function and decreasing oxidative stress and apoptosis. However, the effect of different source of mitochondria has not been tested for the preservation of cartilage allografts stored at 4°C. In this study, we compare the use of mitochondria isolated from rabbit and rat as media supplementation or pre-storage treatment. We hypothesized that the presence of mitochondria isolated from rabbit and rat cardiac muscle in vitro would boost chondrocyte viability in rabbit articular cartilage explants irrespective of the species.

METHODS: Two separate mitochondria isolations were conducted with one gram of cardiac tissue for both rabbit and rat groups. 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). Two final centrifuge cycles were conducted to wash the mitochondria with MESH Buffer without BSA. A BCA was run to measure protein 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 treated in solutions of 2xAA in PBS following dissection. Cartilage explants were randomly sorted into 5 groups: (1) control group, (2,3) rabbit mitochondria (in solution and seeded), and (4,5) rat mitochondria (in solution and seeded). For the rabbit mitochondria treated group, viability and mitochondria count was measured at 6, 13, and 20 days. For the rat mitochondria treated group, viability and mitochondria count were measured at 6, 13, and 27 days. After fluorescent imaging was performed to analyze cell viability and mitochondria count, all images underwent pixel classification using Trainable Weka Segmentation on ImageJ followed by particle analysis. A sample size of 10-14 per group per time point was used for experimentation. Statistical analysis was done using a mixed effect ANOVA model followed by Tukey's or Sidak's multiple comparison.

RESULTS SECTION: Treatment with rat mitochondria whether in solution or seeded did not improve the viability of the cartilage explants. Treatment with matched species (rabbit) demonstrated no significant difference between groups and compared to control for viability or mitochondria count on Day 6. However, at Day 13, the in-solution group for rabbit mitochondria performed significantly worse than both the control and seeded treatment group. At Day 20, both rabbit mitochondria treatment groups were found to be significantly more viable than the control. For the rabbit mitochondria, the morsels seeded with mitochondria were found to have significantly greater average uptake of mitochondria than in solution group at Day 13 and 20.

DISCUSSION: Proof of concept was demonstrated for the use of mitochondrial transfer to increase cartilage viability when isolated mitochondria was from the same species as recipient. The mitochondria count being significantly higher in the seeded group than the in-solution group at Day 13 and Day 20 when significant differences were also found in the percent live cells indicates that further studies should be conducted on the relationship between mitochondria uptake and cartilage viability. The data suggests that the source of the mitochondria possibly impacts treatment efficacy and uptake. Differences in properties between mitochondria from different sources and tissues must also be assessed.

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. The difference in results based on mitochondria source indicates that future mitochondria treatment studies should characterize the mitochondria used for treatment.

IMAGES AND TABLES:

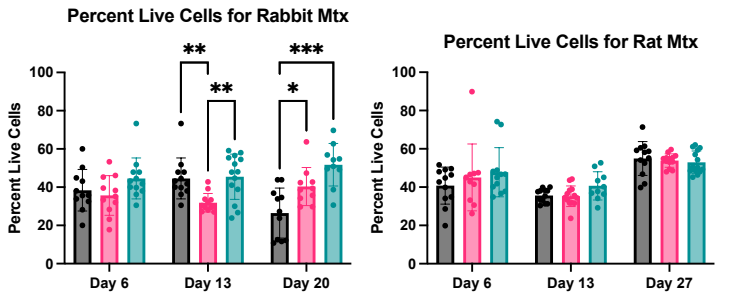

Figure 1 Live cell percentage for 3 treatment groups at 3 time points for rabbit mitochondria and rat mitochondria treatment. Black represents control, pink represents in solution, and blue represents seeded.
* p < 0.0332, ** p < 0.0021, *** p < 0.0002, **** p <0.0001

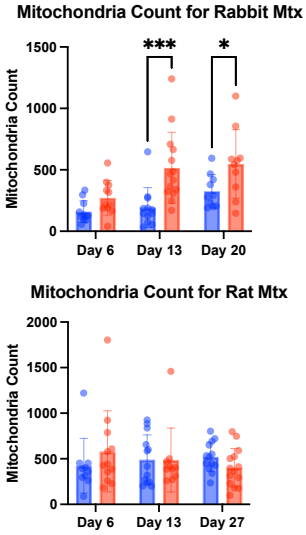

Figure 2 Mitochondria count for rabbit and rat mitochondria treatment at 3 time points. Blue represents solution and red represents seeded.
* p < 0.0332,
** p < 0.0021,
*** p < 0.0002,
**** p <0.0001