

IL-1 β Treatment Increases Mitochondrial Transfer from Mesenchymal Stromal Cells to Cartilage In Situ

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INTRODUCTION: Mesenchymal stromal cell (MSC) therapies for osteoarthritis (OA) have rapidly materialized worldwide, with 64 clinical trials previously conducted, and 122 clinical trials currently underway [1]. Notably, the mechanism of action of MSC therapy for the treatment of OA is unclear. Consequently, the results of clinical trials vary widely. Mechanisms of action that have been previously proposed include differentiation, trophic effects, and immunomodulation [2]. More recently, *intracellular mitochondrial transfer* from healthy MSCs to diseased recipient cells has been shown to have strong therapeutic effects; however, little attention has been paid to mitochondrial transfer as a treatment for OA [3]. Mitochondrial transfer is an incredibly powerful therapeutic mechanism in which a mesenchymal stromal cell donates its mitochondria to diseased cells or tissues.

Notably, our group was the first to show that mitochondrial transfer can occur through the dense ECM of native cartilage tissue [4]. Our current focus involves delving into the potential factors and mechanisms that modulate in-situ mitochondrial transfer. Previously, our group and others have shown that various cellular stressors (metabolic, inflammatory) increase mitochondrial transfer in monolayer culture [3,4]. However, no studies have investigated the effect of these stressors on mitochondrial transfer in cartilage tissue. The objective of this study is to determine the effect of IL-1 β treatment on the rate of mitochondrial transfer from MSCs to cartilage.

METHODS: *Tissue harvest:* Cartilage explants, with the articular surface intact, were obtained from the femoral condyles of 2 neonatal bovine stifle joints (n = 4 explants per group, n = 10 micrographs per explant). Explants were 6mm diameter, 2mm thickness. *Culture:* On days 0-7, explants were cultured in low-glucose DMEM; the IL-1 β group was supplemented with 10ng/mL IL-1 β . Media was changed every 2-3 days. *MSC seeding:* Bovine MSCs were isolated from trabecular bone and transduced with mito-mCherry lentivirus [5]. On day 7, the articular surface of cartilage explants were seeded with ~5,000 MSCs (passage 4). Hyaluronic acid was used as an MSC delivery vehicle, as its viscosity aids in improved seeding efficiency. *Imaging and analysis:* On day 10, explants were stained with calcein AM (live cell stain) and imaged on a Zeiss LSM 880 inverted microscope using a 40x objective; 10 micrographs (3-dimensional z-stacks) were collected at random areas of each explant. Mitochondrial transfer was quantified in napari viewer (python) and was defined as mCherry signal outside of a stromal cell, larger than 1 pixel (221nm) and appearing on at least 2 sequential z-slices. Quantification is shown as mitochondrial transfer events per micrograph (micrograph area was 212 μm^2 , z-depth ranged from 30 μm to 50 μm). Statistical analysis was Kruskal-Wallis rank sum test, using averaged values for transfer events per explant.

RESULTS: Control explants had sparse rounded chondrocytes, with stromal cells well spread-out on the articular surface (Figure 1A). Treatment with IL-1 β resulted in noticeable changes to chondrocyte morphology, while stromal cell morphology remained the same (Figure 1B). In both control and IL-1 β explants, long thin cellular extensions resembling tunneling nanotubes could be seen extending from stromal cells. Mitochondrial transfer could be seen within and immediately surrounding chondrocytes (Figure 1C). Looking at an orthogonal view of a z-stack, transferred mitochondria can be seen internalized within chondrocytes (Figure 1D). Quantifying transfer events reveals a 3 fold increase in mitochondrial transfer in the IL-1 β treated group compared to the control group (p = 0.043).

DISCUSSION: The objective of this study was to determine the effect of IL-1 β treatment on mitochondrial transfer from MSCs to cartilage explants. The data from this study shows that treatment of cartilage explants with IL-1 β increases mitochondrial transfer 3-fold compared to control explants. This data is consistent with mitochondrial transfer data from other systems/tissues, which show generally that transfer is increased in disease/stress states [3]. Furthermore, this data is consistent with previous data from our group, which showed that transfer to chondrocytes in 2D culture is increased following treatment with mitochondrial specific stressors (rotenone/antimycin) [4]. IL-1 β , a powerful inflammatory cytokine, exerts broad effects on cartilage tissue; effects include induction of matrix damage, oxidative stress, and upregulation of cell signaling proteins such as connexin-43 [6]. All of these have been implicated in mitochondrial transfer, and may the mechanism for which IL-1 β increases mitochondrial transfer in cartilage [4].

Here we have demonstrated the modulation of mitochondrial transfer from mesenchymal stromal cells to cartilage explants. Mitochondrial transfer for the treatment of osteoarthritis is tantalizing considering a hallmark of osteoarthritis is mitochondrial dysfunction [7]. Mitochondrial dysfunction manifests as decreased ATP production, increased oxidative stress, increased mitochondrial membrane permeability, and mtDNA mutations- all of which result in cartilage degradation [7]. Studies have shown that the transfer/donation of even a few healthy mitochondria can lead to the sustained restoration of mitochondrial function in a recipient cell [8]. Considering the rapidly expanding interest in stromal cell therapy for osteoarthritis, the ability to modulate mitochondrial transfer from stromal cells to chondrocytes may have significant clinical impacts. Future work will investigate the mechanism(s) of action of mitochondrial transfer, in hopes of developing mitochondrial targeted therapies.

SIGNIFICANCE: This is the first study to show the modulation of mitochondrial transfer in cartilage tissue. As MSC therapy for OA gains traction, further knowledge of mitochondrial transfer mechanisms may inform treatment strategies.

REFERENCES: 1) *clinicaltrials.gov* 2) Wei+ *Int. J. Mol. Sci.*, 2022. 3) Liu+ *Signal Transduct. Target. Ther.*, 2021. 4) Fahey+ *Sci. Rep.*, 2022. 5) Mauck+ *Osteoarthritis. Cartilage*, 2005. 6) Tonon+ *J. Bone Miner. Res.*, 2000. 7) Mao+ *Front. Med.*, 2020. 8) Spees+ *P. Natl. A. Sci.*, 2006.

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Figure 1: A) Maximum intensity z-projection of control explant. View is of the articular surface, looking into the tissue. Scale bar = 50 μm . B) Maximum intensity z-projection of IL-1 β explant. Scale bar = 50 μm . C) Expanded inset from figure 1B, single z-slice showing mitochondrial transfer (circled). Scale bar = 15 μm . D) Orthogonal view of figure 1C, transferred mitochondria inside chondrocyte. Scale bar = 5 μm . E) Treatment of cartilage explants with IL-1 β increases mitochondrial transfer events.

