# Cellular Reprogramming Rejuvenates Synovial Fluid-derived Mesenchymal Stem Cells Obtained from Joints with Osteoarthritis

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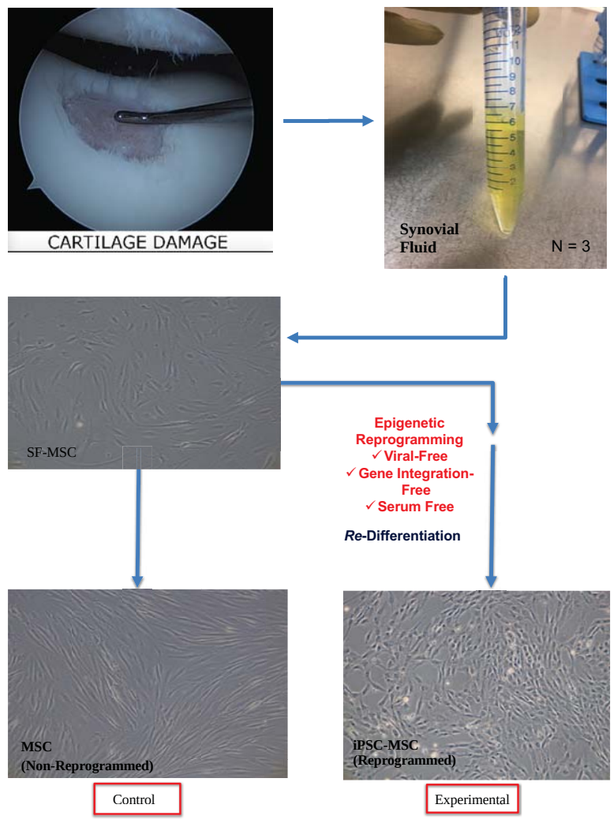
## Introduction

Mesenchymal stem cells (MSCs) hold promise as a therapeutic agent for regenerative medicine. Their translation into clinical application however, is currently limited by properties of the cell that are dependent on an individual's age and overall health status. To overcome the reduced MSC capacity of an aged individual, we hypothesized that epigenetic cellular reprogramming can rejuvenate human synovial fluid-derived mesenchymal stem cells (SF-MSCs). To test this hypothesis, we reprogrammed human SF-MSCs into induced pluripotent stem cells (iPSCs) and subsequently differentiated them into MSCs (iPSC-MSCs). We then compared the activity between iPSC-MSCs (reprogrammed group) to their parental SF-MSCs (non-reprogrammed control group).

## Materials and Methods

After IRB approval, human synovial fluid was collected from the knee joint during arthroscopic surgery for cartilage disease. MSC were isolated and expanded via standard protocol. These parental cells were used to establish subsequent reprogrammed cell lines without altering genetic material (epigenetic reprogramming).

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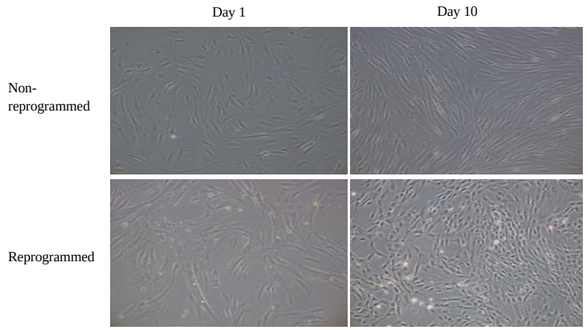
## Results



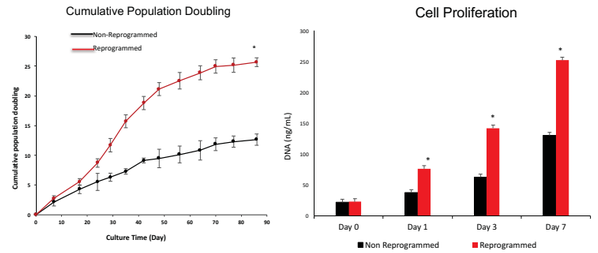




**Figure 1.** Cell Morphology and Growth Characteristics. Above: Cell morphology after 10 day incubation (Nikon 10x). Left: Long-term cell proliferation measured by cumulative population doubling level. Right: Short-term cell proliferation measured by PicoGreen assay. * p-value < 0.01; Student's t-test assuming equal variance.

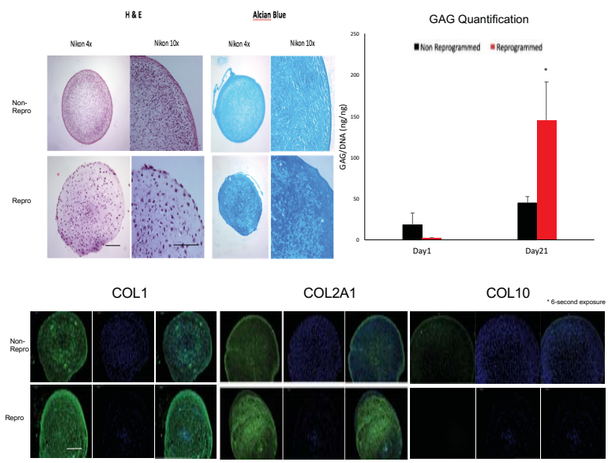



**Figure 2.** Cartilage Differentiation. After reprogramming there was a more organized cartilage architecture (H&E) and greater proteoglycan content (darker blue) on Alcian blue staining (top left). Glucosaminoglycan (GAG) content was significantly greater in the reprogrammed group (top right). Immunoflurescence staining demonstrated increased COL2A1 and decreased COL10 after reprogramming (bottom). These characteristics after reprogramming are similar to native articular cartilage. * p-value < 0.01; Student's t-test assuming equal variance.

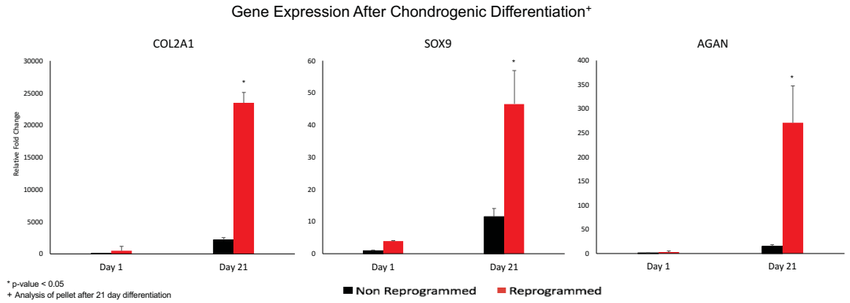



**Figure 4.** Gene Expression After Cartilage Differentiation. Cartilage-tissue related gene expression was significantly upregulated after reprogramming. * Student's t-test assuming equal variance.

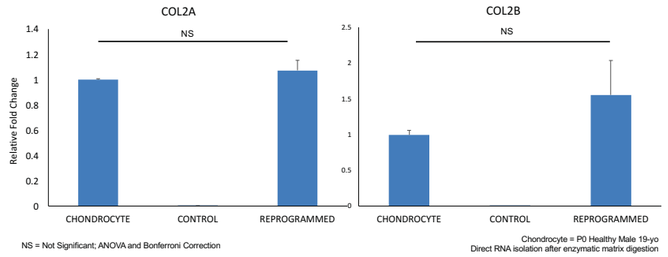



**Figure 5.** Expression of COL2-Subtypes A & B. Reprogramming normalized COL2A & B expression. These genes are expressed early in articular cartilage cell development.

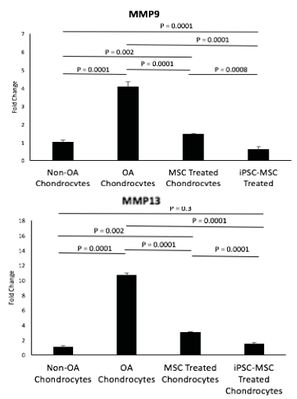



**Figure 6.** Catabolic Modulation of Articular Cartilage. Reprogramming improved MSCs' capacity to regulate genes expressed with cartilage breakdown (ANOVA with Bonferroni Correction).

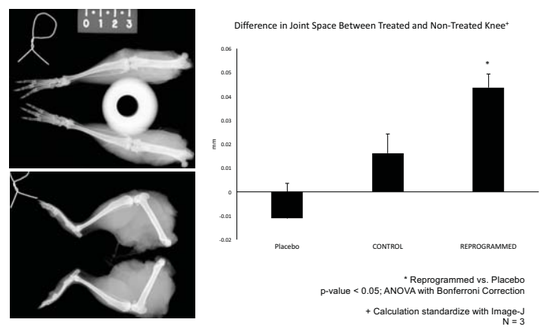



**Figure 7.** In Vivo Data. Nine month old Duncan-Hartley strain 051 guinea pig. This animal model reliably develops degenerative joint disease spontaneously by 7 months of age. Stifles were injected with 50-uL of either saline, 500,000 MSCs (control), or 500,000 reprogrammed cells under fluoroscopic guidance. Faxitron PA and lateral radiographs (left). Joint space as measured using ImageJ, relative to contralateral stifle, after intra-articular injection (right). Joint space loss due to arthritis was significantly less after reprogrammed cell injection.

## Conclusions

- Cellular reprogrammed synovial fluid-derived MSCs, obtained from patients with chondral disease, improves the cells' therapeutic potential.
- Reprogramming improves MSC's potential for tissue regeneration and regulation of chondrocyte inflammation.
- Although early in vivo results are promising, further investigation is required to determine the potential to modulate the joint in vivo.