Transient Exposure of Tissue-Engineered Cartilage Analogs to Synovial Fluid Hematoma after Ankle Fracture Promotes Chondrocyte Death and Alters Gene Expression Toward an Osteoarthritic Phenotype

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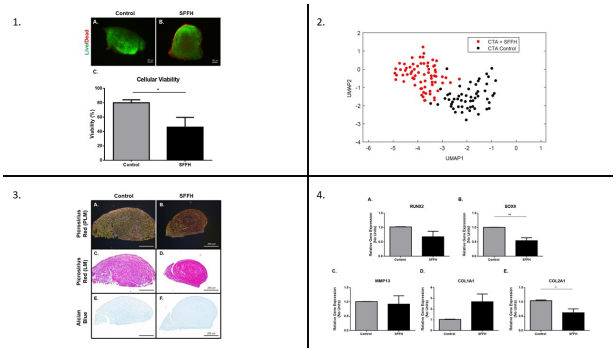
INTRODUCTION: The first stage of fracture healing consists of hematoma formation with recruitment of proinflammatory cytokines and matrix metalloproteinases. Unfortunately, when there is an intra-articular fracture, these inflammatory mediators are not retained at the fracture site, but instead, bathe the healthy cartilage of the entire joint via the synovial fluid fracture hematoma (SFFH). These inflammatory cytokines and matrix metalloproteinases are known factors in the progression of osteoarthritis and rheumatoid arthritis. Despite the known inflammatory contents of the SFFH, little research has been done on the effects of the SFFH on healthy cartilage with regard to cell death and alteration in gene expression that could lead to posttraumatic osteoarthritis (PTOA).

METHODS: SFFH was collected from 12 patients with intra-articular ankle fracture at the time of surgery (Table 1). Separately, C20A4 immortalized human chondrocytes were three-dimensionally cultured to create scaffold-free cartilage tissue analogues (CTAs) to simulate articular cartilage. Experimental CTAs (n=12) were exposed to fresh 100% SFFH for 3 days, washed, and transferred to complete media for 3 days. Control CTAs (n=12) were simultaneously cultured in complete medium. Subsequently, CTAs were harvested and underwent biochemical, histological, and gene expression analysis.

RESULTS: Exposure of CTAs to ankle SFFH 3 days significantly decreased chondrocyte viability by 34% ($p = .027$) (Figure 1.1). Gene expression of both COL2A1 and SOX9 were significantly decreased after exposure to SFFH ($p= .012$ and $p=.0013$ respectively) (Figure 1.4). COL1A1 expression trended upward in the SFFH exposure group while there was no difference in RUNX2 and MMP13 gene expression. Uniform manifold approximation and projection (UMAP) for dimensionality reduction of Picrosirius red staining demonstrated increased collagen I deposition with poor ultrastructural organization in SFFH exposed CTAs (Figure 1.2 and 1.3).

DISCUSSION AND CONCLUSION: Exposure of an organoid model of healthy cartilage tissue to SFFH after intraarticular ankle fracture resulted in decreased chondrocyte viability, decreased expression of genes regulating normal chondrocyte phenotype, and altered matrix ultrastructure indicating differentiation toward an osteoarthritis phenotype. The majority of ankle fracture open reduction and internal fixation does not occur immediately after fracture. In fact, typically these fractures are treated several days to weeks later in order to let the swelling subside. This means that the healthy innocent bystander cartilage not involved in the fracture is exposed to SFFH during this time. In this study, the SFFH caused decreased chondrocyte viability and specific altered gene expression that might have the potential to induce osteoarthritis. These data suggest that early intervention after intra-articular ankle fracture could possibly mitigate progression toward PTOA.



Variable	N (%) / Mean ± SD
Age (years)	53.17 ± 14.6
Sex	
Male	5 (42%)
Female	7 (58%)
Fracture Type	
Trimalleolar	5 (42%)
Bimalleolar	4 (33%)
Fibular (+Deltoid Ligament Tear)	3 (25%)
Time from injury to aspiration (days)	6.25 ± 2.2