Adipose Allograft Matrix (AAM) Improves Cell Viability and Increases IL-10 Concentrations without Altering Extracellular Matrix Gene Expression in Osteoarthritis: Implications for the Use of AAM as an Orthobiologic

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INTRODUCTION: Acellular adipose allograft matrix (AAM) substrate retains collagens and proteins that support host cell infiltration, adipogenesis, and revascularization in soft tissue augmentation applications. AAM, therefore, may serve as a novel 'off-the-shelf' therapy for enthesis regeneration in rotator cuff tears/degeneration by creating a scaffolding for cellular infiltration between the ends of the tear or to serve as a therapy for suprapatellar fat pad restoration in knee fat pad impingement and osteoarthritis. To investigate the potential use of AAM as an orthobiological treatment, pathologically relevant cells and tissue explants were used in co-culture experiments designed to investigate the effects of AAM on the extracellular matrix (ECM) bioactivity of the osteoarthritic cells and tissues at both the transcriptional and translational levels. ECM and matrix metalloproteinases (MMP) gene expression, growth factors, and both MMP and inflammatory cytokine concentrations were then analyzed to determine if AAM impacted ECM remodeling/degradation or impacted the presence of inflammatory molecules present in the osteoarthritic joint. As an inert, acellular matrix, AAM was hypothesized to have no effects on ECM, MMP, or inflammatory cytokine marker activity in the cultured osteoarthritic cells or tissues.

METHODS: All study procedures and protocols were approved by The Ohio State University IRB. Subscapularis (SSC) tendon and humeral head (HH) articular cartilage were collected from patients undergoing reverse total shoulder arthroplasty (n=20), while samples of suprapatellar fat pad (SFP) and femoral condyle (FC) cartilage were collected from patients undergoing total knee arthroplasty (n=14). Following tissue digestion, SSC tenocytes were co-cultured with HH chondrocytes, and SFP adipocytes were co-cultured with FC chondrocytes. Each experiment included a set of cultures plated with media containing 2% AAM and a set plated with control media. This process was repeated using tendon, cartilage, and fat pad explants. After 72 hours, cell and tissue explant culture media were collected. Resazurin and MTT assays were used to check cell viability and cell proliferation, respectively. Expression levels of ECM genes (Col1 Col2, Col3, Col10, COMP, and Aggrecan), MMP-1, -3, and -13, and the transcriptional factors SOX9 and PPARγ were determined using RT-PCR. ELISAs were used to determine MMP-3, MMP-13, IL-1β, IL-6, IL-10, and TNF-α concentrations in the culture media. Sulfated glycosaminoglycan (sGAG) content in culture media and cartilage tissue was determined via DMMB. All data were checked for normality using the Shapiro-Wilk test (α=0.05). Parametric data were analyzed using a paired t-test (α=0.05). Non-parametric data were analyzed using the Mann-Whitney (Wilcoxon Rank Sum) test.

RESULTS SECTION: Tenocyte (~14.0% increase; p=0.015) and chondrocyte (~10.4% increase; p=0.034) cell viability significantly increased and cell proliferation was not significantly altered by AAM. MMP-13 concentration was significantly increased in SSC tenocytes (~131%; p<0.001) and SSC tenocyte/HH chondrocyte co-cultures (14.4% increase; p=0.005) plated with AAM. AAM also significantly increased IL-10 concentrations of SFP explants (~140%; p=0.002) and SFP/FC cartilage explant co-cultures (~105%; p=0.049). ECM (Col1 and Col2) and MMP (-1, -3, and -13) expression and MMP (-1 and -3) and cytokine (IL-1β, IL-6, and TNF-α) concentrations were not significantly impacted by AAM at either the cellular or tissue levels. sGAG concentration was not significantly modulated by AAM.

DISCUSSION: AAM had no significant impact on cell viability or gene expression at either the cellular or tissue levels. The increased MMP-13 concentration found in tenocytes may result from the already increased MMP-13 activity found in the osteoarthritic SSC tenocytes acting on the introduced AAM matrix. Further research is needed to determine why significantly increased MMP-13 concentrations are seen in the tenocytes, but not the chondrocytes or adipocytes. The concentration of the anti-inflammatory IL-10 was significantly increased in both SFP explants and SFP explant/FC cartilage explant co-cultures suggesting AAM may provide protection against inflammation and degradation of native adipose matrix. Future studies will examine IL-10 concentrations in chondrocytes and adipocytes. While limited by the collection of data at one time point which may not be indicative of *in-vivo* conditions, this study provides a foundation for future studies involving pre-clinical animal models or clinical trials investigating the use of AAM as an "off-the-shelf" orthobiological treatment or for use as a cellular scaffold in tissue engineering applications.

SIGNIFICANCE/CLINICAL RELEVANCE: This study addresses the potential use of AAM as an orthobiological treatment for osteoarthritis using cells and explants obtained from relevant pathological tissues. AAM improved cell viability, increased IL-10 concentrations, and had no significant impact on ECM gene expression or inflammatory cytokine concentrations in the osteoarthritic cells or tissues examined.

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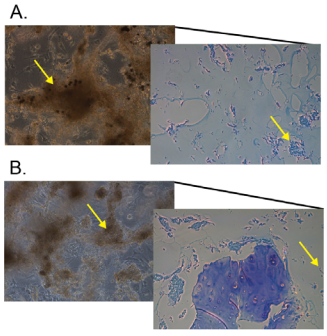
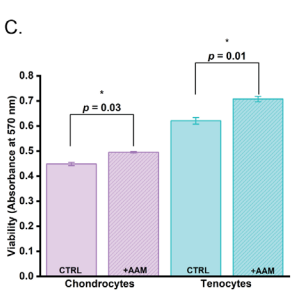
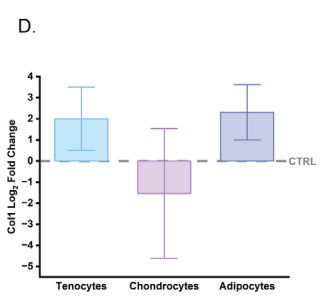


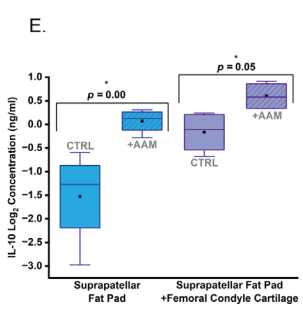
A. Photomicrograph of tenocyte/chondrocyte co-culture with AAM (yellow arrow). Inset: toluidine blue staining of cartilage matrix with AAM (yellow arrow).
B. Photomicrograph of adipocytes with AAM (yellow arrow). Inset toluidine blue staining of fat pad matrix with AAM (yellow arrow)



C. Viability of chondrocytes and tenocytes is statistically significantly increased when cultured with AAM. Data presented as mean ± SD.



D. AAM has no effect on the expression of ECM genes, such as Col1, in tenocytes, chondrocytes, or adipocytes. Data presented as mean ± SD.



E. Box plot showing the anti-inflammatory IL-10 cytokine concentration is increased in fat pad and fat pad/cartilage explant co-cultures with AAM. Squares within the boxes indicate the mean.