Development of a Noncontractile Bioengineered Cartilage Neo-Tissues using Passaged Chondrocytes

Stephanie Richardson-Solorzano[1], Justin Parreno[1]
[1]University of Delaware, Newark, DE
Sgrs@udel.edu

INTRODUCTION: Damage to articular cartilage ultimately results in progression to Osteoarthritis (OA). In OA pathogenesis, inflammatory mediator signaling alters cartilage tissue homeostasis leading to cartilage degradation; chondrocytes reduce matrix expression for Aggrecan (ACAN) and Collagen II (COL2) and elevate degradative enzyme expression such as Matrix Metalloproteinases (MMP) -1, -2, -3, -9, -13. Currently, there are limited disease modifying treatments to prevent OA progression. New models to evaluate the role of inflammatory mediator signaling during OA progression may result in new therapeutic opportunities to treat human OA. Current models of OA pathogenesis use animal (mostly rodent) models to recapitulate disease progression. A drawback to animal models is that such models are not always predictive of the human response (*i.e.*, the response of human chondrocytes may be different from the rodent chondrocyte response). While *in vitro* 2-dimensional human chondrocyte culture is used to study the human chondrocyte responses in OA, a drawback is that these models lack cell-to-matrix interactions. Thus, the generation of new human 3-dimensional (3D) tissue models may aid in elucidating OA pathogenesis. We aim to develop a new model that can mimic the native cartilage environment to study the effect of inflammatory molecules on OA progression. Monolayer expansion (passaging) of chondrocytes enables >400-fold expansion in cell number, which could be used to generate cartilage tissue. Unfortunately, monolayer expansion results in chondrocyte dedifferentiation. In dedifferentiation, chondrocytes spread and alter their actin cytoskeleton from cortical to a stress-fiber organization leading to loss of cartilage matrix expression, and gain of fibroblast matrix expression (i.e. Collagen I). Our previous work has developed methodology to redifferentiate passaged bovine cells enabling the generation of scaffold free cartilage-like tissues in growth factor free conditions. In this study, we aim to redifferentiate passaged human chondrocytes to test our hypothesis that: *the generation of cartilage-like neo-tissues by passaged human chondrocytes can serve as a 3D model to study the effect of inflammatory mediators on chondrocyte homeostasis*.

METHODS: Primary healthy human chondrocytes from subjects between the ages of 20-30 were used; cells were purchased from a commercial source, deidentified, and determined not to required IRB approval. Chondrocytes were expanded in monolayer culture to passage two in media containing fetal bovine serum. At passage 2, cell dedifferentiation was examined by examine gene (qRT-PCR) and protein (WES Capillary) expression as well as imaging via confocal microscopy. To bioengineer scaffold-free neo-tissues from passaged human chondrocytes, passage 2 cells were seeded into agarose molds. After 48 hours, media was switched to 'redifferentiation' media consisting of DMEM supplemented with ITS+ (insulin-transferrin-selenium), proline, pyruvate, dexamethasone, and ascorbic acid. To further stimulate matrix deposition, 10ng/mL TGFβ3 was added with the redifferentiation media. To prevent human passaged chondrocyte mediated contraction of neo-tissue, media was supplemented with 1μm latrunculin at days 0 - 2 and 20μm ROCK inhibitor (Y27632) at days 8-15. Tissues were harvested on days 15 and 20. RT-PCR was used to evaluate gene expression of chondrogenic and fibroblastic genes. Immunostaining of tissue cryosections was used to determine matrix content. To investigate the effect of inflammatory mediators on human cartilage-like neo-tissue, tissues were treated with 5ng/mL IL-1β beginning on day 15. On day 20, tissues were harvested. PCR and immunostaining were used to evaluate the resulting gene and protein expression compared to untreated tissues. Data was analyzed using a t-test (significance set at $p<0.05$).

RESULTS SECTION: In comparison to bovine passaged chondrocytes, human passaged chondrocytes exemplified greater contractile potential. At P2, WES capillary electrophoresis determined that passage human chondrocytes express greater levels of highly contractile actin isoform, alpha smooth muscle actin (αSMA). TGFβ3 exposure further enhances the contractile potential as treatment with TGFβ3 increases αSMA incorporation in passaged chondrocytes (Figure 1). Finally, human passaged chondrocytes, unlike bovine chondrocytes, contract neo-tissues formed in scaffold-free cultures. To prevent contraction of neo-tissues, we exposed cells to latrunculin A for the first 48 hours of culture, which reduces contractile potential of passaged human chondrocytes by reducing stress fiber formation and αSMA expression. After 8 days, we treated cells in 3D culture with ROCK inhibitor (Y27632) which also prevented contraction, but which allows for matrix deposition. To further stimulate matrix deposition, we exposed cells to TGFβ3. While TGFβ3 increases the contractile potential of cells, there was no evidence of tissue contraction when co-treated with Lat/Y27632. TGFβ3 treatment significantly increased chondrogenic mRNA levels for COL2 and ACAN. At day 15 and 20, tissues had greater staining for COL2 and ACAN as compared to untreated counterparts. 3D redifferentiation of passaged human chondrocytes using Latrunculin treatment and TGFβ treatment led to tissues rich in COL2 and ACAN (Figure 2). Redifferentiated passaged cells within neo-tissues responded to inflammatory mediators via alterations in matrix homeostasis as treatment with IL-1β reduced COL2 and ACAN mRNA levels and enhanced MMP-2 and -13 mRNA levels (Figure 3).

DISCUSSION: 3D bioengineered neo-tissue constructs can be formed using passaged human chondrocytes. The formation of stable neo-tissues requires repression of passaged chondrocyte contractility via targeting the actin cytoskeleton and stimulation of matrix deposition using TGFβ3. Exposure of neo-tissues to IL-1β, mimics OA pathogenesis by alteration of chondrocyte homeostasis.

SIGNIFICANCE/CLINICAL RELEVANCE: The generation of cartilage-like bioengineered neo-tissues by passaged chondrocytes serves as an amenable model to study on the effect of inflammatory mediators on human chondrocyte homeostasis, therefore mimicking key aspects of OA progression. This model could be instrumental in developing new insights into disease progression of, and new therapeutic treatments against, human OA.

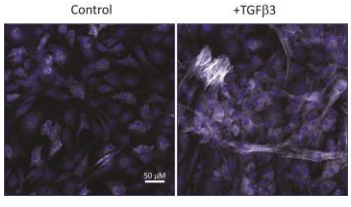

Figure 1. Confocal microscopic images showing nuclei (DAPI; blue) and αSMA distribution in P2 human chondrocytes with 10ng/ml TGFβ3 treatment.

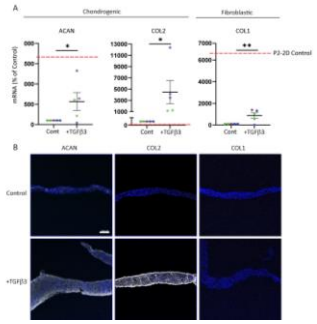

Figure 2. Matrix deposition of P2 chondrocytes within adAM cultures with 10ng/ml TGFβ3 treatment. (A) Gene expression analysis revealed elevated ACAN and COL2. COL1 expression decreased from P2 chondrocytes but increased with TGFβ3 treatment. (B) Confocal microscopic images showing nuclei (DAPI; blue), ACAN, COL2, and COL1 distribution in tissue.

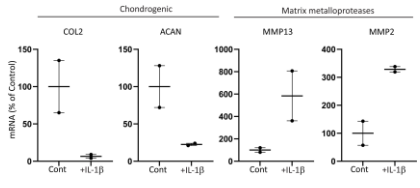

Figure 3. Matrix deposition of P2 chondrocytes within adAM cultures with 5ng/ml IL-1β treatment. Gene expression analysis revealed elevated MMP with decreased chondrogenic gene expression.