Development of a 3D Chondrocyte, Synoviocyte and Macrophage Tri-culture Model for Osteoarthritis Research

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Disclosures: Sezgi Arpag, Fanwei Meng and Xiao Fei Qin are current LifeNet Health employees.

INTRODUCTION: Knee osteoarthritis (OA) is the most common type of arthritis diagnosed and its prevalence will continue to increase as life expectancy and obesity rises. [1] It is now widely accepted that knee OA should be evaluated as a complex organ disease that involves all the major tissue/cell types in the joint. A large body of evidence has demonstrated that cross talking between these tissue/cell types play an important role in OA progression. Although animal models are available to study OA, it is generally expensive and time consuming. A well-tailored *in-vitro* human cell-based model can help reduce and replace animal use, reduce the cost, and provide faster results. The objective of our study is to develop a 3D human chondrocyte/synoviocyte/macrophage co-culture model, which would more closely mimic the *in-vivo* environment of the knee joint for drug discovery/efficacy testing applications.

METHODS: *Establishment of Pellet Culture:* Human primary chondrocytes were expanded in the flasks using Chondrocyte Growth BulletKit for 3 passages (11 days). Chondrocytes were detached using 0.05% Trypsin with EDTA, washed and the suspended cells were spun down at $500g$ for 5 minutes in the low attachment Eppendorf tubes to form the pellets consisting of 180,000 chondrocytes per pellet. Pellets were further cultured for 2 weeks with media change every other day and cryopreserved until use. Chondrocyte pellet viability was assessed by Alamar Blue Assay. To mimic GAG degradation in OA cartilage, pellets were stimulated with 50ng/mL IL-1b and 50ng/mL ADAMTS4 for 24 hours. Chondrocyte pellets were washed and lysed using TE buffer and 150ug/mL Proteinase K for DNA quantification and GAG (Glycosaminoglycan) quantification using 1,9 dimethylmethylene blue (DMB). The GAG quantification data was reported as ug GAG/ug DNA. ***Co-culture of synoviocyte/macrophage:*** Cryopreserved THP-1 monocytes were revived and expanded in the culture for 10 days; Synoviocytes were revived and expanded in the culture for 4 days prior to the co-culture experiments. On Day 0, synoviocytes and THP-1 monocytes were suspended, counted, and seeded together at 1:2 ratio. VitroCol human Collagen-1 hydrogel was prepared according to the manufacturer's recommendations at 1.6mg/mL concentration. Synoviocytes and THP-1 monocytes were mixed with collagen hydrogel, seeded in the 24 well plate and left in the incubator for 30-60 minutes for gelation, followed by monocyte maturation in culture media (RPMI 1640 + 10% FBS + 1% P/S) containing 200nM PMA for 24 hours. On Day 1, Hydrogels consist of synoviocytes and matured THP-1 macrophages were washed 3 times with 2-hour intervals and cells were left in culture media overnight to rest. ***Tri-culture of chondrocyte/synoviocyte/macrophage:*** On Day 2, previously stimulated chondrocytes as described above were added to synoviocyte/macrophage co-culture system inside the transwells. Chondrocyte/synoviocyte/macrophage tri-culture was stimulated with inflammatory cytokine IL-17A at a 100ng/mL concentration except for the baseline "Media Only Control" group. On Day 3, all media was removed and replaced with fresh media containing either 0.5uM Dexamethasone + 100ng/mL stimulant IL-17A (Treatment group), 100ng/mL IL-17A (Stimulation group) or media only (Control group). On Day 4, cell culture media was collected for biological factor quantification using Luminex assays utilizing superparamagnetic beads coated with analyte-specific antibodies. Cells were lysed with TE buffer containing 150ug/mL Proteinase K and lyse supernatant was collected for DNA quantification. All biological factor quantification data was reported as picogram biological factor/ug DNA.

RESULTS SECTION: Chondrocyte pellet viability**:** Alamar blue assay showed that revitalized chondrocyte pellets remained metabolically active (Figure 1). GAG quantification from pellets**:** Stimulation of IL-1b and ADAMTS4 caused a roughly 70% GAG loss from the pellets when compared to the "Media Only" group (Figure 2). Biological factor quantification from co-culture**:** Increased concentrations of pro-inflammatory cytokines, IL-1b and IL-6 were found in the Stimulus group (IL-17A Stim) when compared to the baseline control group (Media only). IL-1b showed an almost 3-fold increase (13.2 vs. 5.4 pg/ug DNA) whereas IL-6 showed a roughly 1.5-fold increase (1577 vs. 1053 pg/ug DNA). The IL-6 concentration secreted by the co-culture was two orders of magnitude higher than the IL-1b. Dex treatment (IL-17A Stim + DEX Tx) was able to reduce the IL-1b and IL-6 concentrations in the co-culture model back to the baseline level (Figure3).

DISCUSSION/CONCLUSION: GAG release from cartilage to synovial fluid is an indication of extracellular matrix (ECM) degradation. IL-1b and ADAMTS4 treatments successfully led to GAG degradation in the chondrocyte pellet, which is one of hallmark features in OA. IL-1b and IL-6 are among the major inflammatory cytokines found in knee OA. Increase of IL-1b and IL-6 concentrations in the IL-17A stimulation group suggests that the IL-17A stimulation causes an inflammatory environment in the co-culture system. Moreover, the cytokine secretion profile of the co-culture system is similar to a previously published ex-vivo culture system using joint tissue explants[2], where the IL-6 concentration was also found to be three orders of magnitude higher than the IL-1b. Treatment of Dexamethasone, a conventional steroid that has been studied to treat OA[3], led to the reduction of both cytokines in the co-culture system. These results suggest that our cell-based model possesses features similar to the ex-vivo explant-based culture system and could be used to study the efficacy of different drugs targeting OA.

SIGNIFICANCE/CLINICAL RELEVANCE: Our 3D culture model could be used for discovery/efficacy testing applications for drugs targeting OA.

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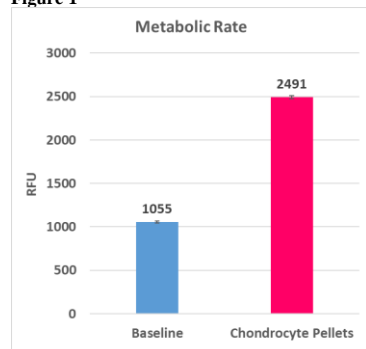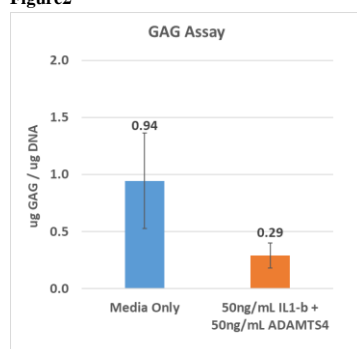
Figure 1

Figure2

Figure 3