In-Vitro & Ex-Vivo Effects of Amniotic Fluid Derived Stem Cell Conditioned Media in End-Stage OA

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INTRODUCTION: Osteoarthritis (OA) of the knee is a chronic disease with limited treatment options and remains the most prevalent joint disorder in the United States. Many of the treatment options on market remain broad in their effect without a clear mechanism of action.

Amniotic membrane and fluid derivatives as a therapeutic for OA have been well documented, with primary clinical studies demonstrating that intra-articular injections may reduce OA progression. Amniotic Fluid Stem Cell Conditioned Media (AFSC-CM) is an acellular preparation that isolates the placental-derived cytokines and growth factors released into growth medium. It is believed that the growth factors and cytokines contained within AFSC-CM exert a paracrine effect to mitigate or slow OA progression, with minimal risk of immunogenicity thus making it a strong candidate for clinical use. This study will examine the in-vitro effect of AFSC-CM on human and porcine chondrocyte cell viability and the ex-vivo effect on the extracellular matrix (ECM) of both human and porcine chondrocyte explants. We hypothesize AFSC-CM will improve chondrocyte proliferation and viability in comparison to control, IL-1 and IL-1 co-cultured with treatment in both human OA phenotype cells and induced OA in porcine cells. We further hypothesize that AFSC-CM will inhibit the catabolic progression that leads to ECM degradation.

METHODS: All human research was approved by the Institutional Review Board at Wake Forest University School of Medicine. Animals used for the study were approved by IACUC at Wake Forest University School of Medicine. Human knee articular cartilage samples (n=4) were de-identified and collected following total knee arthroplasty in patients with end-stage OA. Porcine cartilage (n=4) were collected through sterile dissection after collection of hind limbs from same day pig necropsies. Human and porcine chondrocytes were then cultured in 10% Fetal Bovine Serum (FBS) and treated at baseline once 20% confluent with a low dose (LD=3mg/ml) and a high dose (HD=10mg/ml) of AFSC-CM. Cells were then cultured in 5% FBS and cell counting kit 8 assay (CCK-8) analysis was performed every 48 hours for 6 days. Separate plates were used for day 0, 2, 4 and 6. Articular cartilage explants (n=4 human, n=4 porcine) were collected and treated with 1% mini ITS for 6 days, with timed collection of media for assessment of glycosaminoglycan (GAG) release every 48 hours. Two-way ANOVA with post-hoc Bonferroni correction (IBM SPSS Vs. 29) was performed on optical density (OD) values and GAG content at all time points. Probability level less than 0.05 was considered significant.

RESULTS SECTION: Human chondrocytes showed increased proliferation in the HD treated group (10mg/ml) in comparison to control at every measured time point through day 6 (Day 2 p = <0.001, Day 4 p = <0.001, Day 6 p = 0.021). Increased proliferation was appreciated in porcine cells with both LD and HD AFSC-CM treatment in comparison to IL-1α alone by day 6 (LD p = 0.002, HD p = <0.001). GAG release was decreased in porcine cartilage when treated with AFSC-CM (3mg/ml, 10mg/ml) in comparison to IL-1α after 48 hours (LD p = 0.001, HD p = <0.001). GAG release was also decreased in human articular cartilage treated with AFSC-CM (3mg/ml, 10mg/ml) compared to IL-1β after 48 hours (LD p = 0.007, HD p = <0.001).

DISCUSSION: Treatment of human end-stage OA chondrocytes with AFSC-CM showed increased cell proliferation and viability over time, findings which were replicated in a similar dose-response manner in healthy control porcine samples, where AFSC-CM treatment groups outperformed the IL-1α induced OA group. Similar results were found in both human and pig samples in the IL-1 co-cultured groups, with a dose-dependent response to AFSC-CM treatment increasing cell proliferation when compared to IL-1 alone. These anabolic effects of AFSC-CM on both diseased and healthy cartilage suggests that as a therapeutic, AFSC-CM can be effective both early and late in OA disease progression. In addition to the anabolic effects noted on CCK8, mitigation of GAG release with AFSC-CM treatment in chondrocyte explants suggests its role in preventing catabolic degradation of the ECM in OA. The increased GAG release with IL-1 suggests its greater effect on the catabolic mechanisms affecting the ECM rather than on cell proliferation, thus making the effect of coculturing IL-1 with AFSC-CM on cell proliferation versus catabolism, hard to infer. Regardless, the consistency of the results across species and in particular the dose response of the treatment makes AFSC-CM a promising potential therapeutic.

SIGNIFICANCE/CLINICAL RELEVANCE: OA remains the most common degenerative joint disease in adults, with limited treatment options available to decelerate disease progression. AFSC-CM shows therapeutic promise to restore cartilage anabolism and prevent ECM degradation in human knee samples with end-stage OA.

IMAGES AND TABLES:

Figure 1: Human Chondrocyte proliferation post treatment (n=4). Seeded at density of 10,000 cells/well. Significance between treatment, IL-1β, and control groups displayed by asterisk.

Figure 2: Porcine Chondrocyte proliferation post treatment (n=4). Seeded at density of 5,000 cells/well. Significance between treatment, IL-1, and control groups displayed by asterisk.