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Comparison of Clinically Relevant Adipose Preparations on Articular Chondrocyte Phenotype in a Novel In Vitro Co-Culture Model

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Adipose therapeutics, including isolated cell fractions and tissue emulsifications, have been explored for osteoarthritis (OA) treatment; however, the optimal preparation method and bioactive tissue component for healing has yet to be determined. This in vitro study compared the effects of adipose preparations on cultured knee chondrocytes. De-identified human articular chondrocytes were co-cultured with adipose preparations for 36 or 72 h. Human adipose tissues were obtained from abdominal panniculectomy procedures and processed using three different techniques: enzymatic digestion to release stromal vascular fraction (SVF), emulsification with luer-to-luer transfer (nanofat), and processing in a bead-mill (Lipogems, Lipogems International SpA, Milan, Italy). Gene expression in both chondrocytes and adipose preparations was measured to assess cellular inflammation, catabolism, and anabolism. Results demonstrated that chondrocytes cultured with SVF consistently showed increased inflammatory and catabolic gene expression compared with control chondrocytes at both 36- and 72-h timepoints. Alternatively, chondrocytes co-cultured with either nanofat or bead-mill processed adipose derivatives yielded minimal pro-inflammatory effects and instead increased anabolism and regeneration of cartilage extracellular matrix. Interestingly, nanofat preparations induced transient matrix anabolism while Lipogems adipose consistently demonstrated increased matrix synthesis at both study timepoints after co-culture. This evaluation of the regenerative potential of adipose-derived preparations as a clinical tool for knee OA treatment suggests that mechanically processed preparations may be more efficacious than an isolated SVF cell preparation.

Keywords: adipose therapeutics, adipose stem cells, osteoarthritis, articular chondrocytes, cartilage, inflammation, matrix degradation

Introduction

Osteoarthritis (OA) is the most common form of arthritis and affects nearly half of the world's population aged 65 or older [1]. Knee OA is often comorbid with other conditions such as obesity, diabetes, hypertension, and cardiovascular disease that impair quality of life [2]. Furthermore, OA incidence increases with age; therefore, it is projected that the number of patients with OA will increase with the aging population and cause considerable health care expenses. In addition, the absence of vascularity of articular cartilage, the main tissue affected by OA, results in lowered regenerative capacity, creating greater challenges for combatting this disease [3].

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OA is characterized by a progressive degeneration and destruction of articular cartilage and limited regenerative capacity with conventional treatment [4]. Articular cartilage consists of chondrocytes and their surrounding extracellular matrix (ECM), comprising primarily water, collagen type II, and proteoglycans such as aggrecan, with other non-collagenous proteins and glycoproteins present in lesser amounts. In OA pathology, excessive or abnormal joint loading stimulates joint tissue cells to produce pro-inflammatory factors, such as inducible nitric oxide synthase (iNOS) [5] and cyclooxygenase-2 (COX-2) [6], and proteases that mediate joint tissue destruction.

Key catabolic changes that occur during ECM degradation include increased chondrocyte expression of degradation enzymes, such as matrix metalloproteinases (MMPs), and decreased inhibitors of matrix degradation, such as tissue inhibitors of metalloproteinases [7–9]. Thus, in OA, a pro-inflammatory microenvironment with an unfavorable catabolic-anabolic balance contributes to a degenerative cascade, chondrocyte apoptosis, and ultimately tissue failure.

The standard of care treatment of OA, which includes oral anti-inflammatory or analgesic medication, physical therapy, and corticosteroid and/or visco-supplementation injections, have all proven ineffective for long-term rescue of OA and may potentially hasten the course of degeneration [10–13]. Therefore, these treatments are losing favor within the scientific and medical communities due to their inability to provide a lasting solution that addresses the degeneration of structural proteins in cartilage [10,13]. The inefficacy and impermanence of current OA treatments have necessitated a search for new therapies, with recent approaches focusing on the use of adipose-derived therapeutics for cartilage regeneration.

Adipose tissue is a highly vascularized connective tissue that is rich in regenerative stem cells. The tissue is easily harvested with minimally invasive lipectomy procedures and can be manipulated bedside and reinjected as a single surgical procedure. When utilizing adipose for regenerative experiments, digesting or fragmenting the adipose tissue may be important for concentrating the bioactive regenerative adipose-derived stem cells (ASCs) for optimal outcomes. There exist several ways of processing and isolating ASCs from adipose tissue while minimizing manipulation of its composition for purposes of autologous reinjection; including enzymatic digestion yielding a heterogeneous stromal cell population, called the stromal vascular fraction (SVF).

The SVF includes blood vessel-derived cells such as smooth muscle cells, endothelial cells, pericytes, and most importantly, adipose stem cells. Alternatively, mechanical micro-fragmentation with small pore-sized meshes can be used to reduce the fraction of adipocytes within whole tissue, thus concentrating stromal portions, which reduces potential contamination of residual collagenase and other enzymatic remnants from the final preparation [14,15]. Finally, microparticles of intact tissue can be synthesized with commercial bead-mill kits such as Lipogems® to fragment tissue into nanofat and minimize residual oils released with mesh-fragmentation methods. It is widely hypothesized that the resulting adipose therapeutics release growth factors, modulate inflammatory cytokines, and decrease apoptosis and catabolism of diseased cartilage, thus providing a therapeutic mechanism for combatting knee OA [16,17].

The purpose of the study is to examine the impact of different adipose preparations on chondrocyte phenotypes by indirect in vitro co-culture to determine the interaction of adipose tissues and knee chondrocytes from OA several human donors. We hypothesized that co-culture of cartilage cells with any of these adipose preparations would exhibit a more favorable regenerative gene expression profile than chondrocytes cultured independently, and that the most effective preparation method would induce the highest anabolic chondrocyte gene profile. Therefore, the goal of this study was to determine the candidate adipose tissue preparation(s) that most favorably restores the anabolic to catabolic balance of ECM—suggestive of regenerative capacity in cartilage—as well as to provide rationale for the selection and usage of particular adipose preparations for aiding in knee OA pathologies.

Methods

Level of Evidence: Level 1, Therapeutic Study

Statement of institutional regulatory approval for collection of human tissues

Nonidentifiable human adipose preparations were obtained from full-thickness skin samples discarded from standard of care panniculectomy procedures under University of Pittsburgh IRB exemption #0511186XM. Human chondrocyte lines were donated by Dr. Peter Alexander as frozen cryovials for which patient consent and protocol approval were obtained from the University of Pittsburgh as previously published [18].

Chondrocyte cell culture

Mildly degenerative articular chondrocytes were obtained and purified separately from two de-identified donors who underwent total knee arthroplasty (a 37-year-old male and a 47-year-old female, Mankin score 3–6). These cells were plated from frozen cryovials and expanded in two-dimensional cell culture for 14 days. Cells were grown in ambient oxygen (20%) and carbon dioxide (5%) conditions and using DMEM/F12 media (Life Technologies, Carlsbad, CA), with media changed every 2 days. Once near full confluency (90%), cells were then put into three-dimensional culture at a density of 4×10^6 cells/mL for 48 h through use of biologically inert alginate polymer beads to preserve native chondrocyte phenotype [19]. These chondrocyte-alginate beads were then placed in co-culture with various adipose tissue stem cell preparations for 36 or 72 h as described hereunder.

Adipose preparations: SVF, nanofat, and bead-mill processing

Adipose tissue preparations were obtained through abdominal panniculectomy surgery from human donors ($n = 6$) (age: 44.33 ± 14.77, gender: all female, BMI: 34.09 ± 7.57). Notably, the patients providing adipose tissue through panniculectomy were allogeneic to the cryopreserved chondrocytes.

Stromal vascular fraction. SVF was obtained through enzymatic digestion of whole adipose tissue using a 1:3 ratio of tissue to collagenase enzyme solution comprising

Worthington Type II collagenase (1 mg/mL), Probumin bovine serum albumin (1.05 g/mL), and Hank's balanced salt solution (HBSS; Lonza, Basel, Switzerland). After 45 min of tissue digestion at 37°C, the resulting tissue slurry was filtered through sterile gauze and centrifuged (300 *g*, 5 min, room temperature) to obtain SVF cells.

Nanofat. Nanofat was prepared from lipoaspirate fat obtained using a 3 mm Mercedes tip harvesting cannula connected to a 20 mL leur-lok syringe followed by application of negative pressure. The lipoaspirate was then passed through metal screens of decreasing pore sizes (1, 0.5, and 0.08 mm).

Bead-mill processing. Another form of lipoaspirate preparation was obtained by using a Lipogems kit (Lipogems International SpA, Milan, Italy) to process adipose tissue. Per manufacturer's instructions, tissue fragments were placed in the provided filter shaker with mechanical beads, followed by saline wash in dialysis tubing. After harvest from pannus tissue, all adipose tissue preparations were immediately placed in co-culture with chondrocytes.

Chondrocyte-adipose co-culture system

Beads containing chondrocytes and adipose preparations were then combined in indirect co-culture using transwell baskets or independently as a control. Co-culture duration was either 36 or 72 h. Buoyant adipose tissue preparations were held in the transwell inserts and submerged in culture media through use of a two-part press-fit device custom made in SolidWorks CAD/CAM (Dassault Systems, Waltham, MA). Press-fit devices were 3D printed using biologically inert polymethacrylate plastic (E-shell Envision Tech, Dearborn, MI) and held a metal screen (0.006-inch pore size; McMaster-Carr, Elmhurst, IL). When assembled, the device was added to the novel co-culture system to submerge adipose tissue preparations and to counteract buoyant forces (Fig. 1).

RT-PCR analysis

After co-culture, total mRNA was extracted from the chondrocytes and adipose tissues following methods established in previous studies [20,21]. RT-PCR was performed on isolated mRNA from the chondrocytes and from adipose tissues using the comparative ΔΔCt method using glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) as the housekeeping gene and genes expressed as relative to the controls [22]. Gene expression profiles of chondrocytes and adipose tissues were determined for inflammatory genes inducible nitric oxide (*iNOS*) and cyclooxygenase-2 (*COX-2*), catabolic gene matrix metalloproteinase-13 (*MMP13*), anabolic ECM genes collagen-1 (*COL1*), collagen-2 (*COL2*), aggrecan (*AGC*), and chondrocyte differentiation gene *SOX-9* (Table 1).

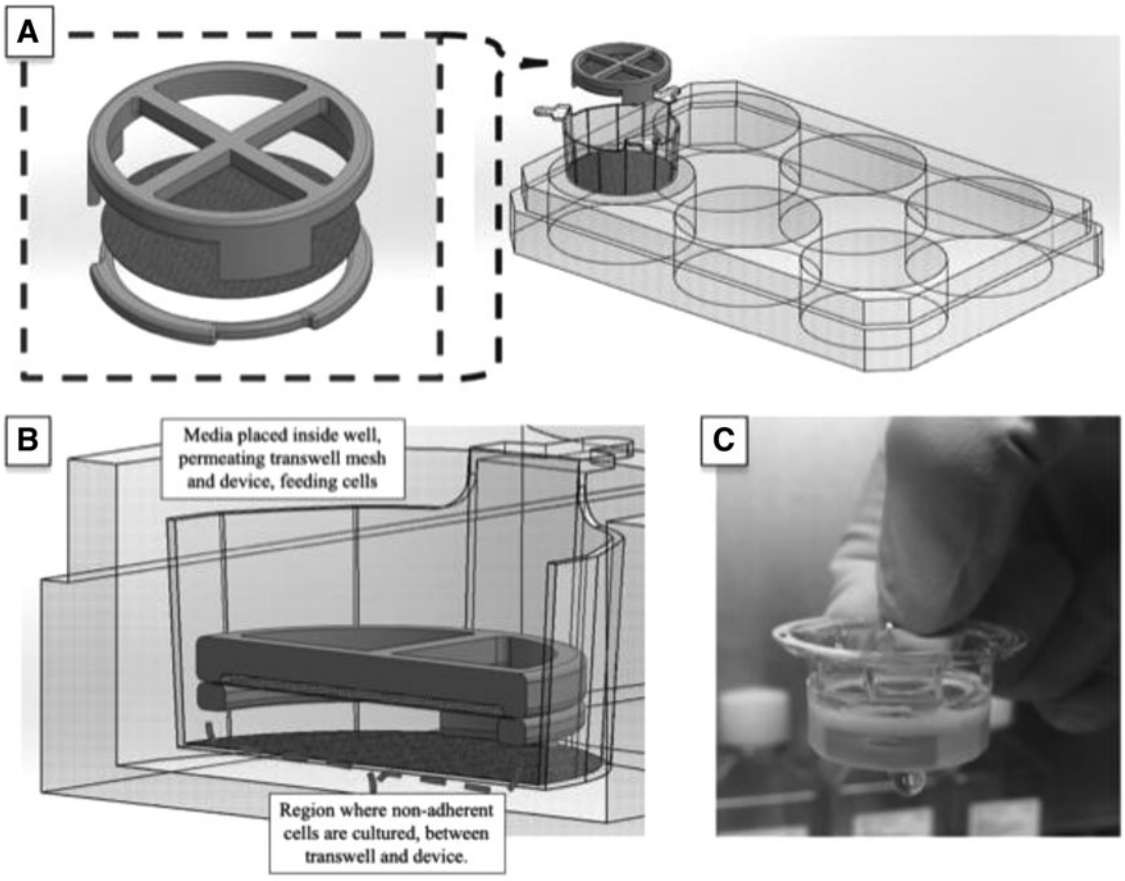

FIG. 1. **(A)** Custom 3-D printed, polymethacrylate transwell inserts were fabricated as two-part press-fit devices entrapping a metal screen. **(B)** Cross-sectional cutaway representation of co-culture system utilized. Chondrocytes beads were cultured in the bottom compartment in the culture well plate and below the transwell insert. **(C)** Adipose cell and tissue derivatives were held inside and above the transwell insert but simultaneously kept submerged through use of a three dimensional-printed metal screen device system.

Table 1. Primer Sequences for Genes of Interest

Human primers	*Forward sequence*	*Reverse sequence*
GAPDH	ACCCACTCCTCCACCTTTGAC	TCCACCACCCTGTTGCTGTAG
iNOS	AGCGGGATGACTTTCCAAGA	CATCTGGAGGGGTAGGCTTG
COX-2	TCCACCAACTTACAATGCTGACTATG	AATCATCAGGCACAGGAGGAAGG
MMP13	TGCTTCCTGATGACGATGTAC	TCCTCGGAGACTGGTAATGG
COL1	GGAAACAGACAAGCAACCCAAACT	GGTCATGTTCGGTTGGTCAAAGATAA
COL2A1	GAACCTGCTATTGCCCTCTG	GAACCTGCTATTGCCCTCTG
Aggrecan (AGC)	AAGAATCAAGTGGAGCCGTGTGTC	TGAGACCTTGTCCTGATAGGCACT
SOX-9	CTGAGCAGCGACGTCATCTC	GTTGGGCGGCAGGTACTG

RT-PCR was performed on RNA extract for chondrocyte groups for all gene primers except for SOX-9. Similarly, RT-PCR was done on RNA extract for all adipose tissue groups for all gene primers including SOX-9. Genes of interest were grouped into categories based on potential effects on cell and tissue inflammation (iNOS and COX-2), catabolism (MMP13), extracellular matrix anabolism (COL1, COL2, and AGC), and chondrocyte differentiation (SOX-9), with GAPDH as the control housekeeping gene.

For statistical analysis, two-tailed Student's *t*-tests were performed pairwise (GraphPad Prism 8) on relative gene expression values between various co-culture and control groups to determine significance. Statistical findings were conducted at a significance level of 0.05 and confidence intervals were also determined.

Results

Inflammatory and catabolic gene expression of chondrocytes co-cultured with adipose preparations

Chondrocytes cultured with SVF consistently showed increased inflammatory and catabolic gene expression compared with control chondrocytes at both 36- and 72-h timepoints. Chondrocytes cultured for 36 h with SVF showed significantly greater expression of both *iNOS* ($P < 0.01$, 95% CI: 6.52–21.69) and *COX-2* ($P < 0.01$, 95% CI: 2.86–10.83) than control group chondrocytes (Fig. 2). The average expression of *MMP13* was also increased in chondrocytes co-cultured with SVF for 36 h relative to control, although this difference was not statistically significant.

At the 72-h co-culture timepoint, both *COX-2* and *MMP13* genes remained significantly upregulated in SVF group chondrocytes compared with the control group chondrocytes (*COX-2*: $P < 0.01$, 95% CI: 2.10–10.05; *MMP13*: $P < 0.01$, 95% CI: 12.9–43.93) (Fig. 3). Chondrocytes co-cultured with SVF also exhibited upregulation of *iNOS* compared with

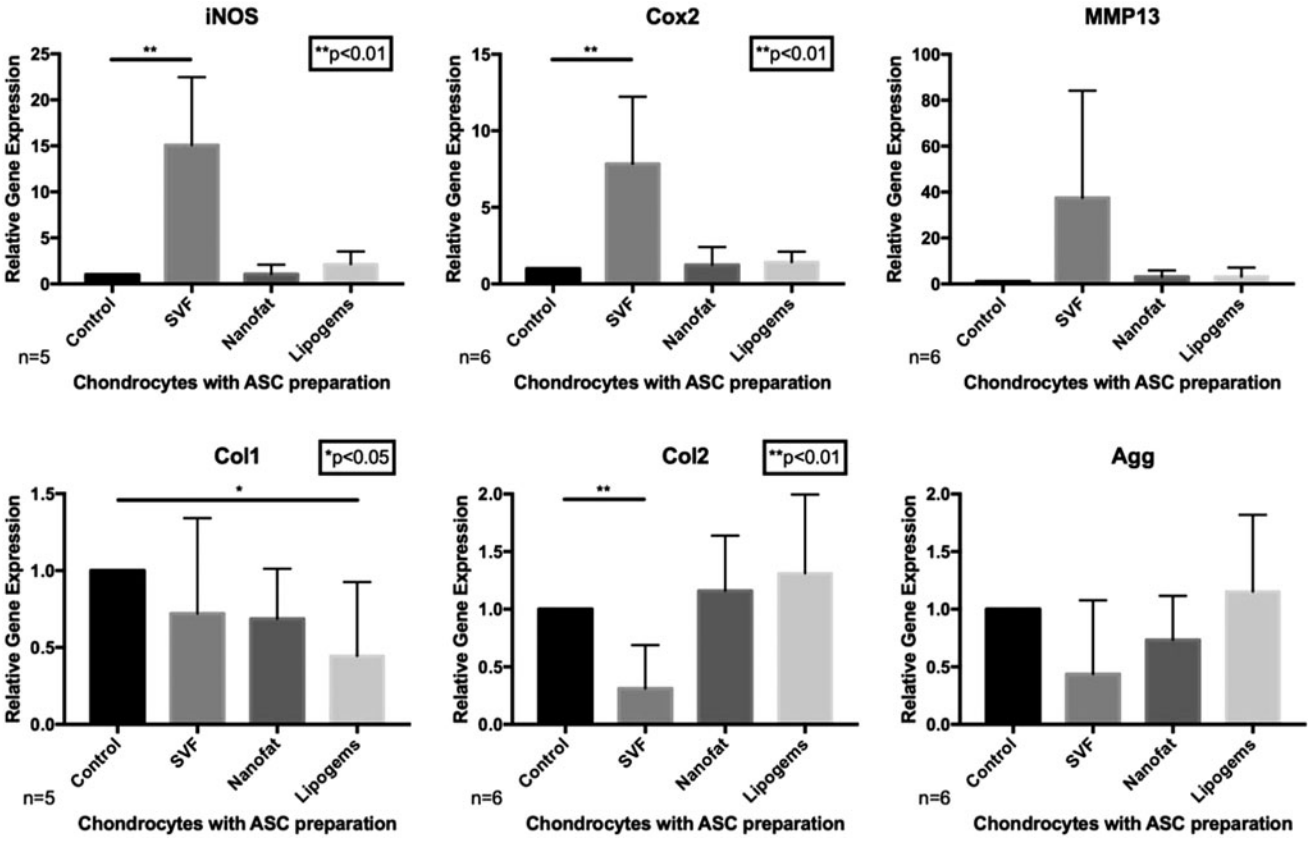

FIG. 2. Comparison of relative chondrocyte mRNA expression, 36-h co-culture.

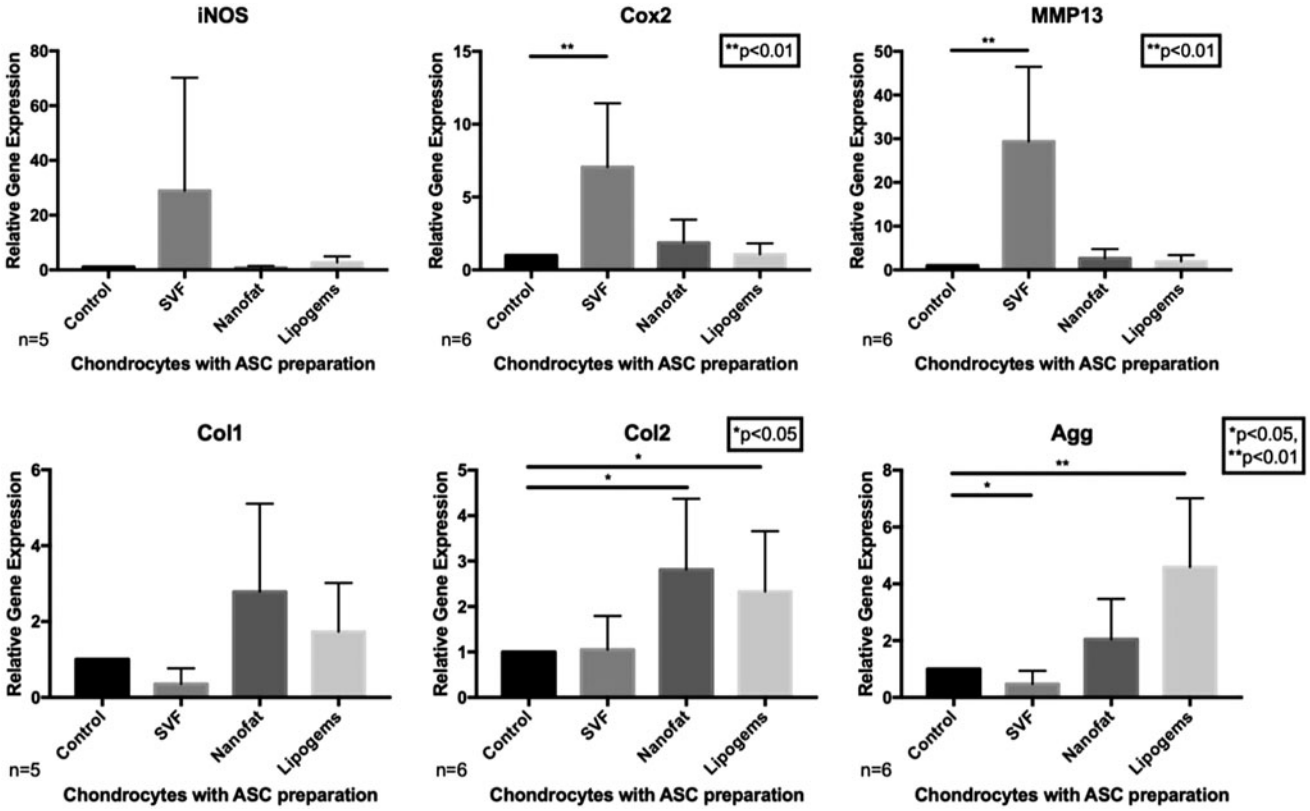

FIG. 3. Comparison of relative chondrocyte mRNA expression, 72-h co-culture.

control chondrocytes at 72 h, although this did not reach statistical significance. Chondrocytes co-cultured with either nanofat or Lipogems did not exhibit significantly different expression of inflammatory or catabolic genes relative to controls at either 36- or 72-h timepoints.

Anabolic gene expression in chondrocytes co-cultured with adipose preparations

Chondrocytes cultured with either nanofat or Lipogems overall exhibited increased anabolic gene expression at 72 h but showed heterogenous levels of anabolic genes at 36 h compared with controls. At 36 h, *COL1* expression was significantly decreased in chondrocytes culture with Lipogems relative to the control chondrocytes ($P < 0.05$, 95% CI: 0.06–1.05) but was increased at 72 h. *COL2* gene expression was also decreased in chondrocytes cultured with SVF relative to the controls at 36 h ($P < 0.01$, 95% CI: 0.35–1.03).

In contrast, co-culture with either nanofat or Lipogems increased chondrocyte *COL2* expression relative to controls at both timepoints, with a statistically significant increase at the 72-h timepoint (nanofat chondrocytes: $P < 0.05$, 95% CI: 0.39–3.23; Lipogems chondrocytes: $P < 0.05$, 95% CI: 0.13–2.54). Similarly, at 72-h aggrecan gene expression was upregulated in the Lipogems group ($P < 0.01$, 95% CI: 1.4–5.8), but was downregulated in SVF co-cultured chondrocytes relative to the control ($P < 0.05$, 95% CI: 0.11–0.94). A summary for chondrocyte anabolic and catabolic changes in gene expression is compiled in Table 2.

Table 2. Chondrocyte Gene Expression After Co-Culture with Adipose Preparations

Chondrocyte Gene	*SVF 36 h*	*SVF 72 h*	*Nanofat 36 h*	*Nanofat 72 h*	*Lipogems 36 h*	*Lipogems 72 h*
Inflammatory/catabolic genes						
iNOS	↑↑	↑	—	↓	↑	↑
Cox2	↑↑	↑↑	—	↑	↑	—
MMP13	↑	↑↑	↑	↑	↑	—
Anabolic genes						
Col1	↓	↓	↓	↑	↓↓	↑
Col2	↓↓	—	—	↑↑	↑	↑↑
Agg	↓	↓↓	↓	↑	—	↑↑

Single arrows indicate statistically insignificant trends, while double arrows indicate statistically significant ($P < 0.05$) changes in expression.
SVF, stromal vascular fraction.

Adipose therapeutic inflammatory gene expression after co-culture with chondrocytes

Adipose prepared with the Lipogems kit and cultured with chondrocytes exhibited increased catabolic gene expression (*MMP13*) compared with identical adipose cultured alone ($P < 0.01$, 95% CI: 0.39–1.37) (Fig. 4). At the 72-h timepoint, SVF cultured with chondrocytes showed significant increase of pro-inflammatory gene expression compared with control SVF for both *iNOS* ($P < 0.01$, 95% CI: 1.22–4.64) and *COX-2* ($P < 0.01$, 95% CI: 1.55–7.11) (Fig. 5).

ECM gene expression in adipose tissue preparations co-cultured with chondrocytes

At early timepoints, SVF co-cultured with chondrocytes exhibited increased *COL2* and aggrecan (*AGC*) gene expression compared with SVF alone (*COL2*: $P < 0.05$, 95% CI: 2.0–14.1; *AGC*: $P < 0.05$, 95% CI: 1.49–22.17) (Fig. 3A). *COL2* gene expression in nanofat adipose tissue was also upregulated in co-culture compared with control at 36 h ($P < 0.05$, 95% CI: 0.46–5.55), whereas *AGC* gene expression in nanofat tissue was downregulated in co-culture compared with control at 72 h ($P < 0.01$, 95% CI: 0.20–0.94). Adipose prepared with Lipogems kits was not significantly affected by chondrocyte co-culture. A summary of adipose gene expression changes is in Table 3.

Chondrocyte differentiation in adipose progenitor cells after co-culture with chondrocytes

To explore the possibility that progenitor or stem cell populations within adipose could differentiate toward chondrocytes and thus replace damaged cells, we also measured chondrocyte differentiation genes in the adipose therapeutics. Results showed that there was a significant decrease in *SOX-9* (a chondrocyte differentiation gene) expression in SVF tissue in co-culture compared with control SVF at 72 h ($P < 0.001$, 95% CI: 0.39–0.76). Meanwhile, expression of *SOX-9* was increased at 72 h in both nanofat ($P < 0.05$, 95% CI: 0.09–2.78) and Lipogems adipose tissues in co-culture compared with their respective control groups.

Donor variability in adipose tissue preparation expression of inflammatory genes

As the adipose tissue was derived from donors of variable health and BMI, we explored the possibility that baseline expression of inflammatory genes in control adipose samples varied. Therefore, the inherent *iNOS* and *COX-2* levels for each adipose preparation type were analyzed at both 36- and 72-h timepoints relatively and across the three different adipose preparations cultured independently. None of the adipose tissue preparations cultured independently exhibited significantly increased inflammatory gene expression relative to each other (data not shown).

Inflammatory gene expression (*iNOS* and *COX-2*) was also analyzed for each adipose tissue in individual culture at the 72-h co-culture timepoint relative to the 36-h co-culture timepoint. This analysis was performed to explore inflammatory gene expression in each adipose tissue group resulting from time spent in the culture system. In the nanofat adipose tissue preparation cultured individually, there was significantly decreased *COX-2* inflammatory gene expression at 72 h relative to 36 h of co-culture ($P < 0.05$, 95% CI:

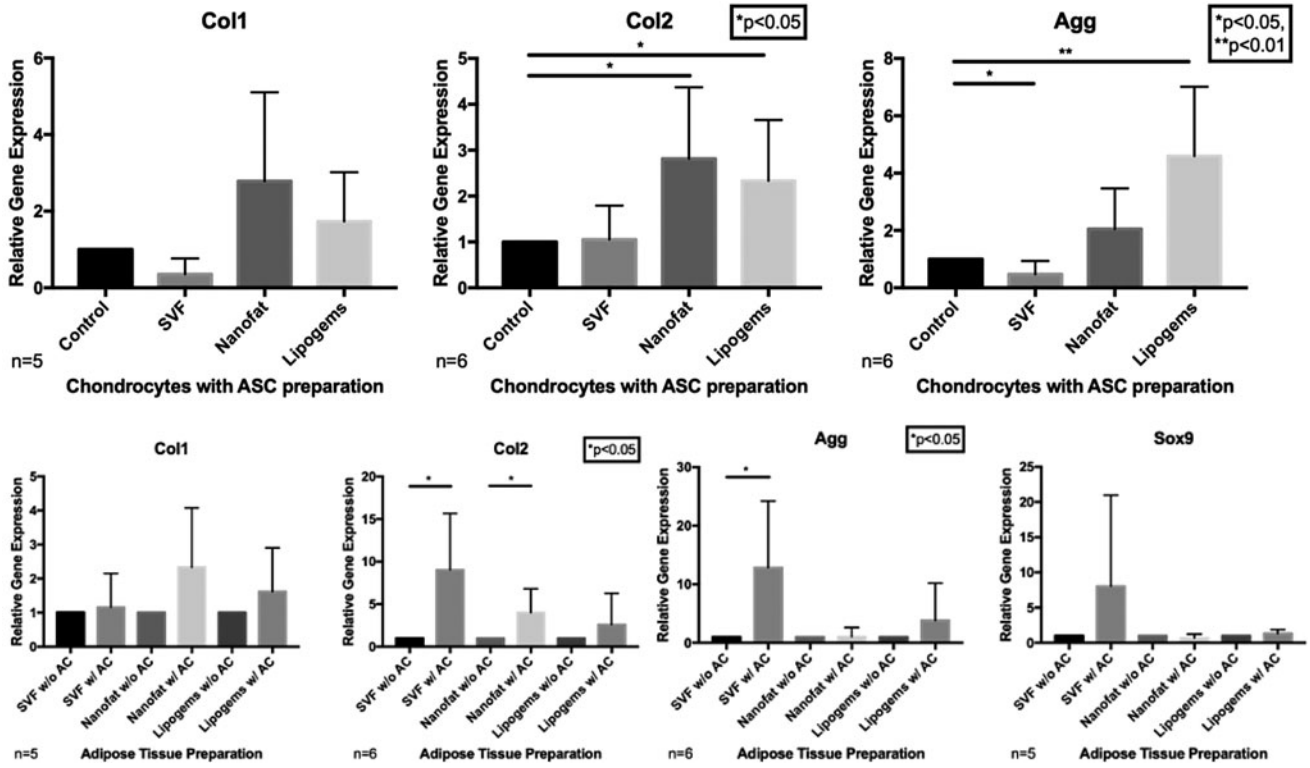

FIG. 4. Comparison of relative adipose tissue mRNA expression, 36-h co-culture.

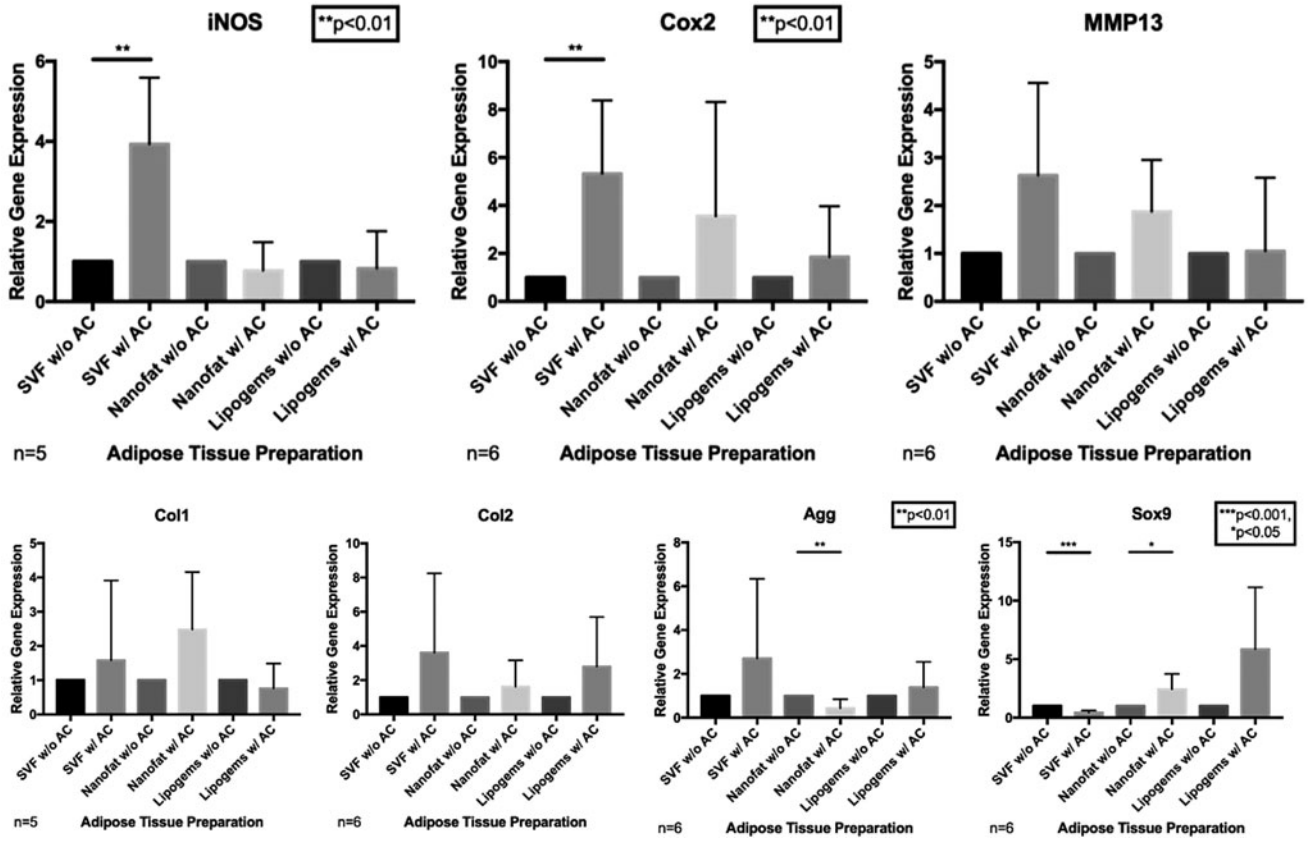

FIG. 5. Comparison of relative adipose tissue mRNA expression, 72-h co-culture.

0.18–0.90). For all other adipose preparations, there were no statistically significant time-dependent changes (72 vs. 36 h) in inflammatory gene expression for the adipose tissues cultured in isolation (Table 3).

Discussion

Overall, chondrocytes co-cultured with SVF displayed a pro-inflammatory profile, whereas chondrocytes alongside either nanofat or bead-mill processed preparations exhibited greater anabolic potential. Across both timepoints, evidence indicates that SVF produces pro-inflammatory and potentially catabolic effects in chondrocytes and may not produce a substantial anabolic or regenerative gene response in vitro.

In addition, increases in pro-inflammatory gene expression (*iNOS* and *COX-2*) in SVF suggest a possible contribution to the inflammatory microenvironment, whereas a decrease in SVF *SOX-9* expression may indicate lower potential for chondrocyte differentiation of progenitors. Thus, co-culture of chondrocytes with SVF appears to increase inflammatory gene response in chondrocytes as well as in the SVF tissue itself. This pro-inflammatory effect might be due to the toxic, oxidative heme groups released from lysed erythrocytes in SVF preparations [23–25]. Therefore, the

TABLE 3. GENE EXPRESSION CHANGES IN ADIPOSE PREPARATIONS AFTER CHONDROCYTE CO-CULTURE

SVF gene expression:	*36 h*	*72 h*	*Nanofat gene expression:*	*36 h*	*72 h*	*Lipogems gene expression:*	*36 h*	*72 h*
Inflammatory/catabolic genes								
iNOS	↑	↑↑	iNOS	↓	↓	iNOS	—	↓
Cox2	↑	↑↑	Cox2	↑	↑	Cox2	↑	↑
MMP13	↑	↑	MMP13	↑	↑	MMP13	↑↑	↑
Anabolic genes								
Col1	—	↑	Col1	↑	↑	Col1	↑	↓
Col2	↑↑	↑	Col2	↑↑	↑	Col2	↑	↑
Agg	↑↑	↑	Agg	—	↓↓	Agg	↑	↑
Chondrocytic differentiation gene								
Sox9	↑	↓↓	Sox9	↓	↑↑	Sox9	↑	↑

Single arrows indicate statistically insignificant trends, while double arrows indicate statistically significant ($P < 0.05$) changes in expression.

use of SVF adipose in co-culture may not produce a substantial in vitro regenerative response in chondrocytes.

Meanwhile, chondrocytes co-cultured with either nanofat or bead-mill processed adipose tissue derivatives yielded minimal pro-inflammatory effects and instead demonstrated potential for increased anabolism and regeneration of cartilage ECM. Nanofat adipose preparations exhibited transient potential for matrix anabolism, whereas Lipogems adipose derivatives consistently demonstrated strong potential of matrix production after co-culture. In addition, both nanofat and bead-mill processed adipose tissue groups exhibited increased *SOX9* expression after co-culture, suggesting potential of chondrocyte differentiation in these adipose tissue preparations [26,27].

None of the adipose tissue preparations cultured individually (without chondrocytes) demonstrated increased pro-inflammatory expression relative to each other, indicating that pro-inflammatory results seen in SVF were likely a result of co-culture interaction with chondrocytes rather than inherent pro-inflammatory properties of the adipose preparation itself. Similarly, there were no statistically significant increases in inflammatory gene expression over time (from the 36- to 72-h timepoints) in the SVF preparations cultured independently. This suggests that the pro-inflammatory profile of the co-cultured SVF adipose tissue group was minimally influenced by duration of in vitro culture. Instead, these isolated adipose tissue findings when coupled with the gene expression profile of co-cultured chondrocytes may indicate a presence of SVF tissue factors acting on chondrocytes to produce pro-inflammatory phenotypes.

This experimental study possessed several inherent limitations. The study pairs adipose tissue and knee chondrocytes of different human donors. Although this co-culture system does not recreate the potential autologous nature of stem cell injections, this limitation is mitigated by the fact that the number of immune cells included in the preparations following the centrifugation and saline wash steps in the adipose preparation protocols and, therefore, the associated immune response is likely minimal in this co-culture system.

This study is also limited by the inclusion of only two different chondrocyte donors. Despite the heterogeneity of the chondrocyte sources, including both genders, each donor's catabolic and anabolic gene expression results analyzed individually maintained very similar trends to the overall findings reported in this study. The only instance of dissimilar results occurred in chondrocytes co-cultured with Lipogem adipose preparation, with *COL1* chondrocyte expression increased in the first donor and decreased in the second donor, when compared with their respective control groups.

This discrepancy may be due to the use of relatively short timepoints, 72 h, whereas substantial anabolic effects of adipose tissues on chondrocytes may require a greater co-culture duration. Still, this short-term study provides insight to the biological effects of the initial phase of stem cell-knee chondrocyte interaction. Finally, caution is always advised when extrapolating in vitro results to complex in vivo disease scenarios. Broadly, the in vitro model described herein does not incorporate the innate immune system, including joint resident macrophages and monocytes, which have been shown to contribute to OA, nor does it incorporate synovial fibroblasts and their multitude of phenotypes. Future study utilizing more sophisticated ex vivo model systems, such as organs-on-chips, should consider adding in these additional cell types for a more thorough analysis of adipose-derived therapeutic effects on OA.

Through use of a novel in vitro co-culture model, these research findings evaluate the interactions of different common adipose preparations and suggest more favorable effects on chondrocytes for micronized adipose and bead-mill processed preparations. In contrast, we observed a greater inflammatory response and a more limited regenerative potential in use of adipose-derived SVF as a clinical tool for knee OA treatment. It is interesting to note the regulatory burden for clinical use of micronized and/or bead-mill processed adipose tissue is significantly lower than for enzymatically isolated SVF in the United States and the European Union.

We recognize that this model does not replicate the complex environment of an intact arthritic joint space, with ongoing mechanical force and presence of multiple tissue types. There may be other factors inherent to that milieu that would impact the clinical efficacy of a biological therapy. Further study may be needed to determine the possible causes of the pro-inflammatory findings of SVF tissue, and to also include other variables relevant to an arthritic joint that may influence the effects of cell and tissue therapies. This study does highlight the different physiological effects of common adipose tissue preparations, as well as demonstrate positive effects in two of the three assessed preparations.

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Author Disclosure Statement

No competing financial interests exist.

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