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The inhibition by interleukin 1 of MSC chondrogenesis and the development of biomechanical properties in biomimetic 3D woven PCL scaffolds

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Abstract

Tissue-engineered constructs designed to treat large cartilage defects or osteoarthritic lesions may be exposed to significant mechanical loading as well as an inflammatory environment upon implantation in an injured or diseased joint. We hypothesized that a three-dimensionally (3D) woven poly(ϵ -caprolactone) (PCL) scaffold seeded with bone marrow-derived mesenchymal stem cells (MSCs) would provide biomimetic mechanical properties in early stages of *in vitro* culture as the MSCs assembled a functional, cartilaginous extracellular matrix (ECM). We also hypothesized that these properties would be maintained even in the presence of the pro-inflammatory cytokine interleukin-1 (IL-1), which is found at high levels in injured or diseased joints. MSC-seeded 3D woven scaffolds cultured in chondrogenic conditions synthesized a functional ECM rich in collagen and proteoglycan content, reaching an aggregate modulus of ~0.75 MPa within 14 days of culture. However, the presence of pathophysiologically relevant levels of IL-1 limited matrix accumulation and inhibited any increase in mechanical properties over baseline values. On the other hand, the mechanical properties of constructs cultured in chondrogenic conditions for 4 weeks prior to IL-1 exposure were protected from deleterious effects of the cytokine. These findings demonstrate that IL-1 significantly inhibits the chondrogenic development and maturation of MSC-seeded constructs; however, the overall mechanical functionality of the engineered tissue can be preserved through the use of a 3D woven scaffold designed to recreate the mechanical properties of native articular cartilage.

Keywords

cytokine; articular cartilage; tissue engineering; textile; stem cell; mesenchymal stem cell; differentiation; fiber; inflammation; fabric

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Introduction

Articular cartilage shows little capacity for repair in response to traumatic injury or degenerative conditions such as osteoarthritis. In this regard, there has been increasing interest in the development of tissue engineering strategies for cartilage repair or regeneration using autologous chondrocytes or stem/progenitor cells such as adult bone marrow-derived mesenchymal stem cells (MSCs) or adipose derived stem cells (ASCs). However, there is little evidence of the long-term clinical success of such procedures as compared to less complex surgical methods such as microfracture [1]. While the reasons for these failures are not fully understood, cell-seeded scaffolds generally do not possess appropriate biomechanical functionality at the time of implantation, and they may require significant maturation time *in vitro* before achieving mechanical properties that can withstand joint loading *in vivo* (e.g., [2, 3]). In many cases, the repair site shows incomplete cell differentiation and a lack of formation of hyaline cartilage *in vivo* [4].

One potential cause of graft failure that is not often considered is that injured and osteoarthritic joints exhibit significantly higher levels of pro-inflammatory cytokines. For example, interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF- α), as well as pro-catabolic enzymes and mediators such as metalloproteinases (MMPs), aggrecanases, prostaglandins, and nitric oxide are overexpressed in these joints and can cause tissue degradation, pain, and inflammation [5-7]. While the effects of IL-1 on chondrocytes have been characterized extensively (e.g., [8]), recent studies have also demonstrated the deleterious effects of IL-1 on the chondrogenesis of ASCs [9] and MSCs [10-15], as well as tissue engineered cartilage comprising chondrocytes seeded in scaffolds of agarose [16, 17] or collagen [18, 19]. Furthermore, IL-1 and TNF- α inhibit the integrative repair of cartilaginous tissues such as the meniscus [20], via upregulation of MMPs [21] and the inhibition of cell proliferation [22]. Thus, the inflammatory environment of the injured joint may have similar effects on a tissue-engineered construct *in vivo*, particularly in severe degenerative conditions such as osteoarthritis.

The development of biomaterial scaffolds that provide appropriate functional biomechanical properties of cartilage, even prior to new tissue formation, may have significant advantages in rapidly restoring and maintaining tissue function in an inflammatory joint environment [23]. In this regard, we have developed a three-dimensional (3D) woven porous scaffold from poly(ϵ -caprolactone) (PCL) that mimics the nonlinear, anisotropic, compressive, and inhomogeneous mechanical properties of articular cartilage [24]. This scaffold can be readily seeded with cells and can be formed into 3D anatomical shapes to resurface large defects [25-27]. Since PCL degrades slowly, we hypothesized that the combination of the biomimetic scaffold and chondrogenically induced MSCs would provide both the initial functional properties of articular cartilage, and also provide long-term load-bearing properties, even in the conditions in which chondrogenesis was potentially inhibited by pro-inflammatory factors such as IL-1.

The goals of this study were to examine the effects of IL-1 on the chondrogenic differentiation of MSCs within a 3D woven PCL scaffold that possesses biomimetic cartilage mechanical properties. We hypothesized that the use of this 3D woven scaffold would allow continuous maintenance of the biomechanical properties of the construct during *in vitro* culture, even in the presence of the pro-catabolic and anti-anabolic effects of IL-1. We further examined the hypothesis that chondrogenic differentiation of MSCs would serve a protective role in cartilaginous tissue development in response to a pro-inflammatory environment.

Materials and Methods

3D Woven Scaffold Production

A custom-built weaving machine was used to weave 156 μm diameter multifilament PCL yarns (EMS-Griltech, Domat, Switzerland) into a porous, 3D textile structure. The multilayer architecture consisted of 11 layers of axially oriented yarns, stacked in alternating x- and y-directions, and bound together by an interwoven set of vertically oriented yarns (z-direction) that repeatedly passed through the thickness of the fabric. Yarn spacing within each layer was controlled such that the resulting structure contained interconnected pores measuring approximately 330 μm x 260 μm x 100 μm with a total void fraction of approximately 60% and an overall thickness of approximately 1.4 mm. After weaving, the PCL fabric was soaked in a 4M NaOH bath for 16 hours to clean the fibers and increase their surface hydrophilicity [28]. A 6 mm diameter biopsy punch was used to cut disk-shaped samples from the fabric. Scaffolds were sterilized with ethylene oxide and given a minimum of 1 week to outgas prior to use.

Human Mesenchymal Stem Cells

Human MSCs (hMSCs) were derived from bone marrow aspirates obtained from three healthy adults (two male and one female, average age 39 years) at the Hematopoietic Stem Cell Core Facility at Case Western Reserve University. Informed consent was obtained, and an Institutional Review Board-approved aspiration procedure was used. Briefly, the bone marrow sample was washed with Dulbecco's modified Eagle's medium (DMEM-LG, Gibco) supplemented with 10% fetal bovine serum (FBS) from a selected lot [29]. The sample was centrifuged at 460g on a pre-formed Percoll density gradient (1.073 g/mL) to isolate the mononucleated cells. These cells were resuspended in serum-supplemented medium and seeded at a density of 1.8×10^5 cells/cm² in 10 cm diameter plates. Non-adherent cells were removed after four days by changing the medium. For the remainder of the cell expansion phase, the medium was additionally supplemented with 1 ng/mL of recombinant human fibroblast growth factor-basic (rhFGF-2, Peprotech, Rocky Hill, NJ), and was replaced twice per week. The primary culture was trypsinized after approximately two weeks, and then cryopreserved using Gibco freezing medium.

Tissue Engineered Constructs

hMSCs were thawed and plated at 5500 cells/cm² and cultured in DMEM-LG supplemented with 10% FBS, 1 ng/mL of rhFGF-2 (Roche Diagnostics, Indianapolis, IN), and 1% penicillin–streptomycin–fungizone (Gibco). Medium was completely replaced every 3 days until cells reached 80% confluence, after which they were passaged and replated. For this study, cells were used after the fourth passage (P4). The PCL scaffolds were placed in 24 well ultra-low attachment plates (Corning, NY) and seeded with 3×10^5 P4 hMSCs at a density of 10×10^6 cells/mL. The scaffolds were then placed in a humidified incubator at 37 °C and 5% CO₂ for 60 min to allow cell attachment to the scaffold. Subsequently, 1 mL of medium was added per well. Three different culture media were used in this study: (i) “basic culture medium” consisting of DMEM-HG supplemented with 1% ITS+ premix (Collaborative Biomedical, Becton-Dickinson, Bedford, MA), 100 nM dexamethasone (Sigma), 100 U/mL penicillin, 100 U/mL streptomycin and 37.5 $\mu\text{g/mL}$ L-ascorbic acid 2-phosphate, (ii) “chondrogenic culture medium” consisting of basic culture medium plus 1 ng/mL of recombinant human transforming growth factor beta (TGF- β_3) (R&D Systems, Minneapolis, MN), and (iii) “pro-inflammatory culture medium” consisting of chondrogenic medium supplemented with 0.1 ng/mL of recombinant human interleukin-1 α (IL-1) (R&D Systems). This concentration was based on concentrations of IL-1 measured in the synovial fluid of osteoarthritis patients [30]. These three media formulations were used to make four different test groups: 1) control, 2) chondrogenic, 3) pro-inflammatory, and 4) chondrogenic.

pro-inflammatory (chondrogenic culture for days 0 to 28, followed by pro-inflammatory culture for days 28 to 56) (Fig. 1). Medium was completely replaced for all groups every 2-3 days and constructs were harvested at 14, 28, 42, and 56-day time points. Cell-loaded scaffolds were also harvested immediately upon seeding (day 0) to assess baseline properties. Each group included 7 samples per time point; 5 were used for mechanical testing first, followed by biochemical analysis, and 2 were used for histology and immunohistochemistry.

Mechanical Testing

Confined compression tests were performed on 3 mm diameter cylindrical test specimens, cored from the centers of the constructs with a biopsy punch, using an ELF 3200 series materials testing system (Bose, Minnetonka, MN). Specimens ($n = 5$ per group) were placed in a 3 mm diameter confining chamber in a bath filled with phosphate buffered saline (PBS) and a compressive load was applied using a solid piston against a rigid porous platen (porosity of 50%, pore size of 50–100 μm). Following equilibration of a 10 gf tare load, a step compressive load of 30 gf was applied to the sample and allowed to equilibrate for 2000s. Aggregate modulus (H_A) and apparent hydraulic permeability (k) were determined numerically by matching the solution for axial strain to the experimental data for all creep tests using a three-parameter, nonlinear least-squares regression procedure [31] assuming intrinsic incompressibility of the tissue [32]. Unconfined compression tests were performed by applying sequential strains of 4, 8, 12, and 16% to the specimens ($n = 5$ per group) in a PBS bath after equilibration of a 2 gf tare load. Strain steps were held constant for 900s, which allowed for the specimens to reach equilibrium. Young's modulus (E) was determined by linear regression on the resulting equilibrium stress-strain plot.

Histology and Immunohistochemistry

Disk-shaped specimens were fixed in 4% paraformaldehyde with 100 mM sodium cacodylate buffer (pH 7.4) for 24 h at 4 °C. From this point on, the constructs were further treated in a fully enclosed tissue processor (Leica ASP300S, Leica Microsystems, Bannockburn, IL). Briefly, constructs were dehydrated in graded ethanol steps, cleared in xylene, and embedded in paraffin wax under vacuum. Embedded paraffin blocks were cut into 8 μm thick sections using a Reichert-Jung microtome and mounted on SuperFrost microscope slides (Microm International AG, Volketswil, Switzerland). Samples were stained for sulfated glycosaminoglycans (s-GAGs) and collagenous matrix using a 0.1% aqueous safranin-O solution and 0.02% fast green solution, respectively, while also using hematoxylin as a nucleus counter-stain. Human osteochondral tissue was used as a positive control. For immunohistochemical analysis (IHC), sections were processed with a fully automated staining system (Bond-Max, Leica Microsystems, Bannockburn, IL). Monoclonal antibodies were used to identify type I collagen (ab6308; Abcam, Cambridge, MA), type II collagen (II-II6B3; Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA), and chondroitin 4-sulfate (2B6; kind gift from Dr. Virginia Kraus, Duke University Medical Center). Human osteochondral samples were used as positive controls, and negative controls were prepared to rule out nonspecific labeling by omitting the primary antibody incubation step.

Biochemical Analyses

Standard assays for DNA, ortho-hydroxyproline (OHP, an index of total collagen content), and s-GAG (an index of proteoglycan content) were performed ($n = 3-4$ bisected constructs per time point per group). Values obtained for all day 0 constructs ($n=6-8$) produced from hMSC-seeded PCL scaffolds were pooled, averaged, and used as a basis for comparison for subsequent (14, 28, 42, and 56-day) constructs. After measuring wet weight, constructs were diced and digested in papain for 12 h at 60°C. DNA was measured using the Quant-iT™

PicoGreen® dsDNA assay (Molecular Probes, Eugene, OR). s-GAG was measured using the Dimethyl-methylene blue assay (DMB) using chondroitin 4-sulfate as a standard and reading the optical density on a plate reader at 595 nm. To measure total collagen, papain digests were hydrolyzed in HCl at 110 °C overnight, dried in a desiccating chamber, reconstituted in acetate–citrate buffer, filtered through activated charcoal, and OHP was quantified by Ehrlich's reaction. Briefly, hydrolysates were oxidized with chloramine-T, treated with dimethylaminobenzaldehyde, read at 540 nm against a trans-4-hydroxy-L-proline standard curve, and total collagen was calculated by using a ratio of 10 mg of collagen per 1 mg of 4-hydroxyproline. The conversion factor of 10 was selected since immunohistochemical staining showed that type II collagen represented virtually all of the collagen present in the constructs [33].

Statistical Analysis

Two-factor analysis of variance (ANOVA) with Tukey's HSD post-hoc test was performed to compare the results of mechanical and biochemical tests for each construct between time points ($\alpha = 0.05$).

Results

Mechanical Testing

At time zero, constructs exhibited an aggregate modulus of ~0.4 MPa (Fig. 2A). Constructs cultured in chondrogenic medium demonstrated a 31% average increase in aggregate modulus over control values ($p < 0.05$) at 14 and 28-day time points (Fig. 2A). Chondrogenic culture conditions also resulted in a reduction in apparent hydraulic permeability of 3 orders of magnitude to approximately $0.0008 \text{ mm}^4/\text{N}\cdot\text{s}$ ($p < 0.05$) (Fig. 2B).

The presence of IL-1 during the initial 4-week period of chondrogenesis abrogated the effects of TGF- β_3 , effectively reducing aggregate modulus to that of control constructs with values of approximately 0.5 MPa (Fig. 2A). IL-1 also prevented the TGF- β_3 -induced decrease in permeability as evidenced of k approaching the value of control at $\sim 1 \text{ mm}^4/\text{N}\cdot\text{s}$ (Fig. 2B).

Under chondrogenic culture conditions, the aggregate modulus increased significantly during the culture period (Fig. 2C), with a significant 51% average increase at days 14 and 28 to values of approximately 0.75 MPa. However, this increase in modulus was not observed in constructs that were cultured in the presence of the pro-inflammatory medium containing IL-1. For constructs cultured in chondrogenic medium for 28 days prior to IL-1 exposure for a subsequent 14 days (i.e., the chondrogenic, pro-inflammatory condition), there were no differences observed in either aggregate modulus or permeability when compared to constructs that were continually cultured in chondrogenic conditions for up to 56 days (Fig. 2A&C).

Histology and Immunohistochemistry

After 28 days of culture in basic control medium, histological analysis of tissue-engineered constructs revealed a sparsely distributed extracellular matrix (ECM) that stained weakly for collagen (via Fast Green) (Fig. 3). Chondrogenic culture conditions containing TGF- β_3 resulted in a dense ECM rich in both s-GAGs (as stained by Safranin-O or chondroitin-4-sulfate) and collagens (as stained by Fast Green) that accumulated and completely filled the interstitial voids within the scaffold.

Constructs cultured in pro-inflammatory conditions with IL-1 stained positively for the presence of collagens, but showed little or no staining for s-GAGs (Fig. 3). Similar results were observed using immunohistochemical labeling for type II collagen and chondroitin 4-sulfate at day 28 (Fig. 3).

Constructs that had been cultured in chondrogenic conditions 28 days before the addition of IL-1 to the cultures (i.e., chondrogenic, pro-inflammatory group) (Fig. 4B) stained positively for type II collagen with similar intensity to constructs cultured in chondrogenic conditions without IL-1 for the entire 56 days (Fig. 4A). In contrast, constructs cultured in chondrogenic conditions for the duration of the 56-day (Fig. 4C) period revealed markedly darker labeling for chondroitin 4-sulfate than those constructs exposed to IL-1 for days 28 to 56 (Fig. 4D).

Biochemical Composition

Constructs cultured in chondrogenic conditions showed an increasing trend in collagen content (normalized to DNA) over the first 28 days, but no statistically significant changes were detected between groups up to this time point (Fig. 5A). However, significant increases in total collagen/DNA were observed in both the chondrogenic and chondrogenic, pro-inflammatory conditions from day 42 to 56 (Fig. 5A, $p<0.05$) to values of approximately 0.8 $\mu\text{g}/\text{ng}$ and 0.6 $\mu\text{g}/\text{ng}$ respectively. This equates to a 25.3% increase in collagen content in chondrogenic only cultures relative to cultures that were chondrogenically induced but received the IL-1 insult for the final 28 days of culture (i.e., chondrogenic, pro-inflammatory condition). Total s-GAG/DNA was 4-fold higher for constructs cultured in IL-1 conditions at day 28 in comparison to those cultured in control conditions. At the same time point, the s-GAG/DNA content of constructs cultured in chondrogenic conditions was 7-fold higher when compared to control conditions (Fig. 5C, $p<0.05$) and continued increasing up to day 56 to a final value of $0.0334\pm0.002 \mu\text{g}/\text{ng}$. By day 56, constructs that were maintained in chondrogenic conditions for the entire study contained 58.7% more s-GAG/DNA than did chondrogenically-induced constructs that were exposed to IL-1 conditions from day 28 onward.

Total collagen content (normalized to the dry weight of the construct) accumulated steadily over time in chondrogenic conditions reaching a maximum of $56.03\pm5.45 \mu\text{g}/\text{mg}$ at day 56 (Fig. 5B). However, in the presence of IL-1 in the culture at day 28 (chondrogenic, pro-inflammatory group), collagen content peaked at day 42 and showed no significant change at day 56 (Fig. 5B). Total s-GAG/dry weight for constructs cultured in chondrogenic conditions peaked at day 42 ($3.07\pm0.24 \mu\text{g}/\text{mg}$) and decreased by 25% at day 56. For constructs initially cultured in chondrogenic conditions and changed thereafter to pro-inflammatory conditions at day 28, total s-GAG/dry weight reached a maximum of $2.18\pm0.22 \mu\text{g}/\text{mg}$ at day 42 and decreased by 64% at day 56 (Fig. 5D, $p<0.05$).

Discussion

Results of this study show that levels of IL-1 encountered *in vivo* under pathophysiologic conditions have a significant, deleterious effect on chondrogenesis and the development of mechanical properties in MSC-based cartilage constructs. However, the extent of this effect is highly dependent on stage of chondrogenesis at which the MSCs are exposed to IL-1. Under chondrogenic conditions, the MSC-seeded 3D woven PCL scaffolds show a significant increase in mechanical properties after 4 or 8 weeks in culture. The presence of IL-1 during the initial 28-day culture period strongly inhibited accumulation of ECM components, preventing any increase in compressive mechanical properties over baseline levels. However, constructs cultured for 4 weeks in chondrogenic medium prior to IL-1 exposure maintained their composition and biomechanical properties after IL-1 was added to

the medium, although these specimens exhibited lower collagen and proteoglycan content than those that were not exposed to IL-1.

Our findings are consistent with previous studies showing that IL-1 can inhibit chondrogenesis of adult stem cells such as ASCs [9] or MSCs [10, 12-15] in hydrogel or pellet culture. In these cells, IL-1 appears to act through the activation of nuclear factor kappa B (NF- κ B), as inhibition of this pathway using a dominant-negative suppressor of I- κ B [15], curcumin [13], or resveratrol [34] abrogates the effects of IL-1 on MSCs. Furthermore, relatively high concentrations (100 ng/ml) of bone morphogenetic proteins (BMPs) such as BMP-2 or BMP-9 can partially overcome the effects of IL-1 [10]. Interestingly, other physical and physicochemical factors such as hypoxia [14] or dynamic mechanical loading [16, 35] can also inhibit the deleterious effects of IL-1 on chondrogenesis or tissue repair in bioartificial culture systems.

An important advance of the current study is the measurement of functional mechanical properties of the construct engineered using MSCs, and the effects of IL-1 on the maturation of these properties. We have previously reported the development and characterization of 3D woven textile scaffolds engineered with predefined mechanical properties that mimic the anisotropic, viscoelastic, and nonlinear tension-compression behavior of native articular cartilage [24]. Additionally, we have shown that the use of PCL fibers to weave this structure confers long-term maintenance of initial properties while also supporting cell attachment and tissue synthesis [26, 27, 36]. Biomechanical testing of the MSC-seeded 3D PCL constructs formed in the present study shows an increasing trend in compressive stiffness with culture time, independent of culture conditions (Fig. 2). Consistent with earlier studies, this occurs as accumulating ECM binds the constituent fiber bundles of the scaffold and limits their ability to move and reorient under applied compressive loading, effectively becoming a hybrid composite material, which stiffens the entire structure [36].

Previous studies have also focused on the functional mechanical properties of tissue-engineered cartilaginous constructs using various biomaterials with MSCs as a cell source. For example, MSCs or ASCs cultured in agarose, gelatin, hyaluronic acid, poly(l-lactic acid), Puramatrix, and silk derived scaffolds demonstrated inferior mechanical properties to articular cartilage and also inferior properties relative to primary chondrocytes used in the same hydrogel [3, 37-42]. Conversely, recent studies by Mauck and colleagues have demonstrated greatly improved mechanical properties in MSC-based constructs, effectively acquiring compressive properties that approach that of articular cartilage [43-45]. In these studies, several strategies have been employed to increase the functional properties of the engineered tissue, including transient exposure to growth factors, compressive mechanical stimulation, and the use of *in vivo* bioreactors [43-45]. Particularly, MSCs seeded in agarose gels and transiently exposed to TGF- β_3 resulted in an equilibrium Young's modulus of ~200 kPa *in vitro* [43], and implanting MSC-laden hyaluronic acid hydrogels with TGF- β_3 -loaded microspheres in subcutaneous pockets in nude mice results in a value of ~400 kPa for the equilibrium Young's modulus after 8 weeks *in vivo* [45]. Both of these studies represent marked improvements in cartilage tissue engineering using stem cells as a cell source as the target for compressive moduli lies in the range of 400 – 800 kPa [46, 47]. These data are also consistent with the values obtained for equilibrium Young's modulus in our current study, in which a rapid accumulation of mechanical properties is noted as tissue consolidates the matrix, thereby significantly increasing the Young's modulus to ~400 kPa after only 2 weeks of culture.

Our data further show that ECM assembly on the 3D PCL scaffold was enhanced by a relatively low, continuous dose of 1 ng/ml TGF- β_3 , and that this dose significantly improved the mechanical properties of the tissue-engineered constructs over an 8-week culture period.

Under these conditions, MSCs produce a robust ECM high in proteoglycan and type II collagen content that penetrate and fills the entire scaffold space. However, the presence of a physiologically relevant dose of IL-1 inhibited the anabolic effect of TGF- β_3 by inhibiting the accumulation of collagen and s-GAG, consequently reducing the compressive mechanical properties. Nonetheless, the 3D woven scaffold still provides functional properties similar to those of native cartilage under these catabolic conditions.

Surprisingly, constructs cultured for 4 weeks in chondrogenic conditions prior to the administration of the catabolic agent IL-1 were protected from the IL-1-mediated decrease in mechanical properties. In these chondrogenically “pre-cultured” constructs, s-GAG/DNA content is lower in comparison to scaffolds cultured continuously in chondrogenic medium after week 4. The mechanisms responsible for the apparently lower sensitivity of chondrogenically differentiated MSCs to IL-1 (as compared to that of undifferentiated MSCs) is unknown and may involve several factors. For example, the accumulation of new ECM within the scaffold is likely to significantly reduce the diffusion coefficient and partition coefficient of solutes within the construct [48], thus effectively reducing the concentration of IL-1 to which MSCs are exposed. Alternatively, the sensitivity of MSCs to IL-1 may change during the differentiation process, either through altered expression of cell surface receptors or antagonists of IL-1 [11, 49-52]. For example, previous studies have shown that IL-1 itself can induce IL-1Ra production in various cell types [53, 54]. Importantly, growing evidence indicates that MSCs may in fact play an anti-inflammatory role when reintroduced *in vivo* [50, 55] and enhance repair by producing IL-1 receptor antagonist (IL-1Ra) [51].

Conclusions

In summary, our findings show that IL-1 has significant deleterious effects on chondrogenesis, matrix accumulation, and the maturation of mechanical properties in MSC-seeded 3D woven constructs. However, the impact of the inhibitory effects on the development of functional properties is significantly reduced by the presence of a biomimetic scaffold that exhibits mechanical properties similar to those of native cartilage. Further studies are necessary to determine the mechanisms by which IL-1 acts on MSCs at different stages of differentiation.

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Figure 1. Experimental timeline depicting culture conditions of each test group over time. Mechanical, biochemical, and compositional properties of all groups were assessed at each time point.

Figure 2.

(A-C) Mechanical properties of constructs at day 0, 14, 28, 42 and 56. Groups not sharing the same letter are statistically different from each other (ANOVA, $p < 0.05$). Data represented as mean + SEM.

Figure 3. Histology and Immunohistochemistry of constructs at day 28 for control, chondrogenic, and pro-inflammatory groups (cross-sectional views). Porcine osteochondral tissue (bottom row) was used as positive control for all staining protocols. Scale bar = 0.5 mm.

Figure 4. Immunohistochemical staining of type II collagen and chondroitin 4-sulfate at day 56 for: (A,C) continuous chondrogenic culture and (B,D) chondrogenic culture for days 0 to 28, followed by pro-inflammatory culture for days 28 to 56. Scaffold fibers are seen as white spaces. Scale bar = 0.1 mm.

Figure 5. Biochemical analysis of constructs over time. (A) Total collagen and (C) total s-GAG normalized to dsDNA, and (B) total collagen and (D) total s-GAG normalized to dry weight. Time points not sharing the same letter are statistically different from each other (ANOVA, $p<0.05$). Data represented as mean + SEM.