Mesenchymal Stem Cells in Chondrogenic 3D Culture Are More Sensitive to Pro-Inflammatory Cytokines than Chondrocytes

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Introduction

Articular cartilage is a specialized tissue that functions to transmit loads during daily activities. However, with increasing age or following injury, damaged cartilage can progressively deteriorate, leading to osteoarthritis.[1,2] Combining various cell types and biomaterials in cartilage tissue engineering can generate constructs that mimic the architecture and function of native tissue.[3] However, the response of these engineered constructs to the inflammatory environment present in the joint during OA is not well understood. Moreover, given the persistent deficits in constructs formed from mesenchymal stem cells (MSCs) compared to chondrocytes,[4] it is important to evaluate their potential in an inflammatory milieu. Multiple pro-inflammatory cytokines mediate tissue degradation in OA; two key factors involved in these catabolic processes are IL-1β and TNF-α.[5] The current study sought to determine the sensitivity of engineered cartilage based on MSCs and articular chondrocytes to inflammatory cytokines, and to elucidate the pathways that mediate alterations in cellular response and construct properties.

Methods

Chondrocytes (CH) and MSCs were harvested from matched juvenile bovine knees and seeded in 2% agarose constructs at 20 million cells/mL (MSCs were passage 3; CH were embedded immediately). Constructs (Ø = 4mm; H = 2.25mm) were cultured in chemically defined media with TGF-β3 (CM+) for 21 days.[6] Constructs were then cultured with CM− (no TGF-β3) for 6 days while being treated with 0, 1, 5, or 10 ng/mL of either IL-1β or TNF-α. Media was collected at day 3 (D3), and IL-1β or TNF-α were refreshed during the media change. On day 6 (D6), media was collected and constructs tested in unconfined compression to determine changes in mechanical properties with treatment.[6] Alterations in biochemical content (GAG, DNA) and gene expression were evaluated post-treatment (N=4).[6] Catabolic mediators in the media were measured, including nitric oxide (NO) (Griess assay) and total matrix metalloproteinases (MMP) (Generic MMP kit, Anaspec) (N=2 for media from 5 constructs cultured together). Samples were processed for histology and stained for proteoglycans with Alcian Blue (N=2). Significance was determined using one-way ANOVA and Tukey's post hoc test with $p<0.05$.

Results

Treatment of chondrocyte (CH) and MSC seeded constructs with either IL-1β or TNF-α, resulted in decreases in mechanical properties and biochemical content over six days ($p<0.05$). For IL-1β treated constructs at 1 ng/mL, the modulus dropped to ~50% for CH constructs, while MSC constructs plummeted by ~80% (Fig 1A) compared to controls. At higher concentrations of IL-1β, the modulus of CH constructs was further reduced, while MSC constructs had little mechanical integrity. In comparison, the modulus of 1 ng/mL TNF-α treated CH constructs was slightly reduced (~15%) compared to controls, but at higher concentrations was reduced by ~70% (Fig 1B). TNF-α treated MSC constructs decreased markedly at 1 ng/mL (~95% decrease in modulus). GAG content of these constructs showed similar declines. Analysis of NO levels showed a concentration dependent increase on D3 and D6. NO levels were highest on D3 of IL-1β treatment and reached similar levels for both cell types. For TNF-α treated constructs, NO levels were higher at all concentrations for MSC compared to CH constructs (Fig 2A,B). In addition, more GAG was released to the media for MSC compared to CH constructs in a concentration dependent manner for both IL-1β and TNF-α on D3. By D6, GAG in the media in MSC was comparable to CH constructs (Fig 2C,D). MMP levels also showed distinct differences between CH and MSC constructs, with IL-1β and TNF-α treated MSC constructs having little MMP on D3, but a 25X increase in MMP on D6. In contrast, CH constructs showed a dose dependent increase in MMP with IL-1β on D3, which was maintained at D6 for the 5 and 10ng/mL treatment groups (Fig 3A). Histological staining for proteoglycans showed a loss of matrix following exposure to TNF-α and IL-1β. Decreased staining of proteoglycan in MSC constructs was observed with increasing concentrations of IL-1β and TNF-α (Fig 3B).

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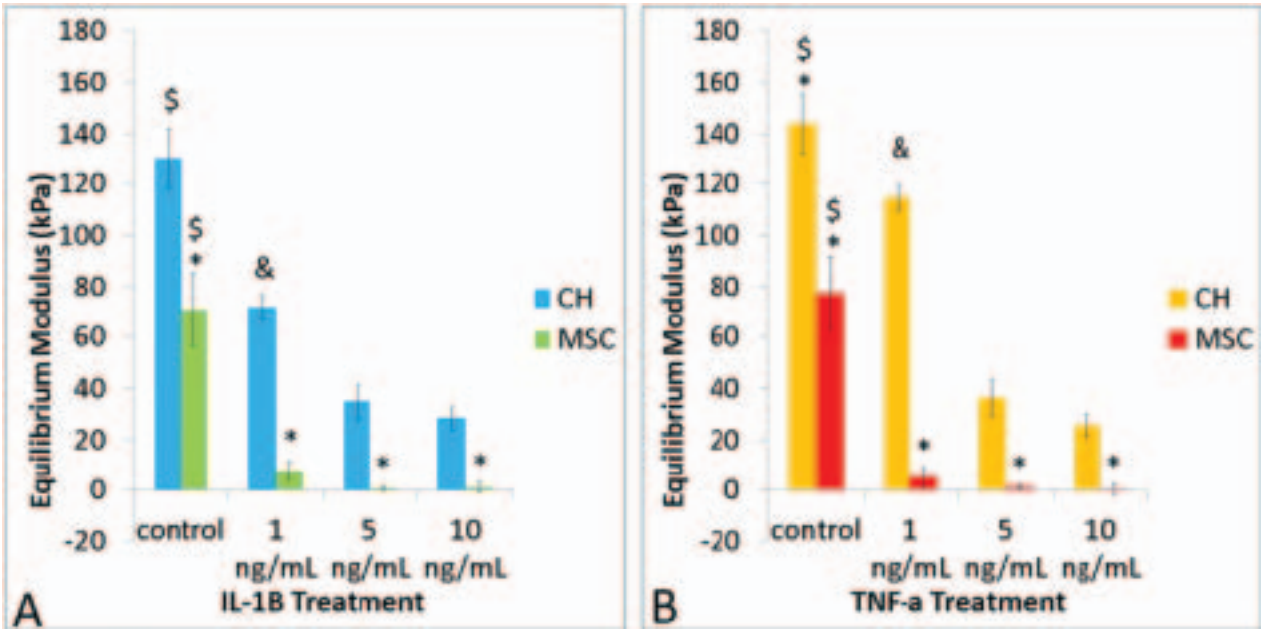

Figure 1. Equilibrium modulus of constructs treated with **(A)** IL-1β or **(B)** TNF-α on D6 shows a severe loss of mechanical properties for MSCs. $p<0.05$: * vs. CH; $ vs. 1, 5, and 10ng/mL.

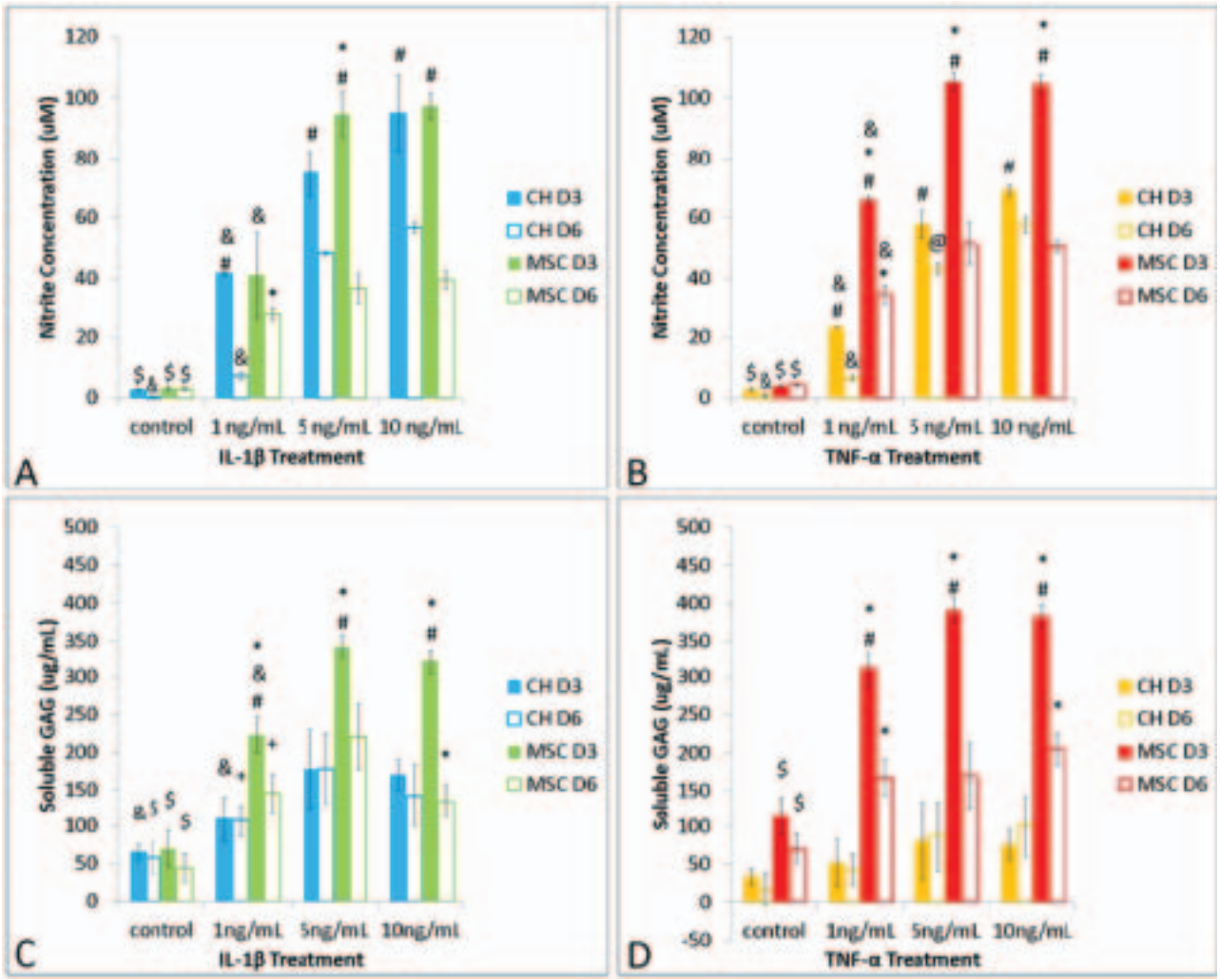

Figure 2. NO levels for constructs treated with **(A)** IL-1β are similar between CH and MSCs, but for **(B)** TNF-α, NO levels are 2X higher in MSCs compared to CH on D3. Soluble GAG for **(C)** IL-1β treatment is 1.5X higher and in **(D)** TNF-α treatment is 4X higher in MSCs than CH on D3. $p<0.05$: * vs. CH; $ vs. 1, 5, 10ng/mL, & vs. 5, 10ng/mL, + vs 5ng/mL, @ vs 10ng/mL (same time point); # vs D6 (same cell type).

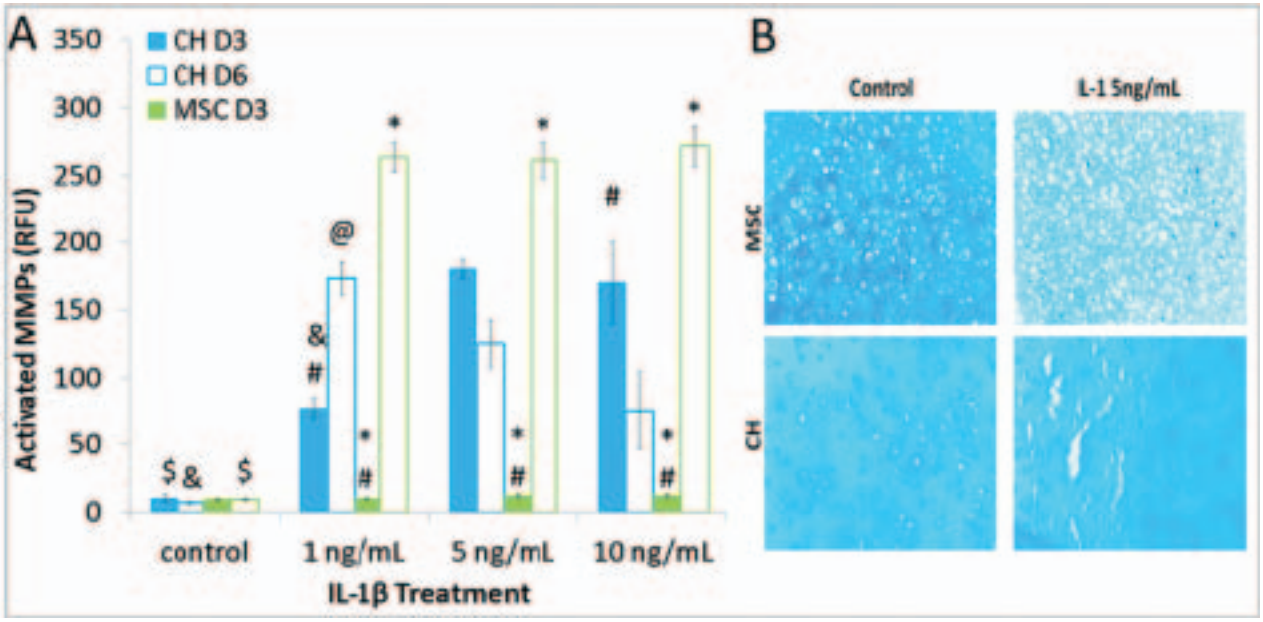

Figure 3. (A) For IL-1B treated constructs MMP production occurs in both cell types, but is highest on D6 for MSCs. $p<0.05$: * vs. CH; $ vs. 1, 5, 10ng/mL, & vs. 5, 10ng/mL, @ vs 10ng/mL (same time point); # vs D6 (same cell type). **(B)** Histology shows that 6 days of treatment with IL-1β 5ng/mL causes severe GAG loss from MSC constructs.

Discussion

This study demonstrated that the action of inflammatory cytokines on engineered cartilage constructs is dependent upon the cell type employed. MSCs were more sensitive to TNF-α and IL-1β compared to CH constructs. This sensitivity resulted in a significant loss of mechanical properties at a lower dose and correlated to increased early NO production and GAG loss to the medium. Interestingly, both CH and MSCs showed increases in MMP activity, though for MSC it occurred later (day 6). This might suggest that the mechanism underlying the differing responses of CH and MSC to these inflammatory cues is related to their differential capacity to manage stress agents and activity of degradative enzymes. It should be noted that the assay employed here measures both active and pro-enzyme forms of MMP and so direct correlations with matrix loss cannot be made. The rapid loss of GAG does suggest heightened aggrecanse activity, and specific analysis of this enzyme is warranted to further explicate the mechanism underlying these findings. One possible rationale for the attenuated mechanical response of CH constructs may be that native cartilage cells are able to, in part, maintain tissue homeostasis even in the presence of mild inflammation or normal joint stress. Future experiments will investigate differences between CH and MSC function under load within an inflammatory environment to better understand the capabilities of MSCs as a cell source for cartilage repair.

Significance

Although MSCs can take on a chondrocyte-like phenotype and produce functional engineered cartilage, these cells are more sensitive than native CH to pro-inflammatory cytokines. These findings are critical as it suggests that the use of MSCs for cartilage repair therapies may be compromised when constructs are exposed to the inflammatory environment characteristic of OA joints.

Acknowledgements

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