Effects of chondrogenic priming duration on mechanoregulation of engineered cartilage anlagen

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Introduction

Endochondral ossification is the primary mode of bone formation during repair and development[1], which starts with a cartilage anlage which undergoes chondrocyte maturation and hypertrophy. Subsequently, neovascular invasion results in remodeling of the hypertrophic cartilage and enables osteoblastogenesis and bone formation. Mechanical forces have a been shown to direct bone formation *in vivo* both in development[2] and in tissue engineered[3] settings in a time-dependent manner. Additionally, endochondral ossification requires various cell types raising the question of which cell types are the critical mechanoresponders and how does the timing of loading impact cells at different maturation stages through endochondral ossification. **The goal of this study was to examine the effect of chondrogenic priming duration on the cell and tissue response to dynamic compression.** First, we determined the effect of priming time and loading on mechanical properties on cells in fibrin hydrogels. Then, we sought to characterize the effects of loading and priming time through biochemical, gene expression, and matrix deposition analysis.

Methods

Human MSCs were encapsulated in fibrin hydrogels and loaded into a bioreactor. Hydrogels were primed in chondrogenic media for a certain length of time before 2 weeks of dynamic loading (Fig. 1). Each hydrogel had a time-matched free-swelling control. At the end of the loading period hydrogels were analyzed for mechanical properties, biochemical content, gene expression, and matrix production.

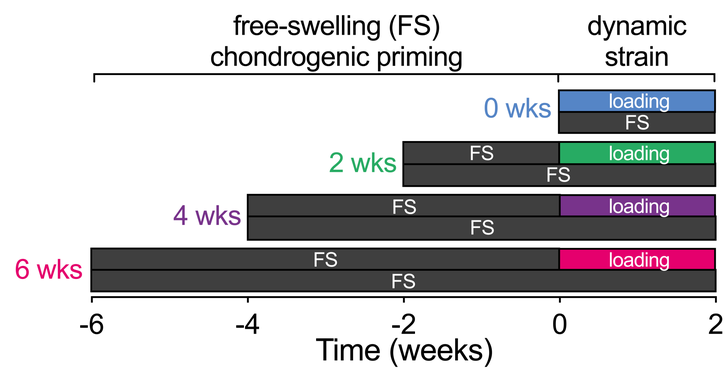

Figure 1. Loading timeline. All samples were collected after their 2-week loading cycle and compared to free-swelling controls at the same time.

Results

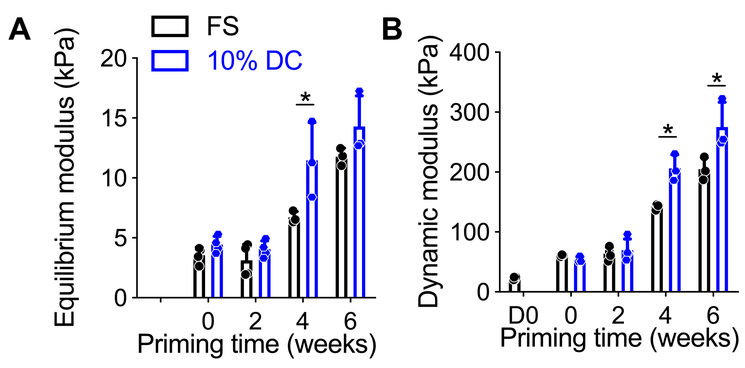

Figure 2. Mechanical properties of chondrogenically-primed hMSC constructs under free-swelling (FS) or 10% dynamic compression (DC) conditions. Equilibrium modulus, E′ (A) and dynamic modulus, E′′ (B) at the end of the final loading cycle, in comparison to time-matched free swelling controls, both increased significantly with time. N = 3 – 4 per group.

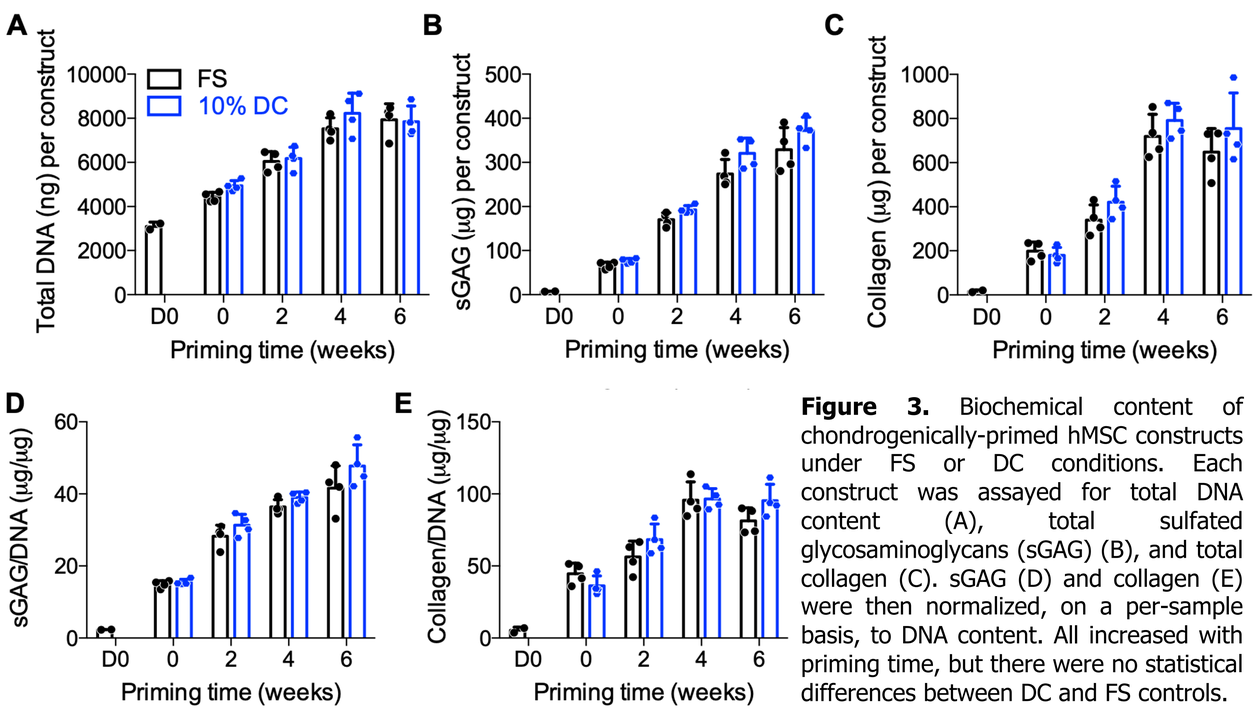

Figure 3. Biochemical content of chondrogenically-primed hMSC constructs under FS or DC conditions. Each construct was assayed for total DNA content (A), total sulfated glycosaminoglycans (sGAG) (B), and total collagen (C). sGAG (D) and collagen (E) were then normalized, on a per-sample basis, to DNA content. All increased with priming time, but there were no statistical differences between DC and FS controls.

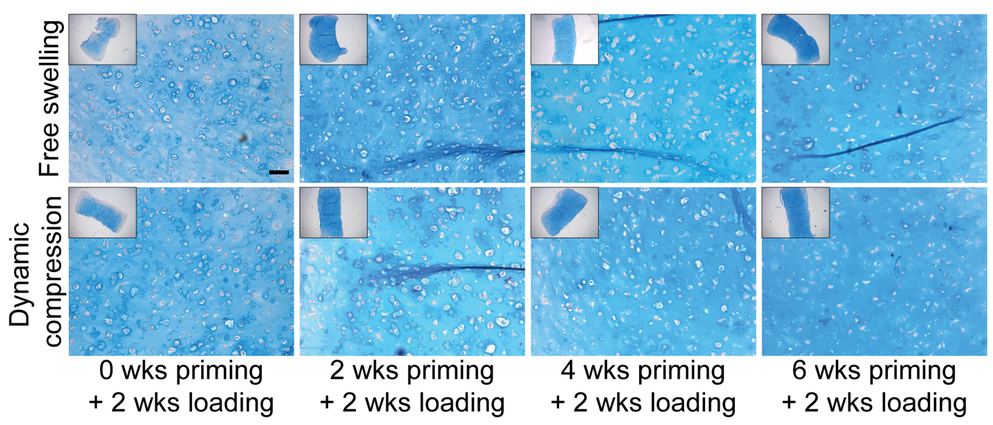

Figure 4. Alcian blue staining of glycosaminoglycans. sGAG content is seen consistently throughout the constructs over time, with modest increase in DC group, suggesting another explanation is necessary for change in mechanical properties over time. Scale bar = 100 µm.

Results

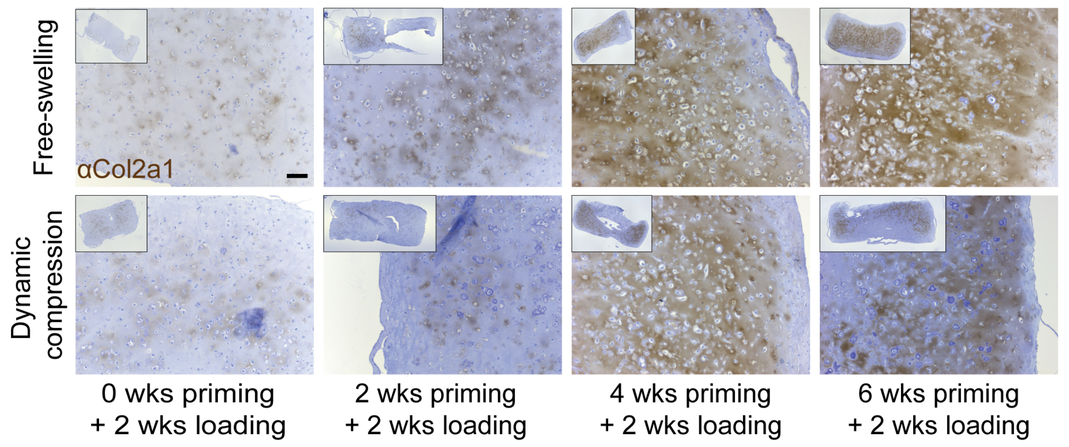

Figure 5. Chondrogenic matrix composition. Immunohistochemistry for Col2a1 with hematoxylin counterstain. At 0 and 2 weeks priming, Col2a1 staining was minimal regardless of loading conditions. In free-swelling constructs after 4 and 6 weeks of priming, Col2a1 staining was largely more prominent in the construct core compared to dynamic compression. Scale bar = 100 µm, N = 4 per group.

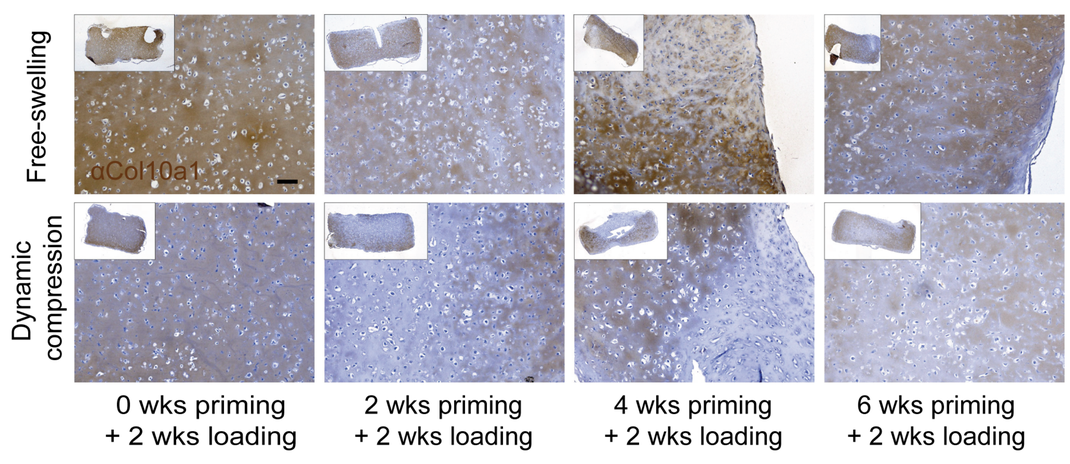

Figure 6. Hypertrophic matrix composition. Immunohistochemistry for Col10a1 with hematoxylin counterstain. Dynamic compression exhibited lower staining under all priming conditions. The FS conditions showed increased staining in the core similar to Col2a1 staining, while DC groups had limited core staining. Scale bar = 100 µm, N = 4 per group.

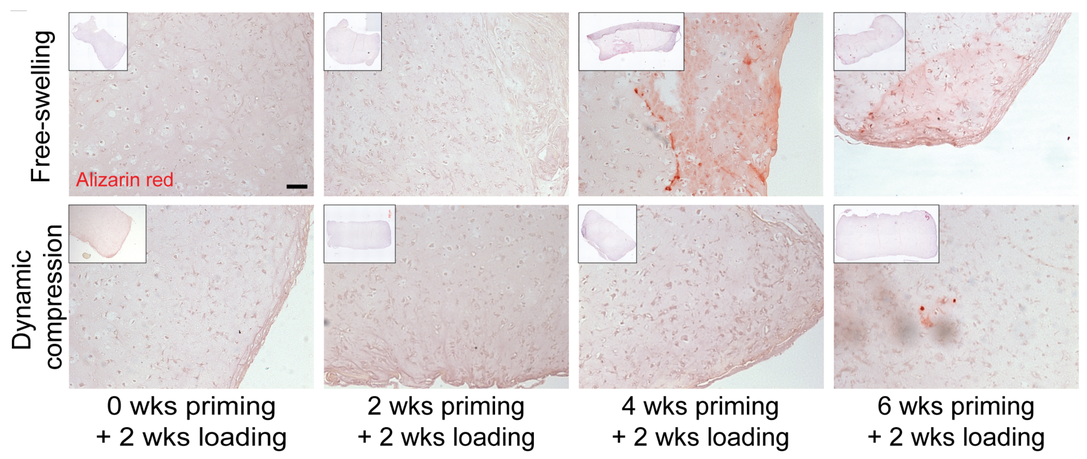

Figure 7. Osteogenic matrix composition. Mineral deposition was evaluated by alizarin red staining on histological sections. Limited alizarin red staining was found only after 4 weeks priming in the FS conditions, whereas the DC group did not see any staining until 6 weeks priming. Scale bar = 100 µm, N = 4 per group.

Discussion

Together, these data demonstrate that the response to dynamic loading depends on the extent of chondrogenic maturation in several ways. First, dynamic loading induced an increase in both elastic and viscoelastic mechanical behaviors, dependent on the extent of chondrogenic priming. Additionally, loading suppressed Collagen type X and mineral deposition with longer priming times. However, in the absence of loading, matrix deposition increased with longer chondrogenic priming times. Under free-swelling conditions we found that Col2a1 deposition was mostly isolated in the core of the hydrogel at 4- and 6-week timepoints, whereas Col10a1 was largely uniform at all priming points. Unlike the free-swelling hydrogels, the dynamic compression group showed minimal staining in the core. **This, along with our previous data[3,4,5], suggest that mechanical loading may alter mechanical properties, dependent on the duration of chondrogenic priming by altering extracellular matrix organization and distribution.** We hypothesize that longer bouts of loading will be needed to observe substantial changes in matrix composition.

References & Funding

[1] Provot+ Osteoporosis, 2013; [2] Felsenthal & Zelzer, Development, 2017; [3] McDermott+ STM, 2019; [4] Thorpe+ Ann. Biomed. Eng., 2010; [5] Luo+ Biomed. Mater., 2015

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