

# The Effects of Long-Term Stimulation on ECM Accumulation in Chondrocyte -Seeded Agarose Constructs

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**Disclosures:** Authors have no conflicts of interest to declare

**INTRODUCTION:** Articular cartilage injury and disease pose significant challenges in orthopedics[1]. Current treatments primarily focus on pain management, necessitating innovative solutions [2] Tissue engineering offers promise, combining cells, scaffolds, and growth factors to generate functional tissue for repair and regeneration [3]. Mechanical stimulation enhances engineered cartilage integrity by promoting ECM production and fiber alignment [4]. However, existing literature lacks consensus on optimal loading parameters for long-term stimulation. This study aims to explore the effects of long-term dynamic stimulation on tissue growth, comparing the effects of various loading parameters. Chondrocyte-seeded constructs undergo dynamic loading in long-term studies, evaluating changes in physical, biochemical, and mechanical properties. Results illuminate loading's potential to maximize growth and minimize catabolism, enhancing cartilage tissue engineering strategies.

**METHODS:** Isolated skeletally mature primary bovine chondrocytes were seeded onto 2% agarose hydrogels, cast in PTFE molds to create cylindrical tissue constructs (3mm x 4mm). Constructs were cultured in Ham's F12 media supplemented with 25 mM HEPES, 20% FBS, 0.2% ascorbic acid, and 1% antibiotics/antimycotics, with media replaced every 2-3 days. The constructs were maintained for two weeks under static, no loading conditions. Following two weeks of pre-culture, constructs were subjected to long-term mechanical stimulation every other day for either 2 or 4 weeks under two various loading conditions. Constructs were stimulated for either 20 minutes at a 5% strain amplitude, or 1 hour at a 2.5% strain amplitude using the Mach-1 micromechanical testing system (Biomomentum). Following the end of each stimulation time course, constructs were harvested and analyzed for changes in physical (histology), biochemical (collagen, proteoglycan and DNA contents) and mechanical properties. Statistical analysis was conducted using one-way ANOVA and Tukey's post-hoc test.

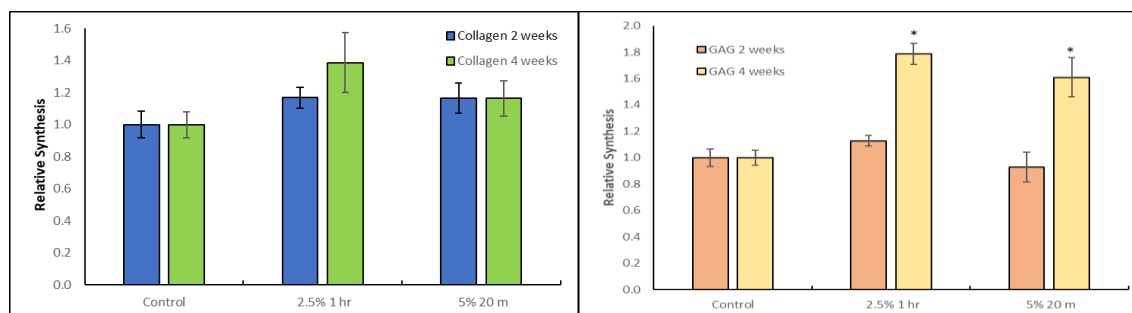
**RESULTS:** Long-term stimulation induced notable biochemical and physical changes. DNA content was decreased significantly in the 2.5% 1-hour group for both timeframes, and in the 5% 20-minute group at 4 weeks (data not shown). Although not significant, collagen accumulation was elevated in the 2.5% 1-hour group at 4 weeks (Figure 1). Conversely, proteoglycan accumulation was elevated under both stimulation conditions after 4 weeks of stimulation (Figure 2). Histological assessment supported the biochemical results with Sirius red (collagen stain) and safranin-O (proteoglycan stain) demonstrating consistent trends with respect to collagen and proteoglycan accumulation (data not shown). Mechanical properties displayed no significant differences between groups at either 2 or 4 weeks (data not shown).

**DISCUSSION:** Mechanical stimulation of chondrocytes has extensively been explored as a means to upregulate tissue formation; however, dynamic mechanical loading simultaneously triggers a catabolic response. For the greatest effect on ECM biosynthesis, chondrocyte metabolism should favor a net anabolic response [5]. This study aimed to elucidate the effects of long-term dynamic compressive loading on chondrocyte-seeded agarose constructs, focusing on biochemical, physical, and mechanical alterations. Guided by previous short-term experiments, constructs were exposed to optimal loading conditions: 1 hour at 2.5% strain or 20 minutes at 5% strain, which displayed maximal increases in ECM synthesis while minimizing MMP-13 activity. The effect of long-term stimulation under these conditions resulted in improvements in ECM deposition, indicating the importance of selecting stimulation conditions that minimize catabolism, and not just those that display an anabolic effect. Unexpectedly, the lack of effect on construct mechanical properties may be due to the breakdown of the agarose scaffold under repeated loading (fatigue)[6]. In conclusion, this research underscores the complex interplay between loading parameters and the chondrocyte response, offering insights into optimizing mechanical stimulation conditions for engineered cartilage constructs.

**SIGNIFICANCE:** Current research exploring the effects of mechanical stimulation on cartilage metabolism has provided no empirical rationale for the selected loading conditions. This research aims to fill this gap by outlining the method for determining optimal conditions to induce a maximal biosynthetic response in long-term stimulated tissues.

## REFERENCES:

- [1] Johnstone et al. (2013). *Eur Cell Mater*, 25(248) e76
- [2] Abramoff et al. (2020) *Med Clin North Am* 104(2), 293-311
- [3] Liu et al. (2021) *Front Bioeng Biotechnol* 9, 770655
- [4] Salinas et al. (2018) *Tissue Eng Part B Rev* 24(5), 335-358
- [5] Song et al. (2021) *Exp Mol Pathol* 118; 104590
- [6] Leddy et al. (2004) *J Biomed Mater Res Part B Appl Biomater* 70B(2), 397-406



**Figure 1:** Relative collagen accumulation at 2 weeks (blue) and 4 weeks (green) under various loading parameters. Data presented relative to static control. Error bars represent mean ± standard error (n=6-8)

**Figure 2:** Relative proteoglycan accumulation at 2 weeks (orange) and 4 weeks (yellow) under various loading parameters. Data presented relative to static control. Statistically significant (\*p<0.05). Error bars represent mean ± standard error (n=6-8).