Effect of Reverse 4-Point Bending on Tissue Engineered Cartilaginous Constructs

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INTRODUCTION: Enhancing quality of tissue engineering cartilage can be achieved through mechanical stimulation [1,4]. Given that native articular cartilage undergoes multifaceted loads due to natural growth and physical activity, there's a growing interest in the mechanical stimulation of chondrocytes. The body of literature regarding mechanical stimulation of tissues is largely focused on compression, shear and tension [2,3], with the effects of these loading modes investigated in isolation [1,5,6]. However, cartilage is typically subjected to these forces in combination and it has been challenging to apply multiaxial loads to cell-seeded tissue engineered constructs. In this study, the unique mechanical stimuli of reverse bending was investigated as a means to simultaneously apply tension and compressive forces on the construct.

METHODS: *Chondrocyte culture:* Isolated primary bovine articular chondrocytes were encapsulated in 2% agarose gels ($10x10^6$ cells/mL) and cast in PLA molds to create 10 mm x 3 mm x 1.5 mm constructs. The constructs were cultured in complete media (Hams F12 media supplemented with 20mM HEPES, 100 µg/mL ascorbate, 20% v/v FBS, and 1% v/v antibiotics/antimycotics) and incubated at 37°C and 5% CO2 for two weeks prior to mechanical stimulation. *Mechanical Stimulation*: Within a bioreactor, constructs were subjected to dynamic 4-point bending (2.5% or 5% strain amplitude at 1 Hz for 20 minutes, 3 times per week) using a Mach-1 Micromechanical Testing System (Biomomentum) with the orientation of the constructs flipped each day to apply reverse bending (Figure 1). *Extracellular Matrix Accumulation and Cellularity*: After 2 weeks of stimulation, constructs were digested by papain (40 µg/mL) for 48-72 hours at 65°C. Digests were assayed for proteoglycan (GAG assay), collagen (hydroxyproline assay) and DNA contents (PicoGreen assay).

RESULTS: Preliminary results suggest that engineered cartilage constructs developed from articular chondrocytes offer a promising improvement in extracellular matrix accumulation after 2 weeks of reverse bending (Figure 2). Interestingly, extracellular matrix accumulation and cellularity were significantly affected by the applied bending strain. Notably, compared to the unstimulated controls, 2,5% bending strains demonstrated superior results in terms of collagen accumulation and cellularity, while 5% bending strains were more conducive to the accumulation of proteoglycans.

DISCUSSION: In our exploration, we observed a direct correlation between the effects of reverse bending and enhanced extracellular matrix accumulation in tissue-engineered cartilage constructs. The introduction of reverse bending, combination of tensile and compressive forces, showed marked improvement to improve tissue formation. Lower bending strains (2.5%) emerged as a potent stimulus for collagen accumulation and perseveration of tissue cellularity, whereas higher bending strains (5%) favoured the production of proteoglycans. This differential response can be attributed to the unique mechanical properties and cellular responses elicited by each strain level. Future endeavors will focus on the optimization of bending strains and the comprehensive evaluation of the resultant constructs, such as histological appearance (H&E, general connective tissue stain; safranin-O, sulfated proteoglycan stain), immunohistochemical staining of specific collagen types (types I, II, and X) and comparison of mechanical properties of the resultant engineered constructs to that of native cartilage [7]. These findings underscore the importance of reverse bending in enhancing the functionality of engineered cartilage. In addition, further studies are needed to validate these findings and explore the long-term efficacy of the engineered cartilage in human articular cartilage.

SIGNIFICANCE/CLINICAL RELEVANCE: This research offers enhanced mechanical stimulation methods for tissue-engineered cartilage that mitigates the challenges of applying multiaxial loads to cell-seeded constructs. By harnessing the potential of reversed 4-point bending, we aim to produce robust cartilaginous tissue suitable for cartilage repair and reconstruction.

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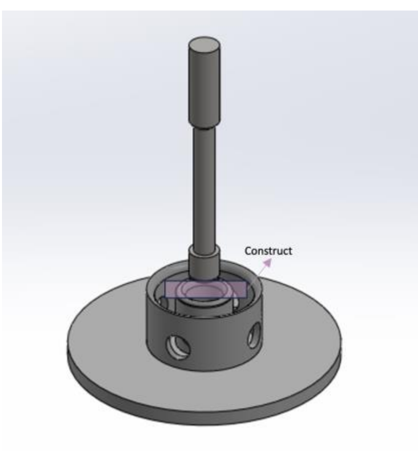

Figure 1: Experimental set up for reverse bending load application inside a 6-well plate in Mach-1 Micromechanical Testing System.

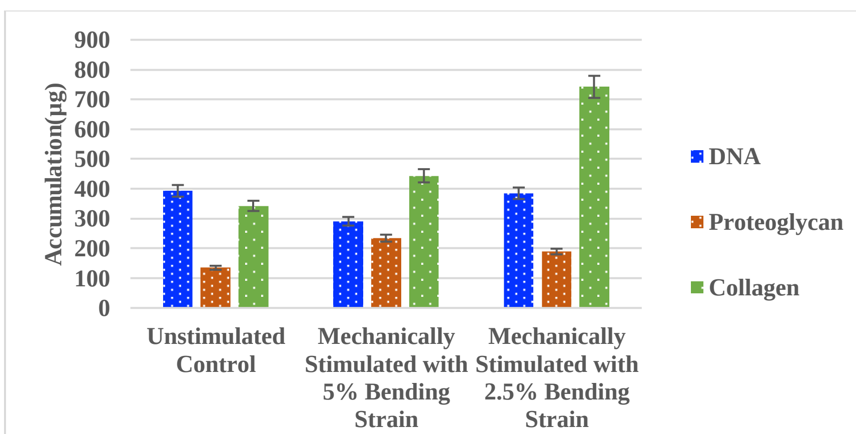

Figure 2: Extracellular matrix accumulation and cellularity after the application of reversed 4-point bending.