The Effect Of Superimposed Vibrations On Chondrocytes Subjected To Dynamic Compressive Loding

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Introduction: Mechanical stimulation is often used as a means to accelerate and improve the growth and properties of tissue engineered cartilage constructs. However, as cartilage cells (chondrocytes) rapidly desensitize to the imposed stimulus [1], the available window to apply mechanical loading is limited, thereby reducing the effectiveness of this treatment. One approach to mitigate the effects of loading-induced cellular desensitization is through the modification of the applied loading protocols. Specifically, cellular sensitivity to mechanical stimuli can be altered by superimposing mechanical vibrations on the loading waveforms. This approach, termed “stochastic resonance”, has been successfully applied to improve sensitivity in other nonlinear biomedical systems including mechano-sensitive cells of the same lineage (e.g. osteocytes [2]). Thus, the purpose of this study was to investigate the effect of stochastic resonance on 3-dimensional chondrocyte cultures subjected to dynamic compressive stimuli.

Methods: *Chondrocyte cultures*: Bovine articular chondrocytes from skeletally mature animals were obtained through sequential enzymatic digestion. Cells were encapsulated in a 2% agarose gel at a concentration of $10x10^6$ cells/mL. Cylindrical constructs measuring 3 mm diameter and 3 mm thickness were cut using a biopsy punch. The constructs were incubated in Ham’s F12 media supplemented with 20 mM HEPES, 20% FBS, 100 µg/mL ascorbic acid, and 2x antibiotics/antimycotics (complete media) for 24 hours at 37°C and 5% CO_2 before mechanical stimulation.

Mechanical Stimulation: The dynamic compressive stimulations were performed using a specialized loading/culture jig that fits a 24 well-plate with a Mach-1 Micromechanical testing system. Dynamic compression was applied at a 1 Hz frequency under a 5% strain amplitude for 20 minutes. Random mechanical vibrations (20-50 Hz bandwidth at 1g) were applied using a LAF10 Voice Coil Actuator located underneath the cultures, alone or in conjunction with dynamic compression. Prior to stimulation, each construct was supplied with 400 µL of fresh complete media.

Quantification of Biosynthesis: Collagen and proteoglycan synthesis were assessed through radioisotope incorporation of [^{3}H]-proline and [^{35}S]-sulphur respectively. Immediately after stimulation, 5 µCi of each isotope was added to each construct and allowed to incubate for 24 hours. At harvest, constructs were washed to remove unincorporated isotope then enzymatically digested by papain for 72 hours at 65°C. Isotope activity was measured through β-liquid scintillation counting and then normalized to DNA content assessed through the PicoGreen DNA assay.

Intracellular Ca^{2+} imaging: Prior to stimulation, constructs were incubated for 90 minutes with 4 µM Fluo-4 dye then in complete media for 20 minutes for de-esterification. Immediately after stimulation, an entire cross-sections of each construct was imaged at 5x magnification with a Leica confocal

microscope (ex/em 495/506 nm) at 5 second intervals for 5 minutes. Images were post processed in ImageJ to locate and examine cells experiencing transients. Ca^{2+} signaling was calculated as the ratio of the number of cells experiencing multiple transients (2 or more) to the number of cells experiencing at least one transient [3]. Signaling was determined overall for each construct as well as regionally, two concentric regions of equal area (core and periphery).

Results:

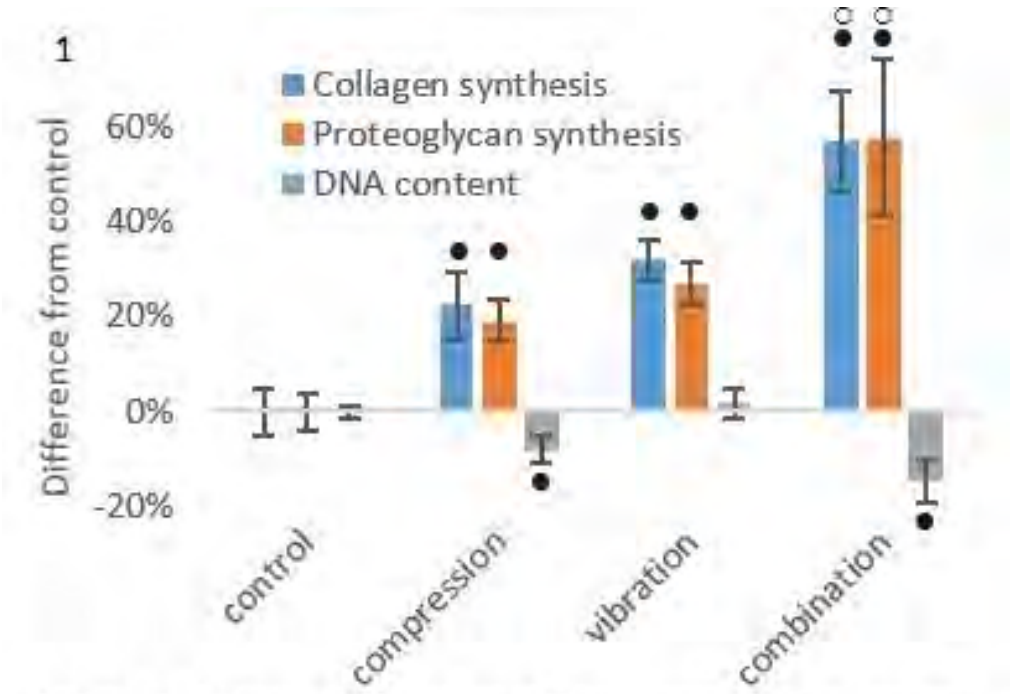


Figure 1: Matrix synthesis and DNA content of constructs subjected to dynamic compression with and without superimposed vibrations ($n \geq 8$). ● Different from control $p<0.05$; ○ Greater than compression $p<0.05$

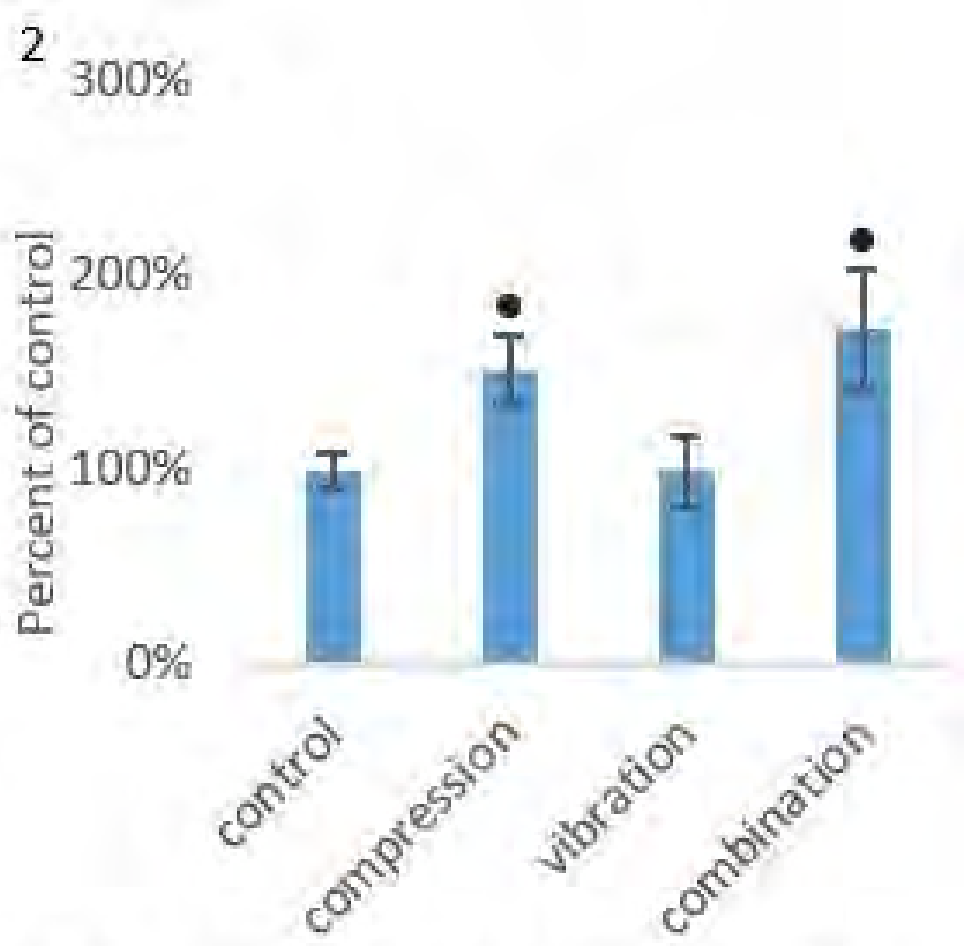


Figure 2: Ca^{2+} signaling ratio calculated over the entire sample cross-section ($n \geq 6$).
● Different from control $p < 0.05$.

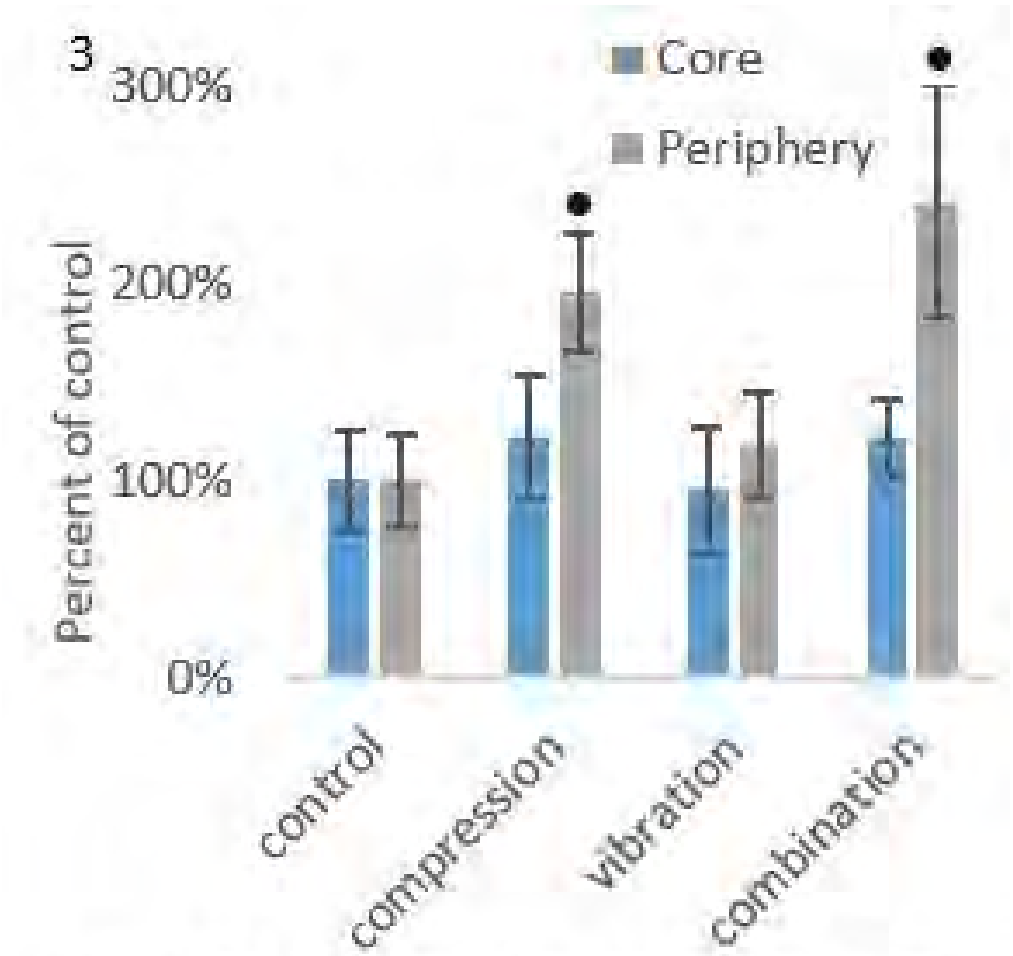


Figure 3: Ca^{2+} signaling ratio calculated over the core and periphery of the sample cross-section (equal areas) ($n \geq 6$). • Different from control $p<0.05$

In the absence of superimposed vibrations, dynamic compressive loading significantly increased matrix synthesis (collagen and proteoglycans) by ~20% ($p<0.05$; Fig.1). Similarly, random vibrations alone also significantly increased matrix synthesis by ~30% ($p<0.05$; Fig.1). The effects of random vibrations and dynamic compression appeared to be additive, with combined loading resulting in a ~55% increase in matrix synthesis (over control) ($p<0.05$; Fig.1). In addition, there was a small decline in construct cellularity with the application of dynamic loading, with or without superimposed vibrations ($p<0.05$; Fig.1). Determined over the entire construct, Ca^{2+} signaling was increased in response to dynamic compression ($p<0.05$), with or without superimposed vibrations; however, random vibrations alone had no apparent effect on Ca^{2+} signaling (Fig.2). When Ca^{2+} signaling was determined as a function of radial location (core vs periphery), changes in Ca^{2+} signaling in response to dynamic compression, with or without superimposed vibrations, appeared to only occur in the periphery of the sample and not at the sample core (Fig.3).

Discussion: The results of this study demonstrate that the application of superimposed random vibrations during dynamic compressive stimulation can elicit an increased biosynthetic response compared to dynamic compression alone. Effect of superimposed vibrations on matrix synthesis appeared to be additive, suggesting that maximal increases in matrix synthesis could be achieved by optimizing both the compressive and vibrational stimuli. Increases in matrix synthesis appeared to be loosely correlated with increased Ca^{2+} signaling determined over the entire construct cross-section. When the signaling response was determined in core and periphery of the construct, changes in Ca^{2+} signaling appeared to exclusively occur in the periphery of the sample. This result is similar to previous studies which have shown that newly synthesized matrix macromolecules are preferentially deposited in the sample periphery as a result of dynamic compressive loading [4]. In response to dynamic compression, greater strains and fluid flow typically occur in the periphery of unconfined cylindrical samples [4]. A confounding observation in this study was that increased matrix synthesis as a result of random vibrations did not result in increased Ca^{2+} signaling. This suggests that mechanical vibrations may act in a different manner on chondrocytes to elicit a synthetic response thus warranting further examination of the underlying signaling pathways responsible.

Significance: The accumulation of matrix molecules in tissue engineered cartilage constructs is vital to their performance after implantation. If the additional synthetic response of mechanical loading with superimposed random vibrations is found to be cumulative over long-term culture, it may be possible to develop robust constructs more efficiently.

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