Applied Dynamic Loading Following chABC Digestion Increases Collagen Production in Engineered Cartilage

+[1]O'Connell, G D; [1]Carapezza M A; [1]Newman, I B; [1]Ateshian G A; [1]Hung, C T
+[1]Columbia University, New York, NY
cth6@columbia.edu

Introduction

Articular cartilage is the load bearing soft tissue of diarthrodial joints, and mechanical loading maintains the integrity of the tissue [1]. Tissue-engineered cartilage constructs cultured in free swelling (unloaded) conditions are capable of achieving native equilibrium compressive modulus and glycosaminglycan (GAG) content within six weeks [2]. Applying physiological levels of dynamic compressive loading in culture increases the modulus by 50% [3, 4]. However, the biochemical composition of dynamically loaded tissue remains similar to unloaded engineered cartilage. Many approaches to cultivating engineered cartilage have been limited by low collagen production in vitro, an impediment for attaining native functional load-bearing properties. Previously we demonstrated that digestion of mature constructs with chondroitinase ABC (chABC) temporarily suppresses the GAG content, increases the collagen content and eventually improves mechanical properties [2, 5]. Four weeks following chABC digestion constructs had mechanical properties that were equivalent to the undigested control (266 ± 62 kPa) and a 40% increase in the collagen content (control = 5.4 ± 0.3% by dry weight) [5]. The aim of this study was to test the hypothesis that the collagen content will be greater for constructs cultured in dynamic loading conditions following chABC digestion.

Methods

Chondrocytes were harvested from juvenile bovine wrist joints. Primary cells were seeded in a slab of 2% w/v agarose with a cell concentration of 30M cells/mL. Constructs were cored from the slab (∅4 mm × 2.34 mm thick) and cultured in chemically-defined media supplemented with 10 ng/mL of TGF-β3 for the first 14 days. At day 14, constructs were digested for 48 hours by adding 0.15U/mL chABC to the culture media. Following the digestion, half of the samples were cultured under dynamic loading. in unconfined compression at 10 ± 1% strain, at 1 Hz for 3 hours a day. Remaining constructs served as control and cultured in free-swelling conditions.

The equilibrium Young's modulus (E_Y) was determined using unconfined compression stress-relaxation at 10% strain. The dynamic modulus was measured from the response to a superposed sinusoidal input of ±1% strain at 0.5 Hz. DNA content was determined using the PicoGreen Kit (Invitrogen Co). GAG and collagen content were determined using the 1,9-dimethylmethylene blue (DMMB) and hydroxyproline assays, respectively. A two-way ANOVA was performed with factors of time and culture condition. Significance was set at $p \leq 0.05$ with n=5 samples per time point and culture condition. A Pearson's correlation was performed on the data from this study and data available in the literature [3] to compare the collagen content normalized by DNA with the dynamic modulus.

Results

By day 42, the mechanical properties of the digested constructs reached the pre-digestion values ($p > 0.05$ vs. day 14; Figure 1A). Constructs that were dynamic loaded following chABC digestion had higher compressive mechanical (equilibrium and dynamic modulus) and matrix content. By day 56, the collagen content of the dynamically loaded constructs was 65% greater than the collagen content of constructs cultured in free swelling conditions ($p < 0.01$; Figure 1B). The collagen content normalized by wet weight reached 2.0 ± 0.2% for the loading group. There was a strong correlation between the collagen content normalized by DNA and the dynamic modulus (Figure 2- solid circles). Histological analyses demonstrate uniform collagen deposition throughout the construct, which was demonstrated by similar staining intensity at the center and the edge of the construct (Figure 3 - top row). The GAG content normalized by dry weight in the loading group was 50% greater than the control ($p = 0.02$). In contrast to the collagen distribution, staining for GAG was more intense in the center portion of the construct (Figure 3 - bottom row).

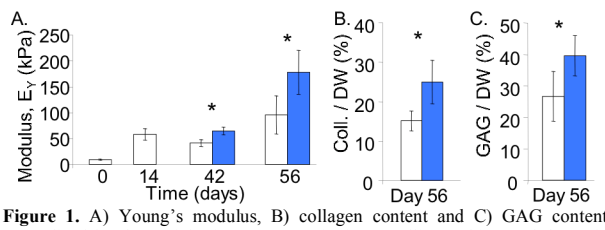

Figure 1. A) Young's modulus, B) collagen content and C) GAG content normalized by dry weight for constructs in free swelling (white) and dynamic loading (blue) culture conditions. *$p \leq 0.03$.

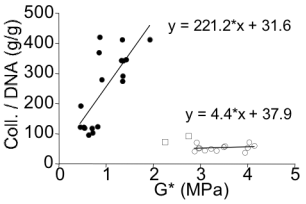

Figure 2. Correlation of collagen normalized by DNA with the dynamic modulus (G*) for data collected in this study (solid circles, r =0.76, p<0.01) and data in the literature (open circles, r=0.19, p=0.5 [3], open squares represents average data [4]).

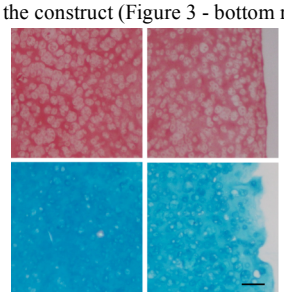

Figure 3. Histological results for an engineered construct under dynamic loading culture conditions (day 56). Picrosirius Red staining for collagen (top row) and Alcian Blue for GAG (bottom row) in the center (left column) and the edge (right column) of the construct. Bar = 100μm.

Discussion

The aim of this study was to improve the collagen composition of engineered cartilage by combining the known benefits of dynamic loading with chABC digestion. Application of dynamic loading for 42 days following chABC digestion greatly improved the mechanical and biochemical properties, thus supporting the hypothesis of this study. Importantly, this study demonstrated that loading engineered cartilage in culture increases collagen deposition towards native values (native = 50% by dry weight). Direct comparison to our previous results [3], with normalized collagen values (Figure 2), suggest that dynamic loading in combination with chABC digestion may increase collagen production better than only dynamic loading or chABC digestion.

Based on the histological results, GAG distribution was inhomogeneous throughout the construct, with more GAGs located in the center of the sample. This gradient is likely due to limited diffusion of chABC from the culture media into the center of the construct [2]. Diffusion of enzymes and nutrients can be improved by incorporating macroscopic or microscopic channels into the scaffold [6]. Recently, we have encapsulated lipid microtubes designed for chABC delivery for spinal chord injury repair [7]. The microtubes provide homogeneous delivery of chABC throughout the scaffold and may improve the depth-dependent matrix distribution. Future work will apply the findings of this study to localized delivery of chABC using microtubes.

Chondroitinase ABC digestion has been shown to increase the collagen production by 40% in free-swelling culture [2]. The findings of this study demonstrate that collagen deposition can be further improved by applying dynamic loading culture conditions to engineered constructs following chABC digestion. Applied dynamic loading may also serve to precondition cells for the post-implantation environment.

Significance

Engineering cartilage constructs with collagen content comparable to native tissue remains a challenge for the field. Increasing collagen content is necessary to provide greater compressive stiffness under dynamic loading. This study shows that dynamic compressive loading of chABC-digested engineered cartilage constructs raises collagen content and compressive moduli closer to native tissue levels.

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References [1] Sun HB, *Ann NY Acad Sci* 2010; [2] Bian+ *Tissue Eng, Part A* 2009; [3] Lima+ *Osteoarthritis Cartilage* 2009; [4] Bian+ *Tissue Eng, Part A* 2010; [5] O'Connell+ *Trans ORS* 2011; [6] O'Connell+ *Trans ASME Summer Bioeng Conference* 2011; [7] Lee+ *PNAS* 2010.