COLLAGEN HYDROLYSATE INCREASES THE MECHANICAL PROPERTIES AND TYPE II COLLAGEN SYNTHESIS OF TISSUE ENGINEERED ARTICULAR CARTILAGE

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INTRODUCTION: Collagen is the major structural protein in articular cartilage, comprising a majority of the tissue dry weight (**1**). This collagen network is vital for providing tensile strength as well as sites for proteoglycan binding and general ECM structure (**1**). Though engineered cartilage has approached the GAG content of the native tissue, such tissues typically possess a total collagen content ~10x less than that of the native tissue, possibly explaining the inability to fully achieve native tissue mechanical properties (**2, 3, 4**). Recently, it has been shown that 2D plated chondrocytes increase production of type II collagen in the presence of collagen hydrolysate, a form of degraded collagen (**5**). Thus, the hypothesis of this study is that the addition of collagen hydrolysate into 3D chondrocyte culture in agarose would improve the functional mechanical properties and collagen composition of *in vitro* engineered cartilage.

METHODS: Construct Preparation: Bovine chondrocytes from 2-3 month old calves were suspended in 2% agarose (Type VII, Sigma) at 60 x 10^6 cells/mL. Disks were cored out (Ø5.0 x 2.3 mm) from the gelled slabs and cultured at 37°C and 5% CO_2 in 30 mL of fully supplemented DMEM (Sigma) with 20% FBS and 50µg/mL ascorbate (Sigma), with media changed daily. Disks were cultured with 0, 0.1, 0.5, 1, or 10 mg/mL collagen hydrolysate (CH) (Gelita, Chicago, USA) added to media daily, with media osmolarity also tested. Cell-free constructs were maintained under all culture conditions as controls. Mechanical Testing: Young's modulus (E_Y) of constructs (n=5-6) was determined on day 0 and 28 by unconfined compression in stress-relaxation tests of 10% strain. Day 0 constructs were tested and processed prior to culture in CH media. Biochemical Analysis and Histology: After mechanical testing, half of each disk was either frozen for biochemical analysis (GAG, collagen) or fixed and prepared for type II collagen immunohistochemistry as described in (**2**). All biochemical content was normalized and is represented as a percentage of the sample wet weights (% ww). Statistics: Statistics were performed on all data using one-way ANOVA with Fisher LSD post-hoc tests, with α=0.05, n=5-6.

Day	CH (mg/mL)	Diameter (mm)	Thickness (mm)
0	0.00	5.06±0.01	2.24±0.02
28	0.00	5.57±0.10*	2.85±0.09*
	0.10	5.47±0.20*	2.85±0.04*
	0.50	5.44±0.10*	2.76±0.06*†
	1.00	5.34±0.12*†	2.73±0.03*†
	10.00	5.14±0.08†	2.36±0.03*†

Table 1. High collagen hydrolysate concentrations modulated construct dimensions. * vs. day 0, † vs. 0 mg/mL group, p<0.05, n=5-6.

RESULTS: The concentrations of collagen hydrolysate used in this study were found to have no effect on media osmolarity (~350 mOsm, p>0.25). Increasing CH concentrations (0.5, 1, and 10mg/mL) were found to affect construct size, with greater concentrations resulting in constructs that more closely retained their original dimensions (**Table 1**). After 28 days in culture, all construct groups increased significantly in E_Y, GAG, and collagen content versus day 0 values (p<0.05). At this time point, CH at concentrations of 0.5 and 1 mg/mL was found to elicit a modest (~12%) but significant increase in E_Y, with 10mg/mL resulting in significant decreases in both E_Y and total collagen content (**Figure 1**, p<0.05). CH at 0.1, 0.5, and 1 mg/mL had no effect on GAG content by day 28 (~1.5 %ww, p>0.10), though 10 mg/mL constructs possessed significantly reduced GAG content (0.69±12 %ww, p<0.05). The majority of type II collagen appeared concentrated pericellularly in 0 mg/mL CH cultured constructs, with some stronger staining along the periphery (**Figure 2**). Qualitative increases in type II collagen immunofluorescence were observed with 0.1, 0.5, and 1 mg/mL CH (**Figure 2**). These increases had an apparent dose-dependent behavior, with less of an increase at 1 mg/mL and severely diminished staining at 10 mg/mL CH (not shown). Cell-free constructs did not exhibit any material or biochemical changes over time in culture.

DISCUSSION: The observed increase in type II collagen production (consistent with [5]) and tissue stiffness in the presence of collagen hydrolysate, a degraded form of collagen, is an encouraging finding for improving the tissue engineering of articular cartilage. The increased presence of type II collagen due to CH would imply that although the total collagen content was not affected, the relative percentage of type II collagen within the constructs was improved, though overall collagen content was still ~10x less than native tissue. The dose-dependent nature of the biochemical data suggests that the chondrocytes are not using the degraded collagen as a building block for new matrix molecules. Otherwise, the 10 mg/mL CH concentration should have resulted in even a greater amount of matrix development. Interestingly, high amounts of CH (i.e., 10 mg/mL) were found to reduce both GAG and collagen content in the engineered constructs. This lack of tissue development would explain why those constructs possessed dimensions close to the original cored parameters. Future studies to optimize the use of collagen hydrolysate will focus on quantifying the resulting collagens affected by the presence of CH along with studying the possible interaction of CH-induced matrix formation with the application of mechanical stimuli.

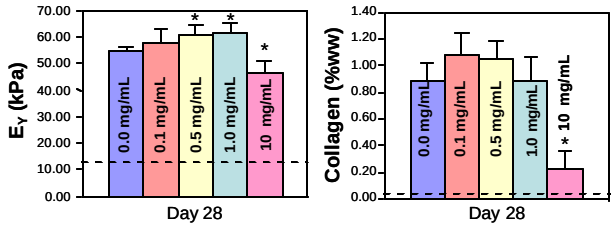

Figure 1. Effect of collagen hydrolysate concentrations on Young's modulus and collagen content. Dotted lines represent day 0 values (E_Y: 13.67±1.8 kPa, Col:0.02±0.07 %ww). *=vs. 0 mg/mL, p<0.05, n=5-6.

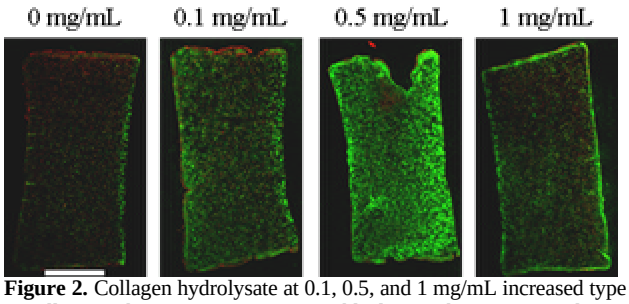

Figure 2. Collagen hydrolysate at 0.1, 0.5, and 1 mg/mL increased type II collagen. This increase was noticeably less with 1mg/mL and was severely diminished at 10 mg/mL (not shown). Scale Bar = 1mm.

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