EFFECTS OF SEEDING DENSITY AND NATIVE PERICELLULAR MATRIX ON THE RESPONSE OF CHONDROCYTES TO DYNAMIC DEFORMATIONAL LOADING

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INTRODUCTION

One approach to the functional tissue engineering of articular cartilage involves the application of physiologic loading to chondrocytes seeded in three-dimensional agarose scaffolds [1,2]. Dynamic deformational loading has been shown to augment the production of matrix macromolecules and enhance the overall material properties of these constructs compared to free-swelling controls [2]. The chondron, composed of the chondrocyte and its pericellular microenvironment, is the functional biomechanochemical unit of articular cartilage. This pericellular matrix is characterized by a cell-associated network of type VI collagen [3]. Previous studies have shown that chondrons in pellet culture exhibit enhanced matrix synthesis compared to cultures prepared with isolated chondrocytes [4-5]. Furthermore, the presence of a pericellular matrix has been shown to alter the response of the chondrocyte to various stimuli including growth factors and osmotic loading [6-8]. Therefore, the presence of this native chondron-associated pericellular matrix may affect the response of the cells in our agarose constructs to deformational loading. In a previous study, we found that chondrons, at low density, did not respond to dynamic deformational loading over four weeks in culture [9]. In the current study, to further examine this phenomenon, we examined the effects of dynamic loading and seeding density on the development of material properties of chondron-seeded agarose constructs over six weeks in culture compared to isolated chondrocyte-seeded agarose controls.

METHODS

Chondrons were isolated from full thickness immature bovine cartilage enzymatically via dispase and collagenase digestion.[10] Chondrocytes were harvested by pronase and collagenase digestion as described previously [2]. The presence of chondrons was confirmed using type VI collagen immunofluorescence (Figure 1). Both chondrons and chondrocytes were encapsulated at a final concentration of 10 or 60 million per ml of 2% (type VII, Sigma) low-melt agarose in phosphate buffered saline. Agarose slabs were cast at 2.25 mm thickness and cylindrical disks cored at 4.76 mm diameter. Disks were cultured in DMEM containing 10% FBS (10 million per ml samples) or 20% FBS (60 million per ml samples), buffers, antibiotics, antimycotics and 50 μg/ml ascorbic acid. Each cell population was divided into a free-swelling group and a dynamically loaded group (10% strain daily for three hours at 1 Hz) [2]. Samples were collected and analyzed on day 0, 14, 28 and 42. A custom mechanical testing device was used to perform stress relaxation and dynamic displacement tests to assess the development of material properties in constructs [2,11]. The equilibrium Young's modulus (E_Y) and dynamic modulus ($|G|$) were determined for these testing regimen, respectively. Type VI collagen immunofluorescence was performed with monoclonal antibody (5C6, DSHB), using a FITC-conjugated secondary antibody (green, Molecular Probes) and propidium iodide nuclear staining (red, Molecular Probes). Two-way ANOVA with Tukey HSD post-hoc test with α=0.05 (n=3 disks) was used for statistical comparisons.

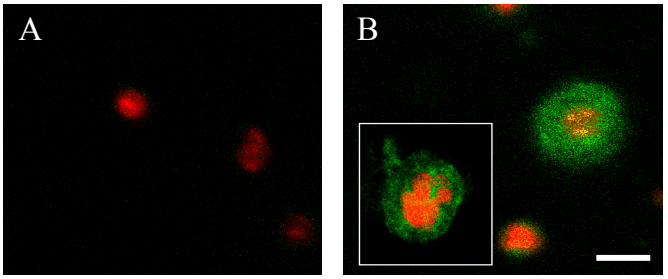

Figure 1. Type VI collagen immunofluorescence at day 0 for chondrocytes (A) and chondrons (B) seeded in agarose hydrogel. Inset: Chondron in intact articular cartilage. Scale bar = 10 μm.

RESULTS

Type VI collagen immunofluorescence showed that on day 0, only chondrons displayed a distinct pericellular matrix. The development of material properties in chondrocyte- and chondron-seeded hydrogel

was examined over six weeks. The material properties at day 0 were similar for chondrocyte and chondron cultures irrespective of seeding density. For the lower density cultures, deformational loading was observed to significantly increase E_Y and |G| for chondrocytes ($p<0.05$) while having no apparent effect on chondron cultures, Figures 2, 3. In contrast, for the higher density cultures, deformational loading significantly enhanced E_Y and |G| for both, which was evident earlier in culture and was more pronounced for the chondrocytes, Figures 2, 3. The relative benefit of deformational loading is more clearly seen by normalizing the E_Y and |G| of the dynamically loaded (DL) groups by their respective free-swelling (FS) groups, Tables 1, 2.

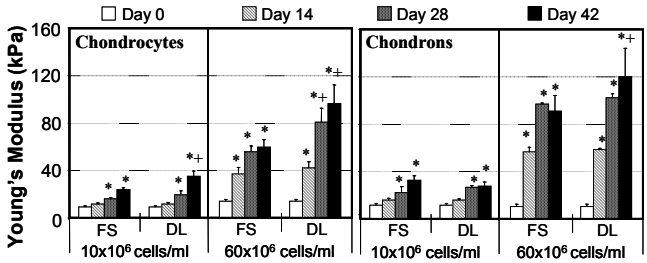

Figure 2. Equilibrium Young's modulus for agarose constructs seeded with chondrocytes and chondrons. * = compared to day 0 ($p<0.05$); + = compared to free-swelling controls at same time point ($p<0.05$).

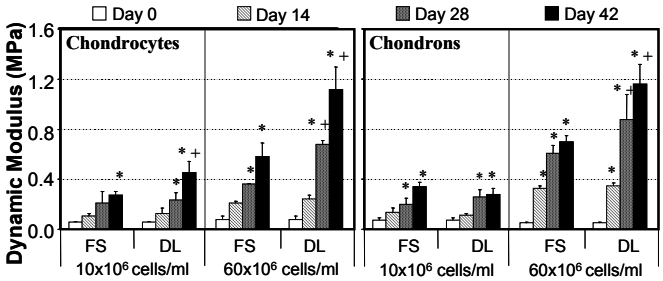

Figure 3. Dynamic modulus for agarose hydrogels seeded with chondrocyte and chondrons. * = compared to day 0 ($p<0.05$); + = compared to free-swelling controls at same time point ($p<0.05$).

DISCUSSION

Chondrons have been encapsulated and studied in agarose hydrogel cultures [5, 8, 9, 13]. To our knowledge, no studies have examined the influence of seeding density and the effects of deformational loading on matrix elaboration in chondron-seeded agarose constructs. In this study, free-swelling cultures of chondrocytes and chondrons appeared to develop at similar rates for the lower seeding density, whereas chondrons at 60 million/ml appeared to grow faster than chondrocytes. This finding is similar to that of Larson et al. (2002) who observed that pellet cultures of chondrons grew more quickly than chondrocyte pellet cultures [4]. In general, deformational loading was more beneficial to the development of chondrocyte-seeded agarose constructs than for chondrons. The mechanisms underlying this difference remain to be elucidated. Interestingly, the pericellular matrix elaborated by isolated chondrocytes acquires enough stiffness in one week to shield the cells from matrix-induced deformation, much like culture native chondrons [12, 13]. This suggests that cell deformation may not be the source of the disparate response between chondrocytes and chondrons to deformational loading. The presence of cell-matrix interactions contributed by the native pericellular matrix may also buffer the cells from the effects of dynamic loading. Although isolated chondrocytes seeded in agarose produce a matrix rich in type II and type VI collagen, this matrix may lack the structural organization of the native pericellular matrix [12, 13]. Therefore, the environment to which the cells are exposed may be different from the native mechanical environment. Our current efforts are focusing on examining the relative concentration and distribution of the various matrix macromolecules in our tissue engineered constructs**,** since these differences may significantly affect the response of the chondrocytes in culture.

Table 1. Equilibrium Young's modulus normalized to free-swelling controls.

	Chondrocytes		Chondrons	
	$10x10^6$ cells/ml	$60x10^6$ cells/ml	$10x10^6$ cells/ml	$60x10^6$ cells/ml
Day 0	1.00	1.00	1.00	1.00
Day 14	1.03	1.14	1.00	1.03
Day 28	1.23	1.44	1.19	1.06
Day 42	1.49	1.61	0.86	1.31

Table 2. Dynamic modulus normalized to free-swelling controls.

	Chondrocytes		Chondrons	
	$10x10^6$ cells/ml	$60x10^6$ cells/ml	$10x10^6$ cells/ml	$60x10^6$ cells/ml
Day 0	1.00	1.00	1.00	1.00
Day 14	1.18	1.16	0.84	1.05
Day 28	1.12	1.89	1.29	1.45
Day 42	1.65	1.92	0.82	1.67

REFERENCES

[1] Butler DL et al (2001) *J Biomech;* 122:570-5;
[2] Mauck RL et al (2000*) J Biomech;* 122:252-260;
[3] Poole CA et al (1988)*, J Cell Sci* 90:635-643;
[4] Larson CM et al (2002) *Matrix Biology* 21:349–359;
[5] Chang J et al. (1997) *Histochem J* 29:515-28;
[6] van Osch et al (1998) *Matrix Biol* 17:413-24;
[7] van Osch et al (1998) *OA Cart* 6:187-195;
[8] Hing et al (2002) *OA Cart* 10:297-307;
[9] Kelly TN et al (2003) *Trans Orthop Soc*, in press;
[10] Lee GM et al (1997) *OA Cart* 5:261-74;
[11] Soltz MA et al (1998) *J Biomech* 31:927-34;
[12] Lee DA & Bader DL (1997) *JOR* 15:181-188;
[13] Knight et al (2001) *Biochim Biophys Acta* 1526:141-146.

ACKNOWLEDGMENT

This work was supported by NIH RO1 AR46568 (CTH) and an associated graduate research supplement (TNK).