TEMPORAL AND SPATIAL DEVELOPMENT OF CONSTRUCT STIFFNESS IN CHONDROCYTE-SEEDED AGAROSE DISKS CULTURED IN FREE-SWELLING AND DYNAMICALLY LOADED CONFIGURATIONS

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INTRODUCTION

Successful tissue engineering of functional articular cartilage constructs requires optimization of the physical environment of chondrocytes in a three-dimensional scaffold [1, 2, 3]. An accurate description of such an environment, however, can be hindered by the unknown temporal and spatial variations of the construct properties developed in culture. Recent studies of chondroctye-seeded PGA scaffolds have demonstrated that matrix deposition begins at the outer edge of the construct and proceeds inward with time in culture [4]. Another study showed that the development of material properties in chondrocyte-seeded agarose disks in free-swelling culture occurs inhomogeneously[5]. While the application of dynamic deformational loading has been demonstrated to elevate the bulk mechanical properties of cell-seeded agarose disks [2, 3], little is known about the spatial development of the material properties within such disks, much less the spatial variation of the physical environment of chondrocytes embedded within. In this study, a technique, which combines video microscopy and optimized digital image correlation [6], was applied to asses the spatial development of material properties in chondrocyteseeded agarose disks cultured in free-swelling and dynamic-loading configurations. The temporal changes in displacement fields resulting from unconfined compression were characterized and spatial variations of construct stiffness determined in the center region of the disks under both culture configurations.

MATERIAL AND METHODS

Sample Preparation: Chondrocyte-seeded agarose hydrogels were prepared as previously described [2]. Briefly, primary chondrocytes were harvested from the carpometacarpal joints of 2-3 month old calves via enzymatic digestion. Cells were encapsulated in 2% agarose (Type VII, Sigma) in PBS at 30×10^6 cells/ml. Disks, $\emptyset 4.76 \times 2.25$ mm, were cored and cultured in 100 mm Petri dishes (20 to 25 disks per plate) with 30 ml of DMEM supplemented with buffers, antibiotics, antimycotics, amino acids, 20% FBS and 50 µg/ml ascorbic acid. Media were changed daily. Dynamic loading (DL) was carried out in a custom deformational loading bioreactor in a volume

of 5 ml DMEM with a loading regime of $\sim 10\%$ strain, at 1 Hz, 3 hours per day, for 5 days per week. Free swelling (FS) controls were maintained in the same amount of media adjacent to the loading device during the loading. Every two weeks, 4 disks were removed for mechanical testing.



Mechanical Testing: Prior to testing, each disk was cut in halves using a custom cutting device. One of the two semi-cylindrical specimens was mechanically compressed in a custom unconfined compression microscopy device mounted on the motorized stage of an IX-70 Olympus inverted microscope (Figure 1a, b). The initial thickness (h_0) of the specimen was measured optically using a calibrated 4× objective (1.66 µm/pixel). For each test, an initial tare strain (5% of h_0) was applied and multiple images of the cross-section acquired by controlling the motorized stage after stress-relaxation reached equilibrium. The specimen was then compressed (5% of h_0) and images and applied stresses were recorded at equilibrium. Multiple images of the cross section were stitched using Panavue ImageAssembler. An optimized digital image correlation technique was applied to the reference and deformed image pairs (Figure 1c, d), producing the displacement fields over the entire cross section. In this study, data for center regions (1/5) of the disks were analyzed and These center regions were further divided into 5 presented.

consecutive layers along the axial direction, and local strain for each layer determined from the displacement fields (Figure 1c). The Young's moduli for the top, middle and bottom layers were determined from these strains and the measured stress at equilibrium. Statistical analyses were performed using two-way ANOVA and Tukey HSD Post Hoc test with α =0.05.

RESULTS

The axial displacement field showed a linear distribution at day 0, but became progressively nonlinear over time in culture (Figure 2). At day 14, the FS samples exhibited softer top and bottom layers, as evidenced by the larger strains (Table 1) or the greater slope of the displacement distributions (Figure 2). By day 28, the softer regions in the top and bottom layers of the FS samples were limited to the extreme outer surfaces, and overall stiffness in both layers was greater than the middle layer. The DL samples, on the other hand, showed consistently stiffer top and bottom layers than the middle layer at both day 14 and day 28. By day 28, the difference in stiffness between the middle layer and the top/bottom layer became significant (p<0.05, Table 1 and Figure 3). Figure 3 summarizes the Young's moduli for the top, middle and bottom 1/5 layers of the center regions for both FS and DL samples. Over a period of 28 days, dynamic deformational loading significantly increased construct mechanical properties. The Young's moduli of the top, middle and bottom layers reached ~68, 58 and 72 kPa for the DL samples compared to ~45, 40 and 43 kPa for FS controls (p<0.01).

DISCUSSION

The free swelling control disks showed softer regions close to the top and bottom surface even by day 28. On the other hand, progressively stiffer regions developed next to such soft regions. These findings may reflect the relative balance between the release of matrix constituents into the media through the surfaces and the accumulation of these constituents to form a densely woven matrix. In this context, dynamic deformational loading appears to enhance matrix accumulation, while lessening the release of constituents at the top and bottom surfaces. It is noted that only data for the center regions of the disks were presented in the current study. It can be further complemented with distribution of displacements, strains and Young's moduli in the outer ring of the disks. A complete description of such spatially-varying deformational behavior of the constructs may aid in the understanding of construct development, which in turn may help to optimize the physical environment of the chondrocytes and improve the growth of the tissue engineered constructs. Further insights can be gained by performing biochemical and histological assays that will permit the correlation between the development of spatial variation in material properties and the spatial distribution of matrix constituents.

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Figure 2. Average displacement distributions in the center regions of the disks resulting from 5% compression. The lines in the plot represent B-spline fitting of the data points.

	Day 0	Day 14 FS	Day 14 DL	Day 28 FS	Day 28 DL
l	-0.0519	-0.0518	-0.0501	-0.0488	-0.0487
(Top)	± 0.0033	± 0.0042	± 0.0079	± 0.0083	± 0.0077
П	-0.0516	-0.0507	-0.0515	-0.0515	-0.0515
	± 0.0040	± 0.0036	± 0.0059	± 0.0064	± 0.0086
lll	-0.0510	-0.0514	-0.0511	-0.0539	-0.0572 (*)
(Middle)	± 0.0043	± 0.0057	± 0.0068	± 0.0052	± 0.0091
IV	-0.0518	-0.0515	-0.0520	-0.0521	-0.0506
	± 0.0036	± 0.0086	± 0.0066	± 0.0055	± 0.0075
V	-0.0515	-0.0516	-0.0495	-0.0491	-0.0478
(Bottom)	± 0.0032	± 0.0067	± 0.0052	± 0.0067	± 0.0069

Table 1. Average strains in the 5 consecutive layers. (* p<0.05 compared to layer I and V in the same group)



Figure 3. Average Young's moduli of the top, middle and bottom 1/5 layers. (* p<0.01 compared to all other groups; ** p < 0.025 compared to day 0, day 14 FS and DL groups; + p<0.05 compared to the top and bottom layers of the same group.)

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