INTRODUCTION: Mechanical stimuli are known to regulate the morphology and differentiated function of connective tissue cells. In particular, hydrostatic pressure has been reported to alter cytoskeletal organization in osteoblast-like cells (1) and chondrocytes (2), and to modulate metabolic activity in both chondrocytes (3-5) and intervertebral disc cells (6). In this study, we characterize the effects of intermittent hydrostatic pressure on the expression of extracellular matrix (ECM) components by human dermal fibroblasts seeded in three-dimensional polymer scaffolds.

METHODS: Cell Culture. Human dermal fibroblasts isolated from neonatal foreskin (Cascade Biologics, Portland, OR) were maintained in MEM with Earle’s BSS (Invitrogen, Carlsbad, CA) supplemented with 10% FBS (Sigma, St. Louis, MO) and antibiotics. Fibroblast cultures from passages 3-8 were used for this study. Thirty microliters of a cell suspension (2.0 x 10^5 cells/ml) were seeded in porous, poly(lactic acid) (PLLA) foams (Kensey-Nash, Exton, PA) (5.0 x 5.0 x 2.5 mm) and cultured for 24 h prior to the application of hydrostatic pressure. Intermittent Hydrostatic Pressurization. Dynamic hydrostatic pressurization was carried out in a custom bioreactor at 37°C (7). Cell-laden constructs were pressurized in sterile heat-sealed plastic bags with 10 ml of media. A pressure of 600 psi (∼4.3 MPa) was applied at a loading frequency of 0.333 Hz for five consecutive hours for five days. RNA Extraction and RT-PCR. Total cellular RNA was isolated by guanidinium thiocyanate-phenol-chloroform extraction using the Trizol reagent (Invitrogen). Reverse transcription and PCR amplification (32 cycles) were performed using the Superscript Preamplification System for First Strand CDNA Synthesis (Invitrogen). Primers for human types I and II collagen and aggrecan were as published (8) while those for β-actin and BMP-2 were designed using a computer-aided software package based on the mRNA sequences deposited in Genbank. The PCR products were resolved on a 1.0% agarose gel in 1X Tris-Acetate-EDTA buffer (Sigma) and visualized by ethidium bromide staining and UV transillumination.

RESULTS: Hematoxylin and eosin staining revealed that dermal fibroblasts adhered to and began to fill the porous structure of the PLLA foams. Cells seeded in constructs subjected to dynamic hydrostatic pressure displayed a rounded morphology in the center of the scaffold while those on the edges exhibited an elongated cell shape typical of fibroblasts (Fig. 1). Although the majority of cells in free swelling controls possessed a fibroblast-like morphology, rounded cells were present but in smaller numbers compared to experimental constructs. After 3 and 5 days of culture, RT-PCR analyses showed elevated mRNA expression levels for type I collagen, characteristic of fibrous tissues, and for type II collagen and aggrecan, typical markers of cartilaginous tissues, in constructs exposed to dynamic hydrostatic pressure (Fig. 2). Additionally, greater expression of BMP-2 was seen in cell-polymer composites with loading. Gene expression data were complimented by immunohistochemical staining which demonstrated increased deposition of type I collagen in pressurized constructs, primarily associated with spindle-shaped cells on the edges of the scaffolds. Further, type II collagen was also detected in constructs subjected to dynamic hydrostatic pressure, but was localized around spherical cells in the center of the scaffold (Fig. 3).

DISCUSSION: This study demonstrates that dynamic hydrostatic pressure enhances the expression of both fibrous and cartilaginous matrix components by dermal fibroblasts seeded in porous PLLA scaffolds. The appearance of cells exhibiting a spherical morphology and producing type II collagen is consistent with previous reports of mechanical load influencing the differentiated phenotype of connective tissue cells (9), particularly those located at the insertion site of ligaments and tendons (10,11). Principal compressive stresses and hydrostatic pressures have been found to be highest at the insertion site and correlate with the presence of rounded cells and fibrocartilage tissue (11). Similarly, the application of hydrostatic pressure in an in vivo system may induce dermal fibroblasts to trans-differentiate along a chondrogenic pathway. The expression of cartilage markers in free swelling controls may be due to the presence of lactic acid, the degradation product of the PLLA scaffolds, which has been shown to enhance cartilage matrix elaboration in dermal fibroblasts (12). In summary, these findings suggest that the mechanical environment may play an important role in governing cell differentiation and may be useful for engineering fibrocartilaginous tissue.

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