INTERVERTEBRAL DISC CELLS EXHIBIT DIFFERENCES IN GENE EXPRESSION IN ALGINATE AND MONOLAYER CULTURE

INTRODUCTION: The intervertebral disc (IVD) is synthesized and maintained by a heterogeneous population of cells derived from the mesenchyme and notochord (1). Phenotypic differences in proteoglycan or collagen biosynthesis and gene expression have been reported among cells from the anulus fibrosus (AF), transition zone (TZ), and nucleus pulposus (NP) in vitro (2-6). Previous studies have examined the metabolism of these cells in both monolayer (7,8) and three-dimensional alginate culture systems (3-6), with evidence of differences between the two systems. The response of IVD cells to biological stimuli has also been studied in these culture systems (9-11), although the influence of the culture system has not been well characterized. Previous studies have demonstrated that long-term maintenance of articular chondrocyte phenotype requires a three-dimensional culture system like alginate or agarose (12-14). When cultured in monolayer, chondrocytes “dedifferentiate”, characterized by decreases in aggrecan and type II collagen synthesis and increases in type I collagen synthesis. In this study, we quantified gene expression for aggrecan and collagen types I and II in cells of the AF, TZ, and NP cultured in monolayer and alginate systems. Studies were performed on both primary cells and those obtained after expansion in monolayer culture. Our results show substantial differences in the gene expression of AF and TZ cells, but not NP cells, when cultured in alginate or monolayer that may relate to specific differences in cell morphology and cytoskeletal organization.

METHODS: Cell Culture. Lumbar IVDs from 4 month pigs were separated into regions corresponding to AF, TZ, and NP. Cells were enzymatically isolated (6) and immediately divided into three experimental groups. Cells from the first group were embedded in 1.2% alginate beads (Sigma) at a density of 1 x 10^6 cells/ml and cultured for 2 weeks (Group alg_1). A second group of cells was seeded in monolayer at an initial density of 20000 cells/cm^2 and subcultured twice at a split ratio of 1:3 (Group mon). A third group of cells was cultured in monolayer as for the mon group and then embedded in 1.2% alginate beads and cultured for an additional 2 weeks (Group alg_2). The average time in monolayer for all cells was 5 weeks. All cells were cultured in Ham’s F-12 medium with 10% FBS in 5% CO_2. At the end of the experiment, cells were released and stored at -80°C. Gene Expression. Total RNA was extracted from the frozen cell pellets and cDNA was synthesized by reverse transcription. Gene expression levels for aggrecan core protein and types α1(I) and α2(I) collagen were measured with porcine-specific, intron-spanning primers in a competitive PCR technique described previously (6). This technique uses specially constructed competitor molecules that act as internal standards during PCR amplification, allowing quantitative measures of gene expression. Statistical Analysis. Data were normalized by act b_1 mRNA levels as a control to test for an effect of culture conditions on gene expression. Data were also normalized by mRNA levels for AF cells within each culture condition to test for differences between AF, TZ and NP cells. A one-factor ANOVA and Duncan’s multiple range post hoc tests were used to test for differences within each factor.

RESULTS: In cells of the AF and TZ, expression of aggrecan and collagen II was decreased in mon cells compared to alg_1 and alg_2, with significant differences for T2 alg_1 cells only (Fig., left). The effect of monolayer culture on collagen I was different in AF and TZ cells, with an apparent increase in mRNA in AF mon cells compared to alg_1, and no change in TZ cells under the same conditions. In contrast to the AF and TZ, gene expression in cells of the NP appeared to be insensitive to culture conditions. Differences were also observed among cells of the AF, TZ, and NP within the alginate culture system (Fig., right). In group alg_1, TZ cells expressed higher levels of mRNA for all genes compared to AF and NP cells, with significant differences for aggrecan and collagen II only. This pattern of gene expression was preserved after expansion in monolayer and reencapsulation in alginate (alg_2), except for collagen I expression in the TZ. Few differences were observed among AF, TZ, and NP cells in monolayer culture (mon).

DISCUSSION: The results of our study suggest that AF and TZ cells undergo a reversible shift in phenotype when cultured in monolayer, while NP cells undergo little change from monolayer to three-dimensional culture. In particular, cells of the AF and TZ decreased expression of aggrecan and collagen II mRNA when cultured in monolayer, in a pattern similar to that of articular chondrocytes (12-14). After reencapsulation in alginate, values for aggrecan and collagen II mRNA increased in these cells, partially reversing the decrease that occurred in monolayer. In chondrocytes, the dedifferentiation process is controlled by a process of cytoskeletal reorganization that occurs in monolayer culture (15). The fact that AF and TZ cells underwent phenotypic shifts in monolayer, but NP cells did not, may be explained by a similar phenomenon. Indeed, isolated AF and TZ cells have been found to have cortical actin distributions that are similar to chondrocytes, while NP cells have a substantially different actin distribution (16). Our results for collagen I expression after monolayer culture, including our observation that all three cell populations continued to express collagen I after reencapsulation in alginate, are substantially different from results for chondrocytes (12-14) and suggest that collagen I expression may be a distinguishing characteristic of IVD cells under certain culture conditions. Finally, differences in gene expression were observed among AF, TZ, and NP cells in alginate that are consistent with phenotypic differences observed previously (2-6). When cultured in monolayer, these differences were not present, suggesting that phenotypic expression and response to biophysical stimuli may be dramatically influenced by the in vitro culture system used to study these cells.

Mean + SEM (n=3-7 per group)

Data are normalized to mRNA values for act b_1 to test for differences among culture conditions (left), and to mRNA values for AF to test for differences among cell type (right) *p<0.05 vs alg_1; **p<0.05 vs TZ.

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