INTRODUCTION:

There is growing evidence to support the hypothesis that intermittent hydrostatic compression can stimulate chondrogenic differentiation of primary bone marrow-derived mesenchymal stem cells [1]. The purpose of this study was to investigate the possibility that the murine C3H/10T1/2 embryonic fibroblast cell line is similarly sensitive to cyclic hydrostatic pressure. It has been previously shown that the C3H/10T1/2 cell line is capable of undergoing chondrogenic, osteogenic, myogenic, and adipogenic differentiation with chondrogenesis being favored when cells are grown as a high-density micromass and supplemented with recombinant human bone morphogenetic protein-2 (rhBMP-2) [2]. It has also been reported that chondrocyte actin microfilaments reorganize in response to hydrostatic compression [3]. Therefore, the effect of microfilament disruption (by cytochalasin D) on the response to pressure was also investigated.

METHODS:

C3H/10T1/2 clone 8 cells from ATCC (Monassas, VA) were grown in DMEM/HAM’s F-12 medium (1:1) supplemented with 10% FBS and 25 ng/ml rhBMP-2. Cells were not allowed to proliferate beyond approximately 70% confluence and were used between the second and third fifth passage. Cells released by trypsin were seeded into 3x4 well sections from a round-bottom 96-well plate at 7.5x10^6 cells per well. Four 12-well sections were placed in flexible plastic pouches, which were filled with medium and heat-sealed so as to exclude air.

Two pouches were subjected to cyclic hydrostatic compression in a custom water-filled chamber connected to a hydraulic cylinder mounted in a MTS servohydraulic testing machine. The pressure chamber consisted of domed, flanged stainless steel base and lid bolted together to compress a Teflon® gasket. The applied hydrostatic compression was sinusoidal: 0.5 to 5 MPa at 1 Hz for 1800 cycles (10 min on/10 min off) each day for 3 consecutive days. Control cultures were placed in a similar water-filled chamber that was not pressurized. Both chambers were immersed in the same 37°C regulated water bath during loading. This experiment was repeated with the duration of loading extended to 7200 cycles.

In a final experiment, cytochalasin D was added to the culture medium to a final concentration of 2 µM during each loading session (to 7200 cycles) and immediately removed by several medium changes thereafter (requiring rescaling of cultures in the plastic pouches prior to loading each day). Controls received identical treatment.

For all experiments, at the conclusion of the 3-day loading period the plates were removed from the pouches and cultured for an additional 5 days under static conditions, during which time the medium was supplemented each day with ascorbic acid to a final concentration of 50 ng/ml. At the end of this 8-day period, the cultures were analyzed for total DNA content by the Hoechst 33258 dye method (DNA Quantitation Kit, Sigma) and sulfated glycosaminoglycan content by the dimethyl methane blue assay (Blyscan™ Glycosaminoglycan Assay, Biocolor Ltd.). In addition, the rate of collagen synthesis was assessed by 24 h macromolecular incorporation of [3H]proline (10 µCi/ml) [4].

RESULTS:

Results are summarized in Figure 1. In all experiments, DNA content was unaffected by pressure. The effect of pressure on sGAG and [3H]proline incorporation were duration-dependent. Cultures exposed to 1800 cycles per day were not substantially different from non-pressurized controls with regard to both parameters. On the other hand, cultures subjected to 7200 cycles of pressure per day had approximately twice the average sGAG content and rate of [3H]proline incorporation as that of non-pressurized controls (p<0.0001 and p<0.02, respectively). The presence of cytochalasin D during pressurization substantially inhibited the increase in sGAG accumulation to the point that it was not significantly different from control (p>0.1) and even further inhibited [3H]proline incorporation to below that of non-pressurized controls (p<0.04).

DISCUSSION:

Exposure of C3H/10T1/2 cells to 1800 cycles of hydrostatic compression to 5 MPa was insufficient to enhance chondroinduction beyond that achieved by rhBMP-2. However, exposure to 7200 cycles significantly increased the accumulation of cartilaginous extracellular matrix over rhBMP-2 baseline controls. The same enhancement in chondrogenesis was not achieved in the presence of the actin disrupting drug cytochalasin D during the longer duration loading, suggesting that microfilaments play an important role in mechanotransduction of hydrostatic pressure. This study demonstrates the usefulness of the C3H/10T1/2 cell model for further investigations into the influence of mechanical factors on cell differentiation. Use of a cell line can be advantageous in terms of availability, population homogeneity, experimental reproducibility, and cost.

REFERENCES:


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