EFFECT OF DYNAMIC HYDROSTATIC PRESSURE ON INTERVERTEBRAL DISC CELLS: A RABBIT MODEL

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Introduction
Long-term occupational exposure to whole-body vibration increases the risk of disc degeneration and the consequent back pain [1]. The pathogenesis of vibration induced disorders is still not completely clear and there is no effective treatment. Although the potential effects of vibrational stress on extracellular matrix (ECM) assembly and degradation are particularly relevant to the clinical findings of the vibration induced disorders, the effects of vibrational loads on disk cells are largely unknown. It has been shown that hydrostatic pressure directly affects the synthesis of collagen and proteoglycan by the intervertebral disc cells [2-5]. However, these studies investigated only the extent of the effect of quasi-static hydrostatic loads. During daily occupational activities intervertebral disk is exposed to dynamic oscillatory hydrostatic loads, characterized by wide frequency spectrum and variable amplitude. In this case a physiological level of altitude and frequency of hydrostatic pressure may be essential for maintaining the matrix of the disc, while an abnormal amplitude and frequency of hydrostatic pressure may accelerate disc degeneration. The ranges of good and bad loading frequencies and amplitudes still need to be discovered. To address this issue we developed a mechanically-active culture system capable of delivering a wide range of loading frequency and amplitude of hydrostatic pressure to cultures of annulus fibrosis cells. The device was used to test the hypothesis that abnormal vibrational loads would induce extracellular matrix (ECM) degradation in such cultures.

Methods
Cells were isolated by sequential digestion of adult rabbit annulus fibrosis with testicular hyaluronidase (1600 u/ml, 60 minutes) followed by collagenase + Pronase E (0.5 mg/ml, 16 hours). First passage cells were seeded in 35 mm culture dishes (600,000 cells/dish) in medium (DMEM/10% serum) and incubated overnight before the first hydrostatic stress treatment. The first passage cells were divided in three groups (7 dishes in each group), Group I was the control group, Group II was exposed to a cyclic hydrostatic pressure of 0.3 MPa amplitude and 1 Hz frequency simulating a low level physiological loading condition, and Group III was exposed to a preload pressure of 0.8 MPa and a cyclic pressure of 1.7 MPa at 20 Hz frequency (maximum of 2.5 MPa) simulating an abnormal loading condition within an in vivo physiological level. Cells cultured in dishes were placed in a hydraulic chamber filled with cell media. The load was transferred to the cells through a fluid media by a piston. The piston-chamber assembly was sealed tight, bleeding any air out of the system (Fig. 1). In this case, the stiffness of the system was much higher than that of the traditional way of load transfer through the air in a pressure vessel. Therefore, we could have a wider range of loading amplitude and frequencies to high levels simulating different low and severe in vivo dynamic loading conditions. The chamber-cell assembly was placed and fixed in a servo-hydraulic MTS mechanical testing system (Fig. 1) and a harvesine compressive cyclic load was applied on the piston by the machine actuator. The cell cultures of different groups were loaded daily for 30 minutes. After 3 days three dishes of each groups were removed for analyses and the remainder four dishes were kept loaded up to 9 days. In this case the effect of loading duration was observed. Cultures were incubated for 16 hours in medium containing H-proline (20 uM) and ascorbate (25 ug/ml) following treatment on days 2 and 8. This labeling medium was removed 3 hours before the final treatment on day 3 or 9. After treatment the cultures were incubated for an additional 36 hours in fresh medium. Medium and cell extracts were analyzed by liquid scintillation counting and DNA was measured in the cell extract by fluorometric assay.

Results
The data in Figure 2 show the total amount of H-proline incorporated at 3 and 9 days (CPM/ug DNA). The histogram shows that incorporation in Group III was~1.4-fold greater than in groups I and II on both days. Statistical analysis (ANOVA and Tukey test) revealed that the differences between group III and groups I or II were significant on both days (p<.05) but that the differences between Groups I and II was not significant. Figure 2 shows the percentage of counts in the medium to the total incorporated (% Released) on days 3 and 9. Although the histogram indicates that this percentage increased slightly from group I to groups II or III, these differences were not significant.

References

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