Fluid Shear Stress Affects Differentiation of Growth Plate Chondrocytes

Denison TA, Doroudi M, Schwartz Z, Boyan BD.
Georgia Institute of Technology.

Statement of Purpose: The right combination of scaffold material and growth factors will likely be essential to the development of quality tissue engineered products. However, some cell types also benefit from an appropriate mechanical stimulus to influence their response to their environment. The dynamic environment of cartilage has made it the attention of studies to optimize mechanical conditions for regenerating tissue engineered cartilage. This study intends to determine what parameters of fluid shear stress can affect the health and developmental activity of chondrocytes in the resting zone of the mammalian growth plate. The growth plate is a naturally developing osteochondral segment, and understanding appropriate loading for cells in specific stages of this endochondral region could improve tissue engineering approaches for this complex interface.

Methods: Rat resting zone chondrocytes from the costochondral growth plate and the murine chondrogenic cell line ATDC5 were used in this study. Cells were cultured in Petri dishes in monolayer and then exposed to shear stress (2, 3.5, 5, or 6.5 dynes/cm^2) in a cone-and-plate shearing apparatus or received no shear as a static control. Cells were sheared for 24 hours and then allowed to recover from shear for up to 24 hours before harvesting the cells for alkaline phosphatase activity, cell number, [^{3}H]-thymidine incorporation, [^{35}S]-sulfate incorporation, or RT-PCR mRNA expression of chondrogenic markers.

Results:

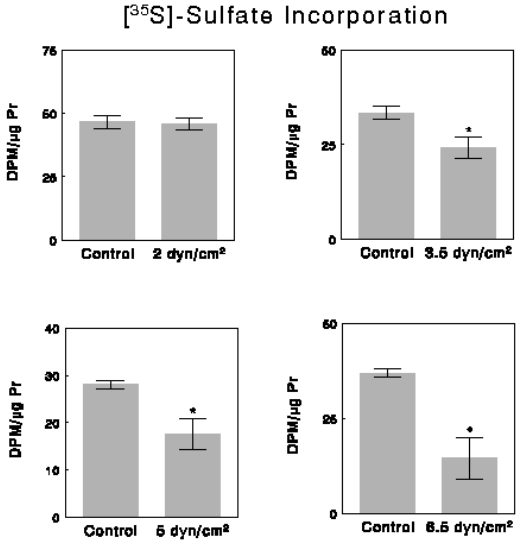

Figure 1 – Sulfate incorporation of resting zone chondrocytes at 24 hours after shear from 2, 3.5, 5, and 6.5 dyn/cm^2. *P<0.05 compared to control.

Resting zone chondrocytes exhibited a reduction in [^{35}S]-sulfate incorporation (Figure 1), alkaline phosphatase (Figure 2), and cell number 24 hours after shear and the effect was dose-dependent on amount of stress.

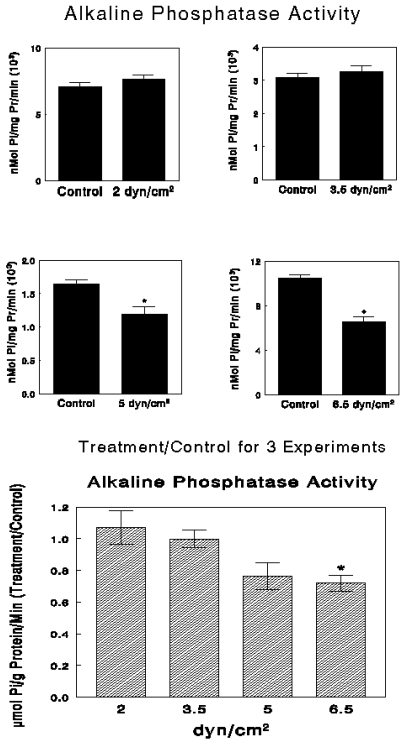

Figure 2 – Alkaline phosphatase activity of resting zone chondrocytes at 24 hours after shear. Also shown is the average treatment/control ratios for 3 experiments at each shear stress. *P<0.05 compared to control or compared to 2 dynes/cm^2 for treatment/control data.

ATDC5 cells responded to shear in a similar manner (data not shown). Resting zone chondrocytes still expressed mRNA for chondrogenic markers by the end of 24 hours of shear. Collagen type II mRNA was expressed consistently from the end of shear up to 12 hours after shear. Aggrecan and cartilage oligomeric matrix protein (COMP) mRNA appear to be slightly increased by 12 hours after shear.

Conclusions: Exposure of rat resting zone cells and ATDC5 cells to shear for 24 hours reduced [^{35}S]-sulfate incorporation and alkaline phosphatase activity. In contrast, levels of type II mRNA remained constant and COMP and aggrecan mRNA were elevated. This indicates that while the fluid shear does cause inhibitory effects on some functions of the cells, other persist throughout shear stress and actually appear to recover to a stronger signal. Further work is needed to determine the appropriate time of recovery and amount of shear that can optimize desirable responses in guiding these cells for controlled differentiation and matrix production.

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