# STEADY SHEAR STRESS STIMULATES BOVINE CHONDROCYTE PROLIFERATION IN MONOLAYER CULTURES

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**Introduction**

Cartilage tissue engineering strategies frequently involve seeding isolated chondrocytes on natural or synthetic scaffolds and then growing these constructs in bioreactors [8]. Construct growth and proliferation of the chondrocytes within it presumably depends on the fluid-induced shear stresses within the bioreactor [8]. While work is underway to characterize the fluid mechanics environment around a construct growing in a bioreactor [5], few studies have attempted to understand how fluid shear stresses may affect chondrocyte proliferation.

In a previous study to characterize matrix protein production by a monolayer of primary human chondrocytes exposed to long term steady shear stresses [4], we also incidentally observed that the monolayer was significantly overgrown. This observation led us to test the hypothesis that *exposure to steady shear stress stimulates primary chondrocyte proliferation.*

**Methods**

Calf knees (4-6 weeks old) were obtained from Lampire Biological Laboratories (Ottsville, PA 18942). Chondrocytes were isolated from the femoral condyle articulating surfaces. Briefly, cartilage tissue was carefully scraped off the articulating surface, cut into small pieces and digested with collagenase (15mg/gm tissue) for 24 hours at 37°C, 5% $CO_2$. The resulting cell suspension was filtered (150μm Nitex mesh) and spun down. Supernatant was discarded and the cells were washed (3x) with PBS (0.138 M NaCl, 0.0027 M KCl, without $Mg^{++}$), and then finally suspended in DMEM with additives (20% FBS, 0.1 mM non-essential amino acids, 0.5 mM proline, 1 mM Na-pyruvate, 4 mM L-glutamine, and 1% penn/strep).

The chondrocytes were then cultured on tissue culture plastic slides at a density of 40,000 cells/$cm^2$ (595000 ± 6455 cells/slide) at 37°C, 5% $CO_2$. Media was changed every other day until the slides were 80%-85% confluent (5-6 days), at which point the cells were serum-deprived (0.2% FBS in media) for 24 hours to stop proliferative activity. The monolayer was then exposed to a steady shear stress of 35 dynes/$cm^2$ (3.5 Pa), using serum-supplemented media (10% FBS), for 4 days. Control slides were grown in static culture with corresponding media changes. At the start of the experiment, slides showing the same level of confluency were paired together.

Cells from eight (n=8) flow and control slide pairs were harvested by trypsinization and cell count was determined using the CyQUANT cell proliferation assay kit (Molecular Probes, Inc., Eugene, OR 97402). Briefly, harvested cells were washed with PBS, spun down to a pellet, and frozen overnight at −70°C after discarding the supernatant. The pellet was thawed to room temperature and lysed to expose the nucleic acids to a fluorescein-conjugated dye. Fluorescence was measured using a standard 96-well plate fluorometer with 485 nm excitation and 535 nm emission filters. A standard curve, using additional chondrocytes, was established to equate fluorescence readings with cell number. Cell number data was analyzed using a two-tailed, paired comparison t-test ($\alpha$=0.05).

**Results**

Fluid induced shear stress significantly increased primary bovine chondrocyte proliferation (p=0.001), as shown in Figure 1. Average cell count from control slides was 2.51 ± 0.68 x$10^6$ versus 3.62 ± 0.56 x$10^6$ for the slides exposed to flow, corresponding to populations doublings of 4.2 and 6.1, respectively, from initial seeding. In all cases the number of cells on the slides exposed to steady fluid shear was more than the number of cells on the control slide.

Morphological observations showed that after 4 days of exposure to fluid shear stress cell monolayers appeared massively overgrown. The statically cultured monolayers were overconfluent only in sporadic patches. Further, cells on the slides exposed to flow did not show a preferred orientation with respect to the direction of flow.

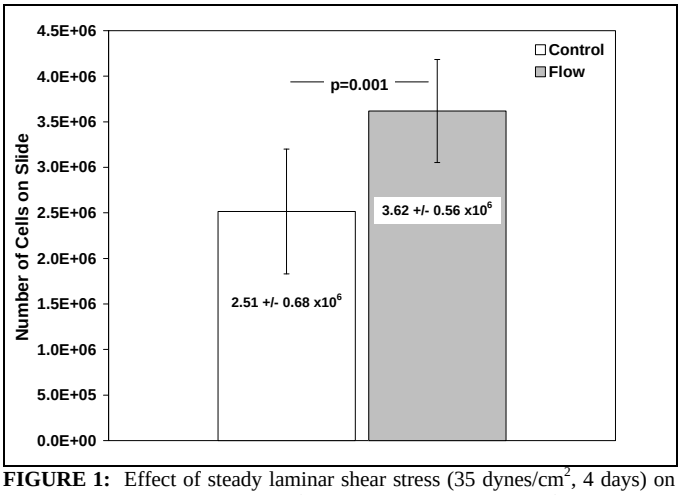


**FIGURE 1:** Effect of steady laminar shear stress (35 dynes/$cm^2$, 4 days) on primary bovine chondrocyte proliferation. A statistically significant increase in cell number (p=0.001) was observed due to shear stress. Cell numbers are expressed as mean ± standard deviation.

**Discussion**

This study has shown that steady fluid-induced shear stress promotes primary bovine chondrocyte proliferation. We believe this is a significant and interesting finding because steady shear stress has been shown to inhibit proliferation in other cell types [3,6,7]. For example, shear stress inhibits proliferation of smooth muscle cells [6,7] and endothelial cells [3] believed to be modulated by TGF-β1 in an autocrine manner [6]. However, TGF-β1 is known to have stimulatory effects on chondrocyte proliferation [2]. At the present time, it is not known if stimulation of chondrocyte proliferation due to shear stress is also due to TGF-β1 upregulation. This question is the subject of our scrutiny in ongoing studies.

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