FLUID-INDUCED SHEAR STRESS MODULATES CX-43 EXPRESSION IN AVIAN TENDON CELLS BUT DOES NOT INDUCE A Ca2+ SIGNAL

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Introduction: Tendons are subjected to mechanical forces including tension, compression and shear, as they transfer muscle force to bone. Tendon cells communicate mechanical signals via connexin-43 (cx-43) gap junctions. The signal is transduced to neighboring cells by chemical mediators, such as Ca^{2+} and IP_3, that pass through the gap junctions and activate signaling pathways in the joining cell. Furthermore, both avian and human tendon cells increase cx-43 expression in response to cyclic stretching. Human tendon cells also respond to fluid-induced shear stress by increasing intracellular calcium concentration ($[Ca^{2+}]_{ic}$). We hypothesized that avian tendon surface cells (ATSC) would respond to fluid-induced shear stress by increasing intracellular calcium and by upregulating connexin-43 expression.

Methods: Cells from flexor digitorum profundus tendons, zone III, of 52-day-old White Leghorn chickens were isolated by sequential enzymatic, mechanical scraping and selective adherence techniques. The surface epitenon cells were plated at 25k cells/cm^2 in DMEM-H with 10% fetal bovine serum (FBS), and 1% penicillin/ streptomycin. To evaluate the $[Ca^{2+}]_{ic}$ response, cells were spot cultured at 2k cells/10 µL on 25 x 75 x 1.5 mm collagen coated, glass Culture Slips™ and grown to confluence and quiescence by halving the medium on days 3 and 5 and using the cells on day 6. The spots were placed across the middle of the slide to prevent the shear stress response confounded by the release of mediators from upstream cells. On the sixth day after culture, the cells were rinsed with Earles' Balanced Salt Solution with HEPES, pH 7.2, Ca^{2+} and Mg^{2+}, incubated at RT in 5 µM FURA-2AM for 90 min, then rinsed with EBSS. The Culture Slips™ were transferred to a FlexFlo™ laminar flow chamber interfaced with a MasterFlex peristaltic pump. The device was mounted on the stage of an Olympus upright fluorescence microscope to permit assessment of intracellular calcium levels using a ratio dye method. Baseline Ca^{2+} was quantified at no flow conditions then shear stress was applied for 2 minutes at 1, 3, 5, 10, 15, and 20 dyne/cm^2. Image I MetaFluor software was used to monitor $[Ca^{2+}]_{ic}$ in 25 to 30 cells per field. The perfusate used was either EBSS, DMEM-H with 2.5% FBS or DMEM-H with 5% FBS. To evaluate the changes in gene expression of cx-43, cells were plated at 25k cells/cm^2 on glass slides and grown to quiescence. On the fifth day after culture, the cells were placed in serum free medium. On day six, the cells were transferred to a novel, 6 bay, laminar flow chamber (Streamer™, Flexcell Corp, McKeesport, PA) and subjected to fluid-induced shear stress with serum-free DMEM-H at 1 dyne/cm^2 for 0, 1, 2, 4 or 6 hours at 37 °C then incubated for a balance of 18 h before collection of total RNA. RT-PCR was used to analyze cx-43 mRNA levels relative to β-actin expression.

Results: ATSCs did not respond to fluid-induced shear stress by increasing $[Ca^{2+}]_{ic}$ (Fig. 1). Moreover, the addition of serum (2.5 & 5%) in the perfusate did not invoke a significant increase in $[Ca^{2+}]_{ic}$ above baseline in response to shear stress (Fig. 1). However, cx-43 expression was upregulated after 1 and 2 h of shear stress but was reduced to control levels after 6 h of shear (Fig. 2). The pixel intensities of the RT-PCR bands for cx-43 relative to β-actin were 70% and 40% greater than control at 1 and 2 h, respectively (Fig 3).

Conclusions: These data indicate that cx-43 is a shear stress-responsive gene that can be upregulated at a physiologic shear stress with message that remains elevated at 18 h post-flow. However, cx-43 mRNA may be down-regulated or mRNA degradation may be enhanced by periods of longer duration shear, since cultures exposed to 4 and 6 h of fluid flow expressed less message than 1 or 2 h sheared samples. This down-regulation or degradation of mRNA levels may indicate that negative modulators that respond to excessive mechanic stimulation are invoked. The data also indicate that an increase in intracellular Ca^{2+} is not involved in the response to fluid-induced shear stress in avian tendon. Thus, another chemical mediator, such as IP_3, ATP or NO, may be involved in the signal transduction mechanism. In conclusion, these are the first data that indicate that cx-43 expression is quickly modulated by shear stress.

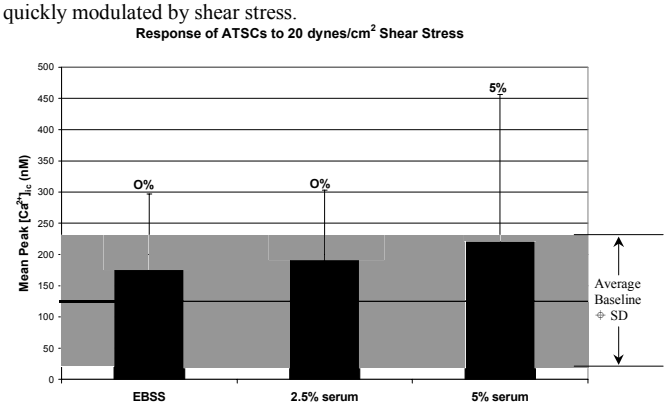

Figure 1. ATSCs do not significantly respond to fluid shear stress by increasing $[Ca^{2+}]_{ic}$. The average baseline ± SD for ATSCs prior to flow is indicated by the dark black line and the gray boxes. The number of % responding cells is indicated above each bar.

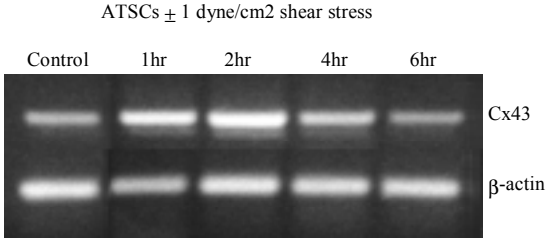

Figure 2. The RT-PCR bands for connexin 43 and β-actin of ATSCs.

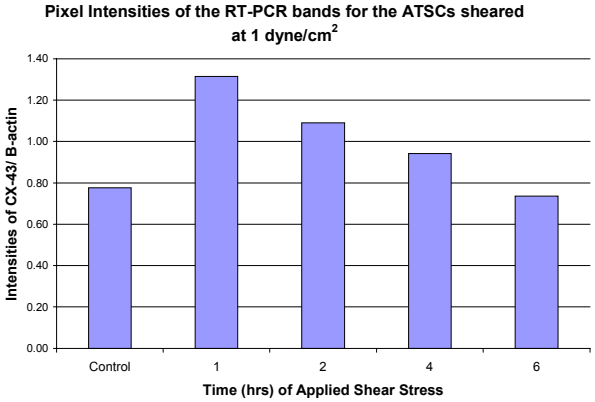

Figure 3. The pixel intensities of the RT-PCR bands for cx-43 relative to β-actin.

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