# FLUID-INDUCED SHEAR STRESS INCREASES [Ca2+]ic IN CULTURED HUMAN TENDON EPITENON CELLS

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INTRODUCTION Tendon, bone and other orthopaedically relevant tissues respond in a positive manner to mechanical stimulation. Motion therapy following surgery is well known to promote increased range of motion, decreased adhesion formation, and decreased time in convalescence. Tendon epitenon cells experience a variety of mechanical forces *in vivo*. Tendon epitenon cells are subjected to cyclic uniaxial longitudinal tension, compression, and fluid-induced shear stress as the tendon slides in its fluid filled sheath. Human tendon epitenon cells are among the many cell types that respond to mechanical stimulation by transiently increasing intracellular free calcium concentration ($[Ca^{2+}]_{ic}$). Tendon cells poked with a micropipette or subjected to equibiaxial cyclic strain have been shown to propagate intercellular calcium waves. Moreover, it is often difficult to isolate the mode of mechanical stimulation to monolayer tendon cell cultures as cyclic tensile strain can be confounded by a component of fluid-induced shear stress. We have employed a parallel plate, laminar flow apparatus that allows for the regulation of shear stress applied to human tendon epitenon monolayer cultures. We hypothesize that human tendon epitenon cells will respond to fluid-induced shear stress with increased signaling through the calcium second messenger pathway.

METHODS Human flexor digitorum profundus tendon cells (epitenon human tendon surface cells) were cultured from discarded tissue taken at surgery, washed free of blood, minced to 0.5 $cm^3$ pieces and treated with 0.5 % collagenase for 30 minutes at 37C. Cells were washed in medium and plated at 25k cells/$cm^2$ in Medium 199 + 10% fetal calf serum (FCS), 0.5 % ascorbate, 20 mM HEPES pH 7.2, 15 mM glutamine, insulin, transferrin and selenium and antibiotics. Cells were subcultured and used between passes 3 and 10. Cells were subcultured onto 75 x 24 x 0.18 mm collagen peptide bonded glass cover slips at 25k cells per $cm^2$ and grown to quiescence by halving the medium with Medium 199 without FCS on days 3 and 5 and using the cells on day 7-9. For studies of calcium concentration in cells, cells were washed in EBSS then loaded with 5 uM FURA-2AM in 0.1% Pleuronic-127 in EBSS for 2 h at RT. Cells were washed with EBSS and the coverslip transferred to the FlexFlo$^{TM}$ chamber (Flexcell Intl. Corp.). A Masterflex peristaltic pump and dampening system were used to deliver flow rates calculated to provide 0, 5, 10, 15 and 20 dynes/$cm^2$ at the monolayer surface. These flow rates were verified using a digital flow meter.

Flow experiments were conducted in EBSS with and without calcium. Single cell intracellular free calcium concentrations were quantitated using Fura-2 ratio, fluorometric imaging techniques and a standardized calcium calibration curve. The average single-cell intracellular calcium concentration for all cells in a field was determined prior to and five minutes following the initiation of flow, which allowed us to determine the shift in baseline $[Ca^{2+}]_{ic}$. Calcium transients were defined as that $[Ca^{2+}]_{ic}$ that increased by 100nM from baseline during the course of an experiment for each flow condition.

RESULTS Human tendon cells at rest had a single cell basal $[Ca^{2+}]_{ic}$ of 40 to 120 nM. Cells subjected to 5 dynes/$cm^2$ had an average preflow baseline of 66nM $[Ca^{2+}]_{ic}$ which increased to a transient level of over 200 nM within 25 to 60 seconds after initiating flow. Following the initial calcium transient, the average $[Ca^{2+}]_{ic}$ plateaued at 118 nM and the percentage cells responding was 83% (figure 1). Cells that were subjected to 10 dynes/$cm^2$ had an average preflow baseline of 62nM $[Ca^{2+}]_{ic}$ which increased to a transient level of over 200 nM within 25 to 60 seconds after initiating flow. Following the initial calcium transient, the average $[Ca^{2+}]_{ic}$ plateaued at 97 nM and the percentage of cells responding was 70% (figure 1). Cells subjected to 15 dynes/$cm^2$ had an average preflow baseline of 68nM $[Ca^{2+}]_{ic}$ which increased to a transient level of over 200 nM within 20 to 30 seconds after initiating flow. Following the initial calcium transient, the average $[Ca^{2+}]_{ic}$ plateaued at 122 nM and the percentage of cells responding was 81% (figure 1). Cells subjected to 20 dynes/$cm^2$ had an average preflow baseline of 48nM $[Ca^{2+}]_{ic}$ which increased to a transient level of over 200 nM within 30 to 60 seconds after initiating flow. Following the initial calcium transient, the average $[Ca^{2+}]_{ic}$ plateaued at 82 nM and the percentage of cells responding was 83% (figure 1).

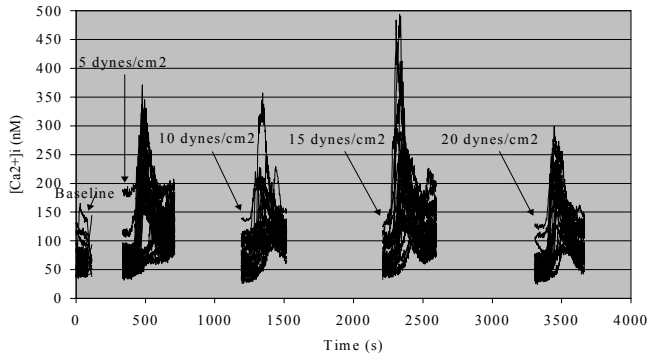


Figure 1: $[Ca^{2+}]_{ic}$ in response to discrete fluid-induced shear stress conditions

In a second set of experiments, cells were subjected to 0, 5, 10, 15, and 20 dynes/$cm^2$ in $Ca^{2+}$-free EBSS. Cells did not increase $[Ca^{2+}]_{ic}$ in response to flow-induced shear stress in $Ca^{2+}$-free EBSS. In a third set of experiments, epitenon cells were subjected to 0, 5, 10, 15 and 20 dynes/$cm^2$ with continuous step increases in flow and a 5-minute hold at each flow rate. Cells initially signaled approximately 30 seconds after flow began, but became refractory to further increases in $[Ca^{2+}]_{ic}$ with increasing flow-induced shear stress when no rest period was provided between flow conditions (figure 2).

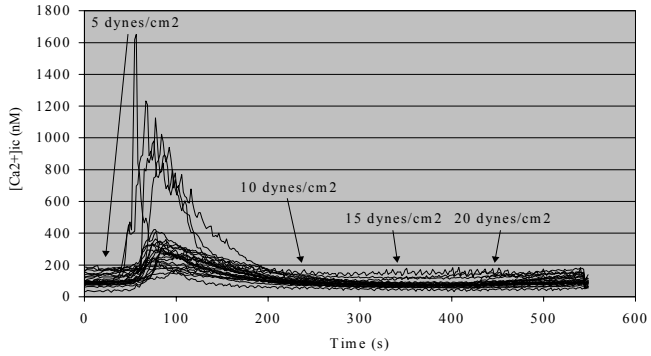


Figure 2: $[Ca^{2+}]_{ic}$ in response to continuous fluid-induced shear stress increase

DISCUSSION Cells in whole tendon experience shear stress as a result of motion in a fluid-filled sheath. Human tendon responds to fluid-induced shear stress *in vitro* by signaling with an increase in $[Ca^{2+}]_{ic}$. Our results demonstrate that, under the shear stress conditions investigated, approximately the same percentage of cells responded with an increase in the $[Ca^{2+}]_{ic}$. The magnitude of transients displayed in response to the increasing levels of shear were also similar, suggesting that lower levels of shear may be more physiologically relevant. Moreover, physiological shear stress patterns might be better represented with pulsatile and oscillatory shear stress waveforms. These results demonstrate that tendon cells are refractory to changes in shear rate following the initial stimulus if no rest period is provided. The shear stress-induced increase in $[Ca^{2+}]_{ic}$ was dependent upon extracellular calcium suggesting a role for shear-sensitive calcium channels in the plasma membrane. Possible directions for future research involve employing more physiologically relevant shear stress magnitudes and waveforms.

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