GENE EXPRESSION AND CYTOSKELETAL TENSIONAL HOMEOSTASIS: EVIDENCE FOR A MECHANOSTAT SET POINT IN TENDON CELLS

Introduction Cells are known to respond to physical signals by way of a mechanotransduction tensility system that connects the cell nucleus to the extracellular matrix through the cytoskeleton. While various in vitro studies have shown the ability of tendon cells to translate physical stimuli into biochemical signals, little is known about how, or if, these cells are (or can be) calibrated to respond to various levels of stress. To explain the response of bone cells to physical stimulus Frost proposed the Mechanostat Set Point theory (1). This theory suggests that bone cells are programmed to sense a certain level of strain-induced signals (the set point). If the signal is below the set point, biological mechanisms are activated which decrease bone mass. If the signal exceeds the set point, biological mechanisms are activated which increase bone mass. A similar system may exist in tendon cells. Investigators have suggested that dermal fibroblasts are capable of generating an internal tensional load through contraction against an extracellular matrix (2). This contraction results in an internal tensional homeostasis within the cytoskeleton, which is thought to permit cells to “recalibrate” themselves in response to persistent changes in extracellular matrix strain. It was our hypothesis that tendon cells are capable of generating internal tensional homeostasis within their cytoskeleton in response to changing external strains. Changing this internal tension either chemically or mechanically would then allow cells to “recalibrate” their set point with regard to gene expression.

Materials and Methods Rat tail tendon cells were seeded into 1 ml collagen gels (2.4mg/ml type I bovine collagen) at a concentration of 400,000cells/ml. The gels were incubated in DMEM with 10% FBS at 37°C and 10% CO2. The gels were allowed to contact the culture dish for 48 hours before treatment. Two experiments were designed to change the internal tensional homeostasis within the tendon cells using either chemical or mechanical methods. The first experiment used cytochalasin D to disrupt the actin cytoskeleton. These experiment gels were treated as follows: Group 1 attached control; Group 2 attached control + cytochalasin D (10μM) for 24 hours; Group 3 released and allowed to contract for 24 hours; Group 4 released and allowed to contract for 10 days; Group 5 released and allowed to contract for 10 days followed by 24 hours incubation in cytochalasin D (10μM); Group 6 released and allowed to contract for 14 days. In the second experiment, the contracting gels were allowed to reach a steady state of contraction around an external 3-point tractional device comprised of three stainless steel pins arranged in a triangular fashion with 10mm between each pin. Subsequent removal of the device would then allow the gel to further contract, changing the internal cytoskeletal stresses. In the second experiment gels were treated as follows: Group 1 attached control; Group 2 released and allowed to contract for 24 hours with 3pt traction pin; Group 3 released and allowed to contract for 10 days with 3pt traction pin; Group 4 released and allowed to contract for 10 days with 3pt traction pin followed by 24 hours of free contraction. At the end of each time period total cellular RNA was extracted from the tendon cells and MMP-1 expression assessed using northern blotting. Five gels per group were evaluated and the experiment repeated 3 times. Additional gels from each group were used to evaluate cell shape and isometric contraction (3). This expression can be inhibited by the reaplication of external loads suggesting that there is a threshold level (set point) of strain, below which MMP-1 gene expression is activated. The results of the current study suggest that cells may be capable of regulating or recalibrating this set point through the generation of internal tractional stresses. Tendon cells extracted from their normal in situ environment were capable of generating an internal, isometric, tractional force against a fixed (attached) collagen matrix. Disruption of this internal stress following gel release or chemical alteration of the actin cytoskeleton activated MMP-1 mRNA expression. Contraction of the gel on itself or around a 3 pt traction device allowed the cells to re-establish internal cytoskeletal tension (and the set point) resulting in an inhibition of MMP-1 mRNA expression. MMP-1 mRNA expression could again be activated in these contracted gels by chemical disruption of the cytoskeleton or removal of the external 3pt traction device. This study documents the ability of tendon cells to regulate MMP-1 mRNA expression through the generation of internal cytoskeletal stresses. This also suggests the existence of a mechanostat set point in these cells which activates MMP-1 gene expression. The ability of tendon cells to internally regulate their mechanostat set point has great implications in the possible pathogenesis and/or prevention of overuse injuries in tendons. In addition, the role of internal cell traction and subsequent set point generation may have significant application in the in vitro generation of tissue engineered tendons.

Results Chemical Alteration of Internal Stresses: (Figure 1) Isometric contraction of the cells in the attached gels produced no measurable expression of MMP-1 (Group 1). However, destruction of the cytoskeleton with cytochalasin D (Group 2) or release of the isometric contraction (Group 3) caused an immediate up-regulation of MMP-1 expression. Upon release the cells began to contract the gels and by 10 days MMP-1 expression was significantly decreased (Group 4). MMP-1 expression in these 10 days gels was immediately upregulated by destruction of the cytoskeleton and release of the internal cellular tension (Group 5). By 14 days the contracted gels reached an asymptotic contracted diameter and MMP-1 expression was effectively eliminated (Group 6).

Discussion Previous studies in our lab have demonstrated that stress deprivation in tendons results in an immediate upregulation of MMP-1 expression (3). This expression can be inhibited by the reaplication of external loads suggesting that there is a threshold level (set point) of strain, below which MMP-1 gene expression is activated. The results of the current study suggest that cells may be capable of regulating or recalibrating this set point through the generation of internal tractional stresses. Tendon cells extracted from their normal in situ environment were capable of generating an internal, isometric, tractional force against a fixed (attached) collagen matrix. Disruption of this internal stress following gel release or chemical alteration of the actin cytoskeleton activated MMP-1 mRNA expression. Contraction of the gel on itself or around a 3 pt traction device allowed the cells to re-establish internal cytoskeletal tension (and the set point) resulting in an inhibition of MMP-1 mRNA expression. MMP-1 mRNA expression could again be activated in these contracted gels by chemical disruption of the cytoskeleton or removal of the external 3pt traction device. This study documents the ability of tendon cells to regulate MMP-1 mRNA expression through the generation of internal cytoskeletal stresses. This also suggests the existence of a mechanostat set point in these cells which activates MMP-1 gene expression. The ability of tendon cells to internally regulate their mechanostat set point has great implications in the possible pathogenesis and/or prevention of overuse injuries in tendons. In addition, the role of internal cell traction and subsequent set point generation may have significant application in the in vitro generation of tissue engineered tendons.

References
1) Frost HM, Anat Rec 219:1-9, 1987;
2) Brown RA et al, J Cell Physiol 175:323, 1998;
3) Amoczky SP et al, Trans ORS (791) 2003

Figure 1. Representative Northern Blot gel showing mRNA expression of MMP-1 from collagen gels from chemical alteration experiment.

Figure 2. Representative Northern Blot gel showing mRNA expression of MMP-1 from collagen gels from mechanical alteration experiment.

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