

# Cyclic Mechanical Stretching of Human Tendon Fibroblasts Increases the Production of Prostaglandin E<sub>2</sub> and Levels of Cyclooxygenase Expression: A Novel In Vitro Model Study

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Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) is a known inflammatory mediator of tendinitis, for which mechanical loading on tendons is believed to be one of the most prominent causation factors. Previous in vitro studies have shown that cyclic mechanical stretching of cells can cause changes in cell morphology and alteration of both DNA and protein syntheses. In our study, a novel system was used whereby tendon fibroblasts are cultured on microgrooved silicone surfaces and are subjected to cyclic uniaxial stretching along their long axes to mimic in vivo conditions. Using this unique model system, the cell shape and alignment can be controlled. Further, this study was designed to test the hypotheses that PGE<sub>2</sub> production increases in a stretching magnitude-dependent manner and that cyclooxygenase (COX) is responsible for the increased PGE<sub>2</sub> production in tendon fibroblasts. Human patellar tendon fibroblasts were cultured on the microgrooved silicone membranes and cyclically stretched at 4%, 8%, or 12% of nominal dish length for 24 hr. PGE<sub>2</sub> production was found to be increased 1.7-fold at 8% cyclic stretching and 2.2-fold at 12% cyclic stretching compared with nonstretched controls. In addition, human tendon fibroblasts had increased expression of both COX-1 and COX-2 for all three applied stretching magnitudes, with the exception of COX-1 at 4% cyclic stretching. Also, cellular PGE<sub>2</sub> production, after 8% cyclic stretching, was significantly decreased with the addition of indomethacin (25 μM), a COX competitive inhibitor, compared with stretched cells without indomethacin treatment. These findings suggest that the increase in PGE<sub>2</sub> production by the human tendon fibroblasts is stretching magnitude-dependent, and that the increase in COX expression contributes to the increased production of PGE<sub>2</sub> after cyclic stretching. As PGE<sub>2</sub> is a known inflammatory mediator of tendinitis, the contribution of COX-1 and COX-2 to

PGE<sub>2</sub> production and their roles in tendon inflammation are clearly indicated.

**Keywords** Cyclooxygenase, Mechanical Stretching, Prostaglandin E<sub>2</sub>, Silicone Microgrooves, Tendon Fibroblasts.

## INTRODUCTION

Tendons are well-organized fibrous connective tissues that are mainly composed of unidirectionally oriented collagen fibers interspersed with elongated fibroblasts. Tendon fibroblasts, which are aligned along the collagen fibers in vivo, are stretched uniaxially along the long axis of the tendon when loaded. Repetitive mechanical loading on tendons is believed to be one of the most prominent causation factors for tendinitis (Almekinders et al. 1995). It has been well recognized that repetitive mechanical loading has been shown to affect cellular structure and function (Ingber 1991), resulting in changes in cell morphology, alterations in cell cycle, and variation of DNA and protein syntheses in many types of cells (Gimbrone et al. 1997; Leung et al. 1976; Liu and Post 2000; Wirtz and Dobbs 2000). Although the etiology of tendinitis is not yet completely known, in vitro models have been used to study its cellular and molecular mechanisms. Interestingly, prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), a known inflammatory mediator of tendons (Almekinders et al. 1995), was found to increase in production after cyclic stretching of tendon fibroblasts in vitro. Furthermore, production of PGE<sub>2</sub> is catalyzed by two cyclooxygenase (COX) isoforms, COX-1 and COX-2, which also are involved in the cellular inflammatory responses (Diaz et al. 1998).

To simulate the in vivo cellular response, we developed a novel in vitro system to mimic those in vivo conditions, including the shape, alignment, and stretching direction of the cells. Tendon fibroblasts are cultured on microgrooved silicone surfaces to control the cell shape and alignment during cyclic stretching,

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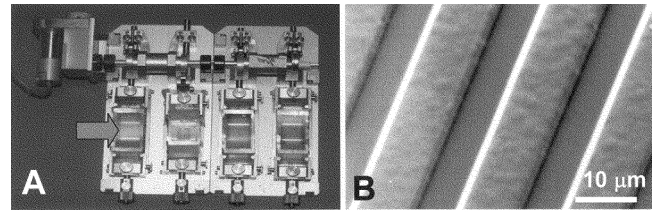
such that the cells are subjected to cyclic uniaxial stretching along their long axis. Using this system, the inflammatory responses of the tendon fibroblasts following cyclic mechanical stretching, specifically, the production of PGE<sub>2</sub>, expression of COX, and the role of COX expression on PGE<sub>2</sub> production can be examined.

Thus, the objectives of this study were first, to evaluate whether human tendon fibroblasts, after being cyclically stretched in the novel model system, have the shape, alignment, and stretching conditions similar to those cells *in vivo*; second, to determine whether cyclic stretching of human tendon fibroblasts results in a cellular inflammatory response, indicated by an increase in PGE<sub>2</sub> production and COX expression. We hypothesize that PGE<sub>2</sub> production by human tendon fibroblasts following cyclic stretching increases in a stretching-magnitude dependent manner. Further, we hypothesize that the increase in PGE<sub>2</sub> production by the tendon fibroblasts is due to, in part, an increase in COX expression.

## MATERIALS AND METHODS

Silicone, a transparent, elastic, and nontoxic material to cells (Wang et al. 2000), was used to make specially designed dishes to grow and stretch human tendon fibroblasts in this study. The silicone dishes were made by an established custom molding process (Wang 2000; Wang et al. 2000, 2001; Wang and Grood 2000). Briefly, smooth glass pieces affixed to a multiple-dish mold made of acrylic (Plexiglass) were used, such that the culture surface (the bottom of the dish) would be a smooth surface. Two silicone components (RTV ME 601A and 601B; Wacker Silicones Corporation, Adrian, MI, USA) were mixed in a ratio of 10:1 and degassed. The silicone mixture was then poured into the mold to create silicone dishes with smooth culture surfaces. In a separate molding process, microgrooved silicone membranes (about 1.5 mm thick) were made by molding silicone fluid against a silicon wafer, on which microgrooves had been fabricated using standard lithographic and reactive ion etching techniques. The obtained microgrooved membranes were cut to  $\sim 3.5$  cm  $\times$  6.5 cm in size. Then, to make the dishes with microgrooved culture surfaces, the smooth surfaces of the silicone dishes were removed and replaced with microgroove membranes, using silicone adhesive (Dow Corning, Midland, MI, USA) to attach them to the bottom of the dish. The microgrooves on the silicone membrane were aligned along the long axis of the silicone dish, i.e., the direction along which it was to be stretched (the stretching direction). The microgrooves had rectangular profiles, with 10- $\mu$ m ridge and groove width, and 3- $\mu$ m groove depth.

The *in vitro* model system consists of a stretching apparatus and silicone dishes with microgrooved culture surfaces. The stretching apparatus was custom-made and described previously (Neidlinger-Wilke et al. 2001). Briefly, the apparatus consists of six stations for the silicone dishes, with each station fixing one end of the dish to a stationary clamp and fixing the other end of the dish to a moving clamp. The stationary clamps hold the



**Figure 1.** Silicone dishes (arrow), mounted on the custom-made stretching apparatus, contained microgrooves oriented along the dish's long axis, i.e., the stretching direction (A). The profile of the microgroove is close to rectangular, and the width of the ridges and grooves was 10  $\mu$ m and the depth was 3  $\mu$ m (B).

dishes in place, while the moving clamps cyclically stretch the dishes by means of six drive shafts that transmit the linear motion from the cam-follower mechanism to the moving clamps. The stretching apparatus is capable of applying uniaxial displacement with varying stretching magnitudes and frequencies to the cells in the silicone dishes (Figure 1). The stretching magnitude is specified by the eccentricity of the circular cams, which are rotated by a DC motor. The stretching frequency can be varied by changing the motor speed with a separate control unit.

Human patellar tendon fibroblasts (HPTFs) were obtained from tendon pieces trimmed from patellar tendon autografts of healthy male and female donors (21 to 38 years) used for reconstruction of the anterior cruciate ligament. The protocol for obtaining the tendon samples was approved by the Institutional Review Board of the University of Pittsburgh Medical Center (assurance of compliance # IORG0000196). In a laminar flow hood, the tendon samples were washed twice with phosphate buffered saline (PBS; Life Technologies, Rockville, MD, USA) and minced in a 100-mm Petri dish. After addition of 5 ml of DMEM containing 10% heat inactivated fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S; Life Technologies, Rockville, MD, USA), the tendon samples were maintained in a humidified atmosphere of 5% CO<sub>2</sub> at 37°C. After the HPTFs grew out of the tendon explants and reached confluence, the cells were subcultured 5 to 7 passages to obtain enough cells for stretching experiments.

The microgrooved surfaces of the silicone dishes were coated with 10  $\mu$ g/ml of ProNectin-F (BioSource International, Camarillo, CA, USA) to promote cell attachment to the silicone surfaces. After coating, the tendon fibroblasts were plated to the silicone dishes at a density of  $2 \times 10^4$  cells/cm<sup>2</sup> and grown in DMEM with 10% FBS and 1% P/S. After incubation for  $\sim 24$  hr, the medium was replaced with fresh DMEM containing 1% FBS and 1% P/S.

To test the first hypothesis that PGE<sub>2</sub> production by human tendon fibroblasts following cyclic stretching increases in a stretching-magnitude dependent manner, the HPTFs were cyclically stretched at 4%, 8%, or 12%, with a constant stretching frequency of 0.5 Hz. Note that 20  $\mu$ M of arachidonic acid (Sigma, St Louis, MO, USA), a substrate for COX and a precursor of PGE<sub>2</sub>, was added to the medium for this set of experiments. After 24 hr of stretching, the cells were left to rest in the

stretching-conditioned medium for an additional 20 hr, which allowed for sufficient PGE<sub>2</sub> to accumulate for detection. Cells on microgrooved silicone surfaces that were not stretched were used for control. The media in the dishes were then collected, and levels of PGE<sub>2</sub> were assayed using ELISA kits (R&D Systems, Minneapolis, MN, USA). After collection of the media, cells in the dishes were trypsinized and counted with a hemacytometer. The cell numbers obtained were used for normalization of PGE<sub>2</sub> measurements.

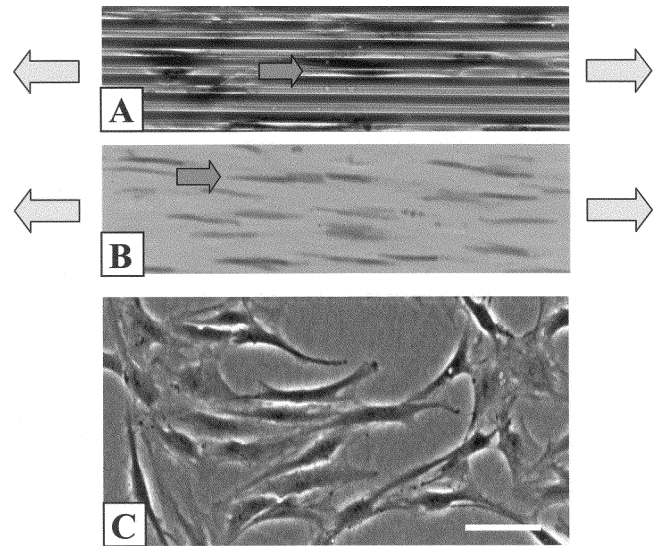
In a separate set of experiments, the tendon fibroblasts were cyclically stretched for 4 hr, followed by 4 hr incubation in the stretching-conditioned medium. A 4 hr stretching duration was chosen on the consideration that within 4 hr of cyclic mechanical stretching, the enzymatic activity of COX is increased, and this increase is maintained for at least 24 hr (Vandenburg et al. 1995). Three separate experiments for each stretching magnitude were performed. COX levels were detected using standard Western blotting technique. Briefly, 20  $\mu$ g of total protein for each sample was separated by electrophoresis using 10% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane. The protein-containing membrane was incubated with a COX-1 or COX-2 monoclonal antibody (1:1000 dilution in PBS; Cayman Chemical Co., Ann Arbor, MI, USA) for 2 hr at room temperature. Then, the membrane was washed extensively and incubated for 2 hr with the secondary antibody, goat anti-mouse conjugated with horseradish peroxidase (1:4000 dilution ratio; Jackson Immunoresearch Lab, West Grove, PA, USA). The COX-1 or COX-2 protein bands on the membrane were detected using the Amersham ECL system (Amersham, Piscataway, NJ, USA). To verify that equal amounts of protein samples were loaded onto the gel, GAPDH, a house-keeping protein, also was probed on the same blots where COX-1 and COX-2 were detected.

To determine whether COX is responsible for PGE<sub>2</sub> production in cyclically stretched human tendon fibroblasts, a third set of experiments was performed where tendon fibroblasts were cyclically stretched at 8% in the presence of indomethacin (25  $\mu$ M), a competitive inhibitor specific to COX. Nonstretched and 8% cyclically stretched cells, both without indomethacin treatment, were used as controls. After 4 hr of stretching, followed by an additional 4 hr of incubation, PGE<sub>2</sub> levels in the stretching-conditioned media were measured with the ELISA kits.

To determine whether the amount of PGE<sub>2</sub> production by the stretched tendon fibroblasts depended on the stretching magnitude, one-way ANOVA was used, followed by Newman-Keuls test for multiple comparisons to determine the effect of a specific stretching magnitude (e.g., 8%) on PGE<sub>2</sub> production by the stretched tendon fibroblasts. A difference between two groups was considered to be statistically significant if the *p* value was less than 0.05.

## RESULTS

In the present model system, human tendon fibroblasts were found to be elongated in shape and were aligned along the mi-

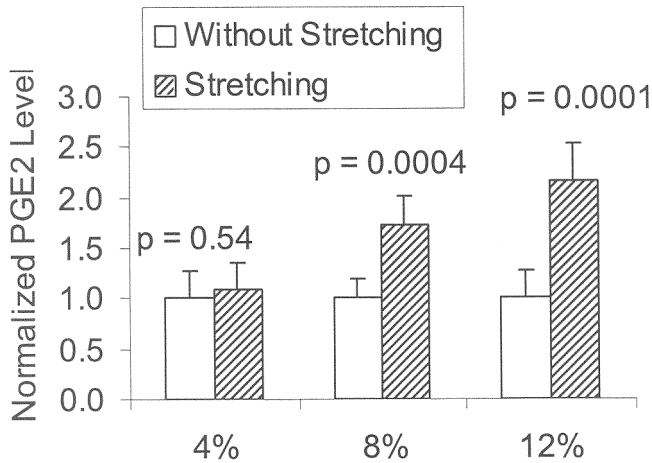


**Figure 2.** The fibroblasts on the microgrooved silicone surface remain elongated and aligned under cyclic uniaxial stretching (arrows) in the direction of the microgrooves (A, notice an arrow pointing to a fibroblast on a ridge of the microgrooves). Tendon fibroblasts in vivo (B, arrow) are aligned with collagen fibers, along the tendon's long axis (H&E staining). Note that these cells on smooth surfaces are randomly oriented with various shapes (C). Bar = 150  $\mu$ m.

crogrooves. Further, the cells remained aligned along the microgrooves after stretching, such that the cells were uniaxially stretched in this system (Figure 2A). Thus, the cell shape, alignment, and uniaxial stretching conditions using this model system appear to mimic those of the tendon fibroblasts in vivo (Figure 2B). Note that the same cells on smooth surfaces had various shapes and were randomly oriented (Figure 2C).

Using this model system, we found that 8% and 12% cyclic stretching of the tendon fibroblasts significantly elevated the levels of PGE<sub>2</sub> ( $p = 0.0004$  and  $p = 0.0001$ , respectively), whereas 4% cyclic stretching was not found to be significantly different ( $p = 0.54$ ) compared with cells without stretching. The PGE<sub>2</sub> production increased 1.7-fold for 8% cyclic stretching and 2.2-fold for 12% cyclic stretching compared with unstretched cells. Further, the PGE<sub>2</sub> levels were increased by 1.6-fold for 8% versus 4% cyclic stretching and 1.9-fold for 12% versus 4% cyclic stretching (Figure 3), demonstrating that the increase in PGE<sub>2</sub> levels are stretching-magnitude dependent. Note that for each stretching condition, three sets of separate experiments (total  $n = 6$ ) were performed. To compare the results from the three experiments, the PGE<sub>2</sub> levels were normalized with the cell numbers. On average, PGE<sub>2</sub> levels before normalization were 1237 pg/10,000 cells with 12% stretching and 551 pg/10,000 cells without stretching.

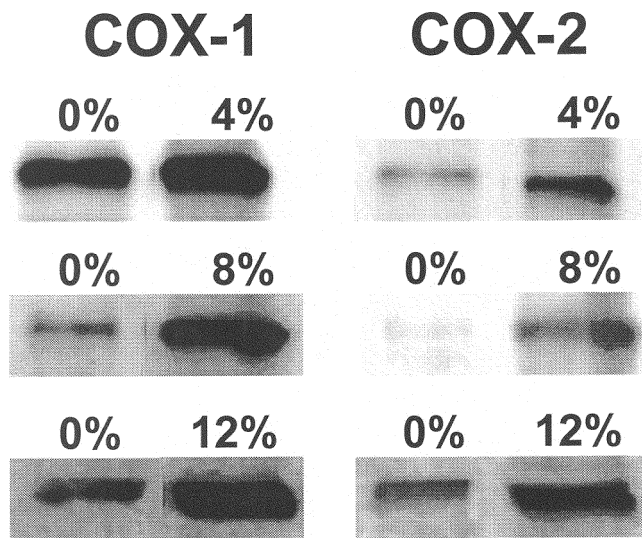
Concurrent to the finding that cyclic stretching increases PGE<sub>2</sub>, this study also found a marked increase in both COX-1 and COX-2 expression levels following 8% and 12% cyclic stretching of the tendon fibroblasts. However, with 4% cyclic stretching, only COX-2 but not COX-1 levels was increased (Figure 4). Similar results with Western blot were obtained for



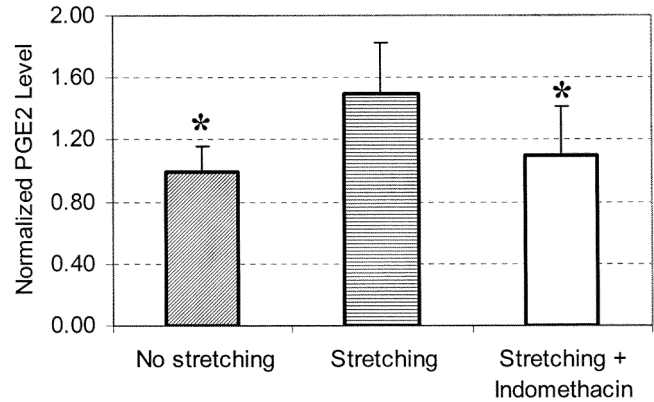
**Figure 3.** Tendon fibroblasts significantly increased the production of PGE<sub>2</sub> at 8% and 12% in a stretching-magnitude dependent manner, but apparently not at 4%. HPTFs were cyclically stretched at 0.5 Hz for 24 hr and incubation for 20 hr in the presence of exogenous AA (20  $\mu$ M).

the three separate experiments performed at each stretching magnitude (4%, 8%, or 12% cyclic stretching).

Because there was a concurrent increase of PGE<sub>2</sub> and COX following cyclic stretching, an additional experiment was performed to verify that the increase in COX expression contributed to the increase in PGE<sub>2</sub> production. We found that with indomethacin (25  $\mu$ M) treatment, a specific COX inhibitor, PGE<sub>2</sub> levels following 8% cyclic stretching were found to be significantly decreased ( $p < 0.05$ ) compared with those without indomethacin treatment (Figure 5). Moreover, PGE<sub>2</sub> levels from



**Figure 4.** At 4% stretching, COX-1 levels were similar to the nonstretched controls, but COX-2 levels were increased. At 8% and 12% stretching, however, both COX-1 and COX-2 levels were dramatically increased. The figure shows representative Western blots from cells cyclically stretched at 0.5 Hz for 4 hr, followed by 4 hr incubation in the stretching-conditioned medium.



**Figure 5.** Production of PGE<sub>2</sub> by the HPTFs was significantly inhibited with indomethacin (25–50  $\mu$ M) treatment compared with the stretched cells without indomethacin treatment ( $p < .05$ ). Further, the PGE<sub>2</sub> production by the indomethacin treatment group was not significantly different from the cells without stretching and without the indomethacin treatment ( $p = .53$ ). The stretched cells were subjected to 8% stretching at 0.5 Hz for 4 hr, followed by 4 hr of incubation in the stretching-conditioned medium. The bar represents  $\pm$ SD ( $5 \leq n \leq 10$  for each stretching condition).

the cyclically stretched cells with the indomethacin treatment were not significantly different from those cells without stretching and without indomethacin treatment ( $p = 0.53$ ). Note that to compare the results for these experiments, the PGE<sub>2</sub> levels were normalized with respect to the control, that of the cells without stretching, and the respective cell numbers. Prior to normalization, PGE<sub>2</sub> levels for the cyclically stretched cells ranged from 231 pg to 7485 pg for 10,000 cells.

## DISCUSSION

Using a novel model system, the inflammatory responses of human tendon fibroblasts subjected to cyclic mechanical stretching were examined in vitro. The unique advantage of this model system is that it can control shape and alignment of the tendon fibroblasts during stretching. In this respect, these tendon fibroblasts subjected to mechanical stretching in vitro indeed mimic those cells in vivo, thus fulfilling the first objective of the study.

The effects of repetitive mechanical stretching on human tendon and ligament fibroblasts have been reported in literature (Almekinders et al. 1993, 1995; Yamaguchi et al. 1994). In this study, the cell shape, alignment, and stretching direction are controlled by means of the microgrooved silicone surfaces. Because cell shape influences cellular functions (Chen et al. 1997), the ability to control cell shape during stretching could yield different cellular responses. Further, under controlled biaxial (Neidlinger-Wilke et al. 2001; Vandenburg et al. 1995) or uniaxial (Neidlinger-Wilke et al. 2001; Vandenburg et al. 1995) stretching of cells on smooth silicone surfaces, the cells could experience different loading conditions from in vivo (Wang and Grood 2000) due to the fact that cells align away from the stretching direction (Wang et al. 1995, 2000, 2001). This indicates the need to control these parameters. As the tendon fibroblasts in this model mimic the shape, alignment, and stretching

conditions of those cells in vivo, their inflammatory responses of these cells in response to mechanical stretching may be more realistic.

Using this novel system, tendon fibroblasts increased PGE<sub>2</sub> production in a stretching magnitude-dependent manner (Figure 3) compared with cells without stretching, confirming the first hypothesis. This finding is supported by a study on human periodontal ligament cells (Yamaguchi et al. 1994). Further, the second hypothesis also was confirmed in that cyclic stretching also increased COX-1 and COX-2 expression (Figure 4). Since PGE<sub>2</sub> production is regulated by two rate-limiting enzymes, phospholipase A<sub>2</sub> (PLA<sub>2</sub>) and COX, the arachidonic acid (AA) exogenously added in culture ensured substrate abundance (Almekinders et al. 1995) for the COX enzyme and would hence negate the effect of PLA<sub>2</sub>, an enzyme catalyzing the production of AA, on PGE<sub>2</sub> production. Further, cyclic stretching at 8% or 12% increased both COX-1 and COX-2 expression levels in the tendon fibroblasts, whereas 4% stretching only increased COX-2 levels.

Although these results are consistent with the notion that COX-2 is inducible in the human tendon fibroblasts (Diaz et al. 1998), COX-1 also appears to be inducible in the cells subjected to large cyclic stretching. Moreover, there was a concurrent increase in COX and PGE<sub>2</sub>, as well as suppression of stretching-induced PGE<sub>2</sub> production to basal levels when the cells were treated with indomethacin, a COX specific inhibitor shown to suppress PGE<sub>2</sub> production (Endo et al. 1995; Ogushi et al. 1999) (Figure 5). With these results, we may be certain that the increase in COX-1 and COX-2 expression is at least partially responsible for the increased production of PGE<sub>2</sub> by the stretched tendon fibroblasts.

It should be noted that the *nominal* applied strain does not represent the strain experienced by the cells, since deformation occurs at the clamp region of the silicone dishes. Also, there is an incomplete transfer of substrate strain to the cells (Wang 2000). For example, endothelial cell strain was shown to be about 77.2% of the nominal substrate strain (Wang et al. 2001). Further, a previous study estimated that 4% strain in a tendon straightens the collagen fibers, whereas 8% and 12% strain potentially relate to micro- and macroruptures, respectively (Curwin and Stanish 1984). Therefore, we suspect that the tendon fibroblasts in this model system experience much smaller strains, which also may preclude the possibility of damaging the fibroblasts due to large strains imposed on the cells.

There are a few significant advantages of the current model systems over many existing systems. First, cell alignment is controlled in the same direction as that of applied stretching, similar to in vivo situation. Second, the tendon fibroblasts are subjected to uniaxial stretching instead of biaxial stretching (see Almekinders et al. 1995). Finally, the shape of the tendon fibroblasts is controlled so that the cells are uniformly elongated in shape, which mimics the shape of the tendon fibroblasts in vivo. Taken together, these advantages make it possible to properly interpret experimental results from stretching of tendon fibroblasts.

Since repetitive mechanical loading on tendons is believed to be one of the most prominent causation factors of tendinitis (Almekinders et al. 1995), the elevated production of PGE<sub>2</sub>, a known inflammatory mediator, found in this study, is encouraging. Further, since prostaglandins have been shown to affect cell proliferation (Elias et al. 1985a, 1985b), the production of extracellular matrix proteins (Barile et al. 1998; Diaz et al. 1993), and inflammatory and immune responses (Betz and Fox 1991; Harada et al. 1982), elevated levels of PGE<sub>2</sub> also may affect proliferation and collagen synthesis of the tendon fibroblasts in vivo.

The biosynthetic production of prostaglandins involves the conversion of free AA by COX to prostaglandin H<sub>2</sub>, the upstream metabolite for prostaglandins. The two COX isoforms, COX-1 and COX-2, are variably expressed in many types of cells. But it is generally thought that COX-1 is expressed constitutively in many type of cells, whereas COX-2 is induced in response to inflammatory stimuli, such as cytokines and bacterial products (Cryer and Feldman 1998). This study, however, demonstrated that at large stretching magnitudes (8% and 12%), the expression levels of both COX-1 and COX-2 are increased. This appears to be consistent with the fact that both COX-1 and COX-2 are induced and contribute to the prostaglandin production in response to lipopolysaccharide in humans (McAdam et al. 2000). Determining the role of each enzyme in tissue pathophysiology is under intensive study. Further studies are suggested to investigate the relative contribution of COX-1 and COX-2 to PGE<sub>2</sub> production and the effect of PGE<sub>2</sub> on proliferation and collagen synthesis of tendon fibroblasts, which are two important cellular responses in the development of tendon pathophysiology, such as tendinitis.

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## REFERENCES

- Almekinders, L.C., Banes, A.J., and Ballenger, C.A. (1993). Effects of repetitive motion on human fibroblasts. *Med. Sci. Sports Exerc.* 25(5):603–607.
- Almekinders, L.C., Baynes, A.J., and Bracey, L.W. (1995). An in vitro investigation into the effects of repetitive motion and nonsteroidal antiinflammatory medication on human tendon fibroblasts. *Am. J. Sports Med.* 23(1):119–123.
- Barile, F.A., Ripley-Rouzier, C., Siddiqui, Z.E., and Bienkowski, R.S. (1988). Effects of prostaglandin E1 on collagen production and degradation in human fetal lung fibroblasts. *Arch. Biochem. Biophys.* 265(2):441–446.
- Betz, M., and Fox, B.S. (1991). Prostaglandin E2 inhibits production of Th1 lymphokines but not of Th2 lymphokines. *J. Immunol.* 146(1):108–113.
- Chen, C.S., Mrksich, M., Huang, S., Whitesides, G.M., and Ingber, D.E. (1997). Geometric control of cell life and death. *Science* 276(5317):1425–1428.
- Cryer, B., and Feldman, M. (1998). Cyclooxygenase-1 and cyclooxygenase-2 selectivity of widely used nonsteroidal anti-inflammatory drugs. *Am. J. Med.* 104(5):413–421.

- Curwin, S., and Stanish, W. (1984). *Tendinitis: Its Etiology and Treatment*. City: The Collamore Press, D.C. Heath and Company.
- Diaz, A., Chepenik, K.P., Korn, J.H., Reginato, A.M., and Jimenez, S.A. (1998). Differential regulation of cyclooxygenases 1 and 2 by interleukin-1 beta, tumor necrosis factor-alpha, and transforming growth factor-beta 1 in human lung fibroblasts. *Exp. Cell. Res.* 241(14):222–229.
- Diaz, A., Munoz, E., Johnston, R., Korn, J.H., and Jimenez, S.A. (1993). Regulation of human lung fibroblast alpha 1 (I) procollagen gene expression by tumor necrosis factor alpha, interleukin-1 beta, and prostaglandin E2. *J. Biol. Chem.* 268(14):10364–10371.
- Elias, J.A., Rossman, M.D., Zurier, R.B., and Daniele, R.P. (1985a). Human alveolar macrophage inhibition of lung fibroblast growth. A prostaglandin-dependent process. *Am. Rev. Respir. Dis.* 131(1):94–99.
- Elias, J.A., Zurier, R.B., Schreiber, A.D., Leff, J.A., and Daniele, R.P. (1985b). Monocyte inhibition of lung fibroblast growth: Relationship to fibroblast prostaglandin production and density-defined monocyte subpopulations. *J. Leukoc. Biol.* 37(1):15–28.
- Endo, T., Ogushi, F., Sone, S., Ogura, T., Taketani, Y., Hayashi, Y., Ueda, N., and Yamamoto, S. (1995). Induction of cyclooxygenase-2 is responsible for interleukin-1 beta-dependent prostaglandin E2 synthesis by human lung fibroblasts. *Am. J. Respir. Cell Mol. Biol.* 12(3):358–365.
- Gimbrone, M.A., Jr., Nagel, T., and Topper, J.N. (1997). Biomechanical activation: An emerging paradigm in endothelial adhesion biology. *J. Clin. Invest.* 100(11 suppl):S61–S65.
- Harada, Y., Tanaka, K., Uchida, Y., Ueno, A., Oh-Ishi, S., Yamashita, K., Ishibashi, M., Miyazaki, H., and Katori, M. (1982). Changes in the levels of prostaglandins and thromboxane and their roles in the accumulation of exudate in rat carrageenin-induced pleurisy—A profile analysis using gas chromatography-mass spectrometry. *Prostaglandins* 23(6):881–895.
- Ingber, D. (1991). Integrins as mechanochemical transducers. *Curr. Opin. Cell Biol.* 3(5):841–848.
- Leung, D.Y., Glagov, S., and Mathews, M.B. (1976). Cyclic stretching stimulates synthesis of matrix components by arterial smooth muscle cells in vitro. *Science* 191(4226):475–477.
- Liu, M., and Post, M. (2000). Invited review: Mechanochemical signal transduction in the fetal lung. *J. Appl. Physiol.* 89(5):2078–2084.
- Neidlinger-Wilke, C., Grood, E.S., Wang, J.-C., Brand, R.A., and Claes, L. (2001). Cell alignment is induced by cyclic changes in cell length: Studies of cells grown in cyclically stretched substrates. *J. Orthop. Res.* 19(2):286–293.
- Ogushi, F., Endo, T., Tani, K., Asada, K., Kawano, T., Tada, H., Maniwa, K., and Sone, S. (1999). Decreased prostaglandin E2 synthesis by lung fibroblasts isolated from rats with bleomycin-induced lung fibrosis. *Int. J. Exp. Pathol.* 80(1):41–49.
- Vandenburgh, H.H., Shansky, J., Solerssi, R., and Chromiak, J. (1995). Mechanical stimulation of skeletal muscle increases prostaglandin F2 alpha production, cyclooxygenase activity, and cell growth by a pertussis toxin sensitive mechanism. *J. Cell Physiol.* 163(2):285–294.
- Wang, H., Ip, W., Boissy, R., and Grood, E.S. (1995). Cell orientation response to cyclically deformed substrates: Experimental validation of a cell model. *J. Biomech.* 28(12):1543–1552.
- Wang, J.H. (2000). Substrate deformation determines actin cytoskeleton reorganization: A mathematical modeling and experimental study. *J. Theor. Biol.* 202(1):33–41.
- Wang, J.H., Goldschmidt-Clermont, P., Wille, I., and Yin, F.C. (2001). Specificity of endothelial cell reorientation in response to cyclic mechanical stretching. *J. Biomech.* 34(12):1563–1572.
- Wang, J.H., and Grood, E.S. (2000). The strain magnitude and contact guidance determine orientation response of fibroblasts to cyclic substrate strains. *Connect Tissue Res.* 41(1):29–36.
- Wang, J.H., Grood, E.S., Florer, J., and Wenstrup, R. (2000). Alignment and proliferation of MC3T3-E1 osteoblasts in microgrooved silicone substrata subjected to cyclic stretching. *J. Biomech.* 33(6):729–735.
- Wirtz, H.R., and Dobbs, L.G. (2000). The effects of mechanical forces on lung functions. *Respir. Physiol.* 119(1):1–17.
- Yamaguchi, M., Shimizu, N., Goseki, T., Shibata, Y., Takiguchi, H., Iwasawa, T., and Abiko, Y. (1994). Effect of different magnitudes of tension force on prostaglandin E2 production by human periodontal ligament cells. *Arch. Oral Biol.* 39(10):877–884.

