INTRODUCTION

Tendinitis is a common problem both in occupational and athletic settings. Nevertheless, the etiology of tendinitis is largely unknown, but it is believed to result from excessive repetitive mechanical loading of tendons, which causes tendon inflammation. However, because of the lack of a reliable experimental model, the biological mechanism responsible for the onset and development of tendinitis remains unclear.

We have developed a novel culture system to study the mechanism of tendinitis. The objective of this study was two-fold. The first was to determine whether tendon fibroblasts in this system had similar shape and alignment to those in vivo so that responses of tendon fibroblasts to mechanical loading can be better simulated in vitro. The second was to test the hypothesis that cyclic stretching of tendon fibroblasts induces high levels of prostaglandin E$_2$ (PGE$_2$). PGE$_2$ is a known mediator involved in tissue inflammation (Almekinders et al., 1995).

MATERIALS AND METHODS

Silicone dishes were custom made and used for growing and stretching cells. The dish had a large well (3 x 6 cm$^2$) in the middle, and contained microgrooves on their culture surfaces, with the groove direction along the dish’s long axis, i.e., the stretching direction. The microgrooves had rectangular profiles, with about 10 $\mu$m ridge and groove width, and 3 $\mu$m groove depth. The silicone dishes were coated with 10 $\mu$g/ml ProNectin-F (Sigma) to promote cell attachment to silicone surfaces. Human patellar tendon fibroblasts derived from surgical tendon samples were used for experiments. The cells were grown in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin (Life Technologies). However, for PGE$_2$ experiments, the cells were grown in DMEM supplemented with 10% FBS only 1% FBS. ELISA kits from R&D Systems were used for measuring PGE$_2$ according to the manufacturer’s instructions. For statistical analysis of data, ANOVA and un-paired t-test were used, with a significance level set at 0.05.

RESULTS

In the present culture system, the tendon fibroblasts were elongated in shape and aligned in the direction of microgrooves, along which cyclic stretching was applied. It is evident that the cell shape and alignment were similar to those in vivo (Fig. 1).

Fig. 1 The shape and alignment of tendon fibroblasts in our model system (A) appear similar to the cells in vivo (B, H&E staining). An arrow points to a cell on a ridge of the microgrooves.

To determine whether the PGE$_2$ production depends on the stretching magnitude, the tendon fibroblasts were stretched at 4%, 0.5Hz for 24 h and then left to rest for 20-24h in the stretching-conditioned medium. Production of PGE$_2$ was not significantly increased, compared with non-stretched cells. However, when the cells were stretched at 8% or 12%, the levels of PGE$_2$ production were significantly elevated in stretched cells versus non-stretched cells. Moreover, when stretching magnitude was increased from 4% to 8%, and to 12%, the PGE$_2$ levels were significantly increased proportionally (Fig. 2).

DISCUSSION

This study showed that the novel culture system can control cell shape, alignment, and the loading conditions, which were similar to those in vivo. Thus, the cellular responses in this culture system may better represent the responses of tendon fibroblasts to mechanical loading in vivo.

Furthermore, this study showed that in response to cyclic stretching, tendon fibroblasts produce PGE$_2$ in a stretching magnitude-dependent manner. However, it appears that once the PGE$_2$ levels were elevated after a certain period of stretching, the levels remained unchanged; that is, further stretching did not produce additional effect on PGE$_2$ production. Since PGE$_2$ is a known inflammatory mediator, the results of this study suggests that tendinitis may result from over-stretching of the tendon fibroblasts in vivo, which releases PGE$_2$ and causes the tendon inflammation often seen in the clinical setting. Therefore, to treat tendinitis, the production of PGE$_2$ by repetitively stretched tendon fibroblasts in vivo should be reduced. One potential strategy might be the inhibition of cyclooxygenase (COX), an enzyme that converts arachidonic acid to PGE$_2$. Using the present culture system, studies are underway to examine the effect of inhibiting COX on the PGE$_2$ production of tendon fibroblasts.

REFERENCES


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