# Transforming Growth Factor-β1 Stimulation Enhances Dupuytren's Fibroblast Contraction in Response to Uniaxial Mechanical Load Within a 3-Dimensional Collagen Gel

Marcus A. Bisson, MD, Kate S. Beckett, MD, D. Angus McGrouther, MD, Adriaan O. Grobbelaar, MMed, Vivek Mudera, PhD

**Purpose** A function of fibroblasts is the generation of cytomechanical force within their surrounding extracellular matrix. Abnormalities in force generation may be the cause of many pathologic conditions including scarring, and some fibroproliferative disorders such as Dupuytren's disease, which is the focus of this report.

**Methods** This work investigated the cytomechanical responses of Dupuytren's-derived fibroblasts to externally applied mechanical force using a culture force monitor model, with and without stimulation with the fibrosis-linked cytokine, transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1). We compared these responses with cytomechanical responses of fibroblasts derived from the transverse carpal ligament.

**Results** Dupuytren's fibroblasts display a significantly greater ability to contract a collagen matrix compared with control fibroblasts, with a maximum generated force of 131 dynes (p < .001). These cells did not exhibit a characteristic plateau phase in the contraction, which indicates a delay in achieving tensional homeostasis from Dupuytren's-derived cells. After being subjected to uniaxial overload and underload, Dupuytren's fibroblasts responded by increased force generation, whereas control fibroblasts responded by a reduction in force in response to an overload, and contraction in response to an underload. These changes were exacerbated by the addition of the profibrotic factor TGF- $\beta$ 1 with a significant increase in generated force for all cell types, in particular during the early phase of fibroblast attachment and contraction, and a positive contraction gradient in response to overloading forces.

**Conclusions** These data suggest that cells derived from this fibrotic disease display characteristic abnormalities in force generation profiles. Their default response to loading or underloading is contraction, or increased force generation. This work highlights the role of TGF- $\beta$ 1 as a mechano-transduction cytokine, which has an influence on the early phase cell of force generation, as well as a role in mechanical responses of cells to external mechanical stimuli. This, in turn, may influence the progression of Dupuytren's disease and the high rates of recurrence seen postoperatively. (*J Hand Surg 2009;34A:1102–1110. Copyright* © 2009 by the American Society for Surgery of the Hand. All rights reserved.)

Key words Fibrosis, TGF- $\beta$ , Dupuytren's disease, tensional homeostasis, mechanical stimulation.

From The Raft Institute of Plastic Surgery, Mount Vernon Hospital, Northwood, Middlesex; The Institute of Orthopaedics and Musculoskeletal Sciences, Royal National Orthopaedic Hospital, University College London, Stanmore; and Wythenshawe Hospital, Southmoore Road, Manchester, United Kingdom.

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This study was funded in part by the British Society for Surgery of the Hand, the Royal College of Surgeons of England, the Kirby Laing Foundation, and the Dunhill Trust. **Corresponding author:** K.S. Beckett, MD, 64 St John's Court, Beaumont Avenue, St Albans, Herts AL1 4TR, United Kingdom; e-mail: katesbeckett@hotmail.com.

0363-5023/09/34A06-0020\$36.00/0 doi:10.1016/j.jhsa.2009.02.008 **F** IBROBLASTS EXIST *in vivo* within a complex 3-dimensional environment. Their mechanical behavior depends on their location and their interaction with the surrounding extracellular matrix. A basic function of these cells is the generation of forces on the matrix, and different mechanisms of force generation have been demonstrated depending on the mechanical environment in which the cells reside.<sup>1</sup> Several models have been developed to investigate the response of fibroblasts to the mechanical environment. These models have been crucial to our understanding of how cells behave in the course of wound healing and scar contraction.

Three-dimensional fibroblast-populated circular collagen gels have been used extensively as experimental models with tractional remodeling demonstrated in free-floating lattices<sup>2–8</sup>; however, it is not possible to quantify the cell-generated forces and mechanical responses of cells to uniaxial changes in mechanical load using a circular model.

The culture force monitor model<sup>9</sup> allows continuous real-time quantification of the force generated by fibroblasts within a rectangular collagen gel and quantification of mechanistic fibroblast responses to an applied uniaxial load. Dermal fibroblasts have been shown to produce characteristic and quantifiable patterns of force generation.<sup>10</sup> Using this model, previous researchers have proposed the theory of tensional homeostasis.<sup>11</sup> Dermal fibroblasts have been shown to develop an equilibrium of balanced mechanical forces within a 3-dimensional collagen matrix. When subjected to an external uniaxial increase in force, however, the cells responded mechanically by decreasing the force held within the matrix, thus reducing the applied force and returning the system toward the level of equilibrium. The opposite mechanical cellular response was reported when the force held within the system was externally decreased, again returning the system toward equilibrium by increasing the cell-mediated force generation. Tensional homeostasis has been reported in the literature only in human dermal fibroblasts.

Dupuytren's disease is a benign fibroproliferative condition characterized by fibrosis of the fascia of the hand and fingers, which affects mechanical function. The diseased tissue is found clinically in 2 main forms. Nodules are fusiform swellings of affected fascia that are hypercellular and can be confused with fibrosarcoma histologically, and cords are tendon-like structures with longitudinally arranged parallel bundles of collagen within a hypocellular matrix.<sup>12</sup> Patients experience disability with the formation of flexion contractures of the digits, which occur as the fibrotic tissue

shortens. This shortening of the tissue is thought to occur because of a combination of continuous cellmediated contraction and matrix remodeling.<sup>13</sup> The generation of force by Dupuytren's fibroblasts is therefore of key interest in the understanding of contracture formation. Several authors have used basic fibroblast-populated collagen lattices to investigate Dupuytren's fibroblast contraction.<sup>14–18</sup> The culture force monitor has recently been used in experiments reporting that Dupuytren's fibroblasts generated a significantly greater force compared with fibroblasts from normal palmar fascia.<sup>19</sup>

Transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) is a ubiquitous polypeptide growth factor thought to have a central role in fibrotic conditions. It has been shown to increase collagen deposition<sup>20,21</sup> and enhance contraction of fibroblast-populated collagen lattices.<sup>18,22–24</sup> TGF- $\beta$ 1 is postulated to promote cellular contraction as a primary mechanoregulatory growth factor, as suggested by Brown et al.,<sup>24</sup> by increasing integrin expression,<sup>23</sup> and also by stimulating cytoskeletal component upregulation, as in the transformation of fibroblasts to myofibroblast phenotypes.<sup>25–27</sup>

We have studied the cytomechanics of Dupuytren's disease–derived fibroblasts to investigate whether inherent alterations in mechanoresponses might provide evidence for a cell-mediated basis in the formation of clinical flexion contractures. The culture force monitor allows real-time assessment of force generation by both nodule and cord-derived fibroblast types. These types have been compared with fibroblasts of a similar non-diseased connective tissue origin (carpal ligament). We examined the response of all 3 fibroblast types to uniaxial overloading and underloading forces, and repeated experiments in overloading cases only with fibroblasts that had been stimulated with TGF- $\beta$ 1.

## **MATERIALS AND METHODS**

#### **Fibroblast culture**

After we received local ethical committee approval, we generated primary fibroblast explant cultures from Dupuytren's nodules, cords, or carpal ligament tissue. Patients were included in the study only if they had not undergone previous surgery for the disease; that is, we excluded recurrent cases. Cell cultures were bathed in normal growth medium, Dulbecco's modified Eagle's medium (Gibco, Paisley, Scotland) supplemented with 10% fetal calf serum (Gibco), penicillin–streptomycin (100 U/mL and 100  $\mu$ g/mL; Gibco), and L-glutamine (2 mmol/L; Gibco), and buffered with 1 mol/L HEPES solution (3.5 mL). Cells were routinely passaged at confluence and for experimental purposes were used at

or below passage 5. We used 5,000,000 fibroblasts for each collagen lattice. Generally, 2 T225 tissue culture flasks were required with cells just subconfluent. Each carpal ligament cell line or nodule and cord cell line was derived from a separate patient and processed independently.

### **Culture force monitor**

We set up the culture force monitor apparatus as previously described<sup>9</sup> and calibrated the equipment. Collagen gels were seeded with fibroblasts in a standard fashion.<sup>10,11</sup> Briefly, fibroblasts were trypsinized, counted, and suspended in normal growth medium at a concentration of 1,000,000 viable cells/100  $\mu$ L (using trypan blue staining to assess viability). Collagen gels were made using type I rattail collagen (2.3 mg/mL; First Link UK Ltd., Birmingham, UK) mixed with  $10 \times$  minimal essential medium (Gibco) at a ratio of 10:1 by volume. The resulting solution was neutralized with NaOH (5 mmol/L, then 1 mmol/L) so that there was a color change from yellow to pale pink. At this point 500  $\mu$ L of fibroblast suspension was added to 4.5 mL of the gel solution and mixed thoroughly, producing a 5-mL gel suspension containing 5,000,000 cells. The solution was poured into a pre-prepared, sterilized rectangular mold (dimensions  $7.5 \times 2.5 \times 1.5$  cm) between flotation bars (Clear Mesh Cat 33030-1; John Lewis Department Stores, Watford, UK). Once gelation had occurred, the gel was floated and mounted onto the culture force monitor. Connection to a desktop computer allowed real-time data collection at a rate of 1 reading per second. We collected data continuously over 20 hours.

At the conclusion of this time period, we applied a series of uniaxial loading or underloading forces across the gels to investigate the responses of the fibroblasts to a rapid change in load. The overloading was achieved by rapidly moving the mounting stage away from the force transducer using a micrometer screw to produce a load increase of 30 dynes. After each overload, the cellular responses were monitored for 30 minutes using continuous data collection before a further overload was applied. In total, 4 overloads were carried out for each cell-seeded gel. We investigated 4 carpal ligament, 9 Dupuytren's nodule, and 10 Dupuytren's cord cell lines. The underloading was similarly achieved by rapidly moving the mounting stage toward the force transducer, which produced a load reduction of 30 dynes, and then following procedures as above for a total of 4 underload periods. Three carpal ligament, 6 Dupuytren's nodule, and 6 Dupuytren's cord cell lines

were investigated for this arm of the experiment. All cell culture experiments were repeated for each cell line.

Cell viability and number within the collagen lattices were confirmed at 24 hours by parallel experiments in which cells were recovered from gels by collagenase-D digest, stained with trypan blue, and counted.

For TGF- $\beta$ 1 stimulation experiments, fibroblasts were grown to 75% confluence, at which point the growth medium was exchanged for TGF- $\beta$ 1-containing medium at a concentration of 2 ng/mL (no. 240-B; R&D Systems, Minneapolis, MN) in 10% fetal calf serum. The concentration of TGF- $\beta$ 1 was based on a dose-response curve from previous work within this laboratory. Cells were incubated without further media changes for 72 hours before the fibroblasts were trypsinized and collagen lattices constructed using the same protocol as described earlier. Culture force monitor experiments were conducted for a 20-hour period, with data collected continuously at 1-second intervals. At the conclusion of this time period, a series of uniaxial overloading forces were applied across these gels to investigate the response of the fibroblasts to a rapid change in force.

Mean contraction profiles were generated for Dupuytren's nodule, Dupuytren's cord, and carpal ligament fibroblasts, both with and without TGF- $\beta$ 1 stimulation. We calculated and compared parameters relating to the profiles and responses to mechanical stimuli. The gradient of contraction at 20 hours was calculated to give a value for rate of contraction in dynes per minute. The gradient was calculated by dividing the difference in the force readings at 19.5 and 20.5 hours by 60 to give a value of rate of change in force in dynes per minute per 5,000,000 cells.

We performed statistical analysis using Sigma Stat software (Jandel Corps., San Rafael, CA), applying unpaired Student's *t*-tests or Mann-Whitney rank sum tests to all sets of data being compared.

## RESULTS

### **Standard contraction profiles**

From the standard mean contraction profiles, Figure 1 shows the mean maximum total force generated at the conclusion of the experiments (20 hours). Nodule fibroblasts generated a mean force of 131.2 dynes (standard error of the mean [SEM]  $\pm$  9.6 dynes), whereas cord fibroblasts generated 116.9 dynes (SEM  $\pm$  13.1 dynes) (p > .38). Carpal ligament fibroblasts generated a mean peak force of 45.4 dynes (SEM  $\pm$  7.2 dynes), significantly less than both nodule and cord (p < .001 and p < .002, respectively). A second parameter used



Carpal Ligament Dupuytren's Nodule Dupuytren's Cord

**FIGURE 1:** Histogram demonstrating the mean peak force generated by fibroblasts derived from carpal ligament (n = 7), Dupuytren's nodule (n = 15), and Dupuytren's cord (n = 16). Error bars represent standard errors of the mean. Note the low level of force generation for carpal ligament compared with the high level of generated force for the Dupuytren's-derived cells. There is a significant difference in force generated between carpal ligament fibroblasts and Dupuytren's fibroblasts (\*p < .002) but no difference in force generation between nodule and cord (\*<sup>†</sup>p > .038).

for analysis was the rate of continued force generation at the conclusion of the experiments. We calculated this by dividing the difference in force readings between 19.5 and 20.5 hours by 60, to give the rate of change in force expressed in dynes per minute per 5,000,000 cells. Figure 2 shows the mean values of the 20-hour gradients; nodule fibroblasts had a positive contraction gradient after 20 hours on the culture force monitor (0.1 dynes/min, SEM  $\pm$  0.02). Cord fibroblasts also continued to contract but at a significantly reduced rate (0.04)dynes/min, SEM  $\pm$  0.005; p < .015) compared with nodule. Carpal ligament fibroblasts plateaued (0.009 dynes/min, SEM  $\pm$  0.005), with no continued increase in force generation. Both nodule and cord gradients at that time point were significantly greater than carpal ligament (p < .001).

#### **Response to mechanical stimuli**

*Overloading:* The second part of the experimental model was to determine the effects of a uniaxial mechanical load applied to the fibroblast-populated collagen gel at the end of the 20-hour period. Figure 3 shows the gradients for each postoverload period in all 3 cell types. In the first period, Dupuytren's fibroblasts, both nodule and cord, contracted, as represented by an increase in gradient in response to the overload (gradients of 0.1 dynes/min; SEM  $\pm$  0.05; p < .01, compared with carpal ligament). In keeping with the results found in the initial contraction profile experiments, Dupuytren's fibroblasts displayed increased contraction in response

to an applied load compared with carpal ligament fibroblasts in the first overload period.

Underloading: After we obtained the results of a series of mechanical overloads, we stimulated the 3-dimensional collagen gels with a series of 4 uniaxial underloads of 30 dynes each. When carpal ligament-seeded collagen gels were subjected to the reduction in force, we recorded a marked cellular response (Fig. 4). Gradients were positive after each underload period ( $0.27 \pm 0.06$ ,  $0.37 \pm 0.16$ ,  $0.46 \pm 0.08$ , and  $0.34 \pm 0.05$  dynes/min, respectively), indicating a mechanical cellular contractile response to the fall in external load. There was no significant difference between gradients in each period.

When Dupuytren's fibroblasts were subjected to a series of uniaxial underloads, results were not significantly different from those of the carpal ligament fibroblasts (Fig. 4). After the first underload period, the mean gradient for nodule was 0.32 dynes/min (SEM  $\pm$  0.09), and for cord 0.39 dynes/min (SEM  $\pm$  0.05). There was no significant difference between gradients for nodule and cord.

## Effects of TGF- $\beta$ 1 stimulation

We tested the effect of TGF- $\beta$ 1 stimulation on contraction force generation and response to mechanical stimuli on all cell types. Experiments were repeated with fibroblasts that had been stimulated for 3 days with TGF- $\beta$ 1 (2 ng/mL). In all 3 cell types tested, there was a significantly increased generation of force throughout the experimental time period (Fig. 5) compared with the same cell types not stimulated with TGF- $\beta$ 1 (p < .01; Fig. 1). The rate of increase in force generation was calculated as described previously at 2 hours, and also at 20 hours. The 2-hour gradient was calculated as the rate of change of force between 60 and 120 minutes, expressed as dynes per minute. Figures 6A and B illustrate the mean 2-hour gradients and mean 20-hour gradients, respectively, for TGF- $\beta$ 1–stimulated fibroblasts.

Figure 5 shows that the mean 20-hour forces generated were significantly increased after stimulation by TGF- $\beta$ 1 in all fibroblast types (p < .05), with a mean force generation for nodule fibroblasts at 280 dynes (SEM ± 27.1). Force generated by cord fibroblasts was not significantly lower than nodule at 214 dynes (SEM ± 25.4), whereas carpal ligament fibroblasts generated the least force at 20 hours, with a mean of 165 dynes (SEM ± 32.7) (p < .05).

Figure 6A shows that at 2 hours, TGF- $\beta$ 1–stimulated fibroblasts displayed a significantly increased rate of contraction comparison with unstimulated fibroblasts (p < .05). Between nodule and TGF- $\beta$ 1–stimulated nodule fibroblasts, there was a 4-fold increase in this early rate of contraction (1.25 dynes/min compared



**FIGURE 2:** Histogram demonstrating the gradient of contraction profile at 20 hours for fibroblasts derived from carpal ligament (n = 7), Dupuytren's nodule (n = 15), and Dupuytren's cord (n = 16). Error bars represent standard errors of the mean. The gradient of contraction is significantly greater for Dupuytren's cells compared with carpal ligament (\*p < .001). In addition Dupuytren's nodules show a greater gradient of contraction compared with Dupuytren's cord fibroblasts (<sup>†</sup>\*p < .015).



**FIGURE 3:** Histogram demonstrating the mean gradients of contraction for carpal ligament (n = 4), Dupuytren's nodule (n = 9), and Dupuytren's cord (n = 10) fibroblast-seeded collagen gels for all of the postoverload periods. Error bars represent standard errors of the mean. There are no significant differences between the groups except in the first postoverload period, which shows a positive gradient for Dupuytren's-derived cells. This difference was significant (p < .01).

with an unstimulated value of 0.36 dynes/min; p < .001). This was also significantly greater (p < .05) than either cord or carpal ligament fibroblast gradients after TGF- $\beta$ 1 stimulation.

The 20-hour gradients (Fig. 6B) after TGF- $\beta$ 1 stimulation exhibited a significant increase for each cell group compared with unstimulated values, although this reached statistical significance only in the cord and carpal ligament fibroblasts (p < .05).

When fibroblasts that had been stimulated with TGF- $\beta$ 1 were exposed to the uniaxial overloading pattern, there was an enhancement of the contractile response observed previously (Fig. 7). The first postoverload response was positive for nodule and cord fibroblasts (0.37 and 0.19 dynes/min compared with unstimulated values of 0.1 and 0.05 dynes/min, re-

spectively), although this just failed to reach significance. Carpal ligament fibroblasts also demonstrated a positive contractile response when stimulated with TGF- $\beta$ 1 (0.14 compared with an unstimulated value of -0.16 dynes/min, p < .05). This contractile response in TGF- $\beta$ 1–stimulated nodule and cord fibroblasts persisted not only into the second postoverload period, but also into the third. Only on reaching the fourth postoverload period did the combined stimulus of rapid uniaxial loading and TGF- $\beta$ 1 incubation fail to produce this pattern in Dupuytren's fibroblasts.

# DISCUSSION

Our study used the culture force monitor to test the cytomechanical responses of Dupuytren's fibroblasts to mechanical stimulation. This fibroproliferative condi-



**FIGURE 4:** Histogram demonstrating the mean gradients of contraction for carpal ligament (n = 3), Dupuytren's nodule (n = 5), and Dupuytren's cord (n = 6) fibroblast-seeded collagen gels for all of the postunderload periods. Error bars represent standard errors of the mean.



**FIGURE 5:** The mean force generated at 20 hours by TGF- $\beta$ 1-stimulated fibroblasts derived from carpal ligament (n = 4), Dupuytren's nodule (n = 8), and Dupuytren's cord (n = 8). Note the increased force generation in all cell types after TGF- $\beta$ 1 stimulation, which is a significant increase compared with unstimulated values in Figure 1 (p < .001 for carpal ligament and Dupuytren's nodule; p < .002 for Dupuytren's cord).

tion is currently believed to result from a combination of cell-mediated contraction and simultaneous matrix turnover, leading to a physically shorter tissue matrix on the volar aspect of the fingers.<sup>13</sup> Clearly, in such a condition any abnormality of cellular contraction could be fundamental in the causation and progression of the contractures. Despite several previous studies investigating contraction in Dupuytren's fibroblasts,<sup>14–18</sup> few previous reports exist on the effect of mechanical stimulation on cytomechanical responses of these cells that are responsible for the remodeling and shortening of the matrix in Duputren's disease.<sup>19</sup>

# **Contraction profiles**

The contraction profiles quantitated over the experimental period showed that Dupuytren's disease fibroblasts consistently generated statistically significantly greater force compared with controls in this 3-dimensional experimental model. Moyer et al.<sup>28</sup> previously reported this result using a free-floating collagen gel model. The low force generation by carpal ligament fibroblasts was demonstrated previously in this laboratory.<sup>19</sup> These properties may be a function of the environment in which the cells are found, such as that in normal tendon or ligament tissue, where there is a dense matrix and relatively low turnover, the fibroblasts have no dynamic demands placed on them. The matrix shields' the fibroblasts<sup>29</sup> from stresses, and hence they are relatively noncontractile, as seen here.

The theory of tensional homeostasis<sup>11</sup> postulates that fibroblast in a 3-dimensional environment will contract to reach equilibrium where balanced forces exist between cellular contraction and the resistance of the environment. This plateau will be the particular group of fibroblasts' preferred tension. This pattern was not observed here in either of the Dupuytren's fibroblast types. In fact, there was ongoing contraction at the experimental end point of 20 hours. These cells may have escaped normal tensional homeostatic controls, or the actual point of tensional homeostasis may be higher and it may take longer to attain this level than in other fibroblasts. It may be that these cells use alternative mechanosensors and contractile elements for this to occur.

Of interest is the finding of no significant difference in force generation between nodule and cord fibroblasts within this study at 20 hours (p > .03). This finding differs from previously published data using this experimental model<sup>19</sup> and others,<sup>28</sup> although patient numbers were higher in this study,



**FIGURE 6:** A Mean 2-hour contraction profile gradient generated by TGF- $\beta$ 1-stimulated fibroblasts derived from carpal ligament (n = 4), Dupuytren's nodule (n = 8), and Dupuytren's cord (n = 8) plotted alongside the mean untreated Dupuytren's nodule (n = 9), cord (n = 10), and carpal ligament (n = 4) contraction gradients. Note the increased gradient in all cell types after TGF- $\beta$ 1 stimulation, which is a significant increase (\*p < .001 for Dupuytren's nodule; \*p < .05 for Dupuytren's cord and carpal ligament (n = 4), Dupuytren's nodule (n = 8), and Dupuytren's cord (n = 8). Note the increased gradient in all cell types after TGF- $\beta$ 1 stimulation, which is a significant increase compared with unstimulated values (p < .011 for carpal ligament; p < .005 for Dupuytren's cord).

which lends weight to our statistics. It may be hypothesized from this result that nodule and cordderived cells are similar in phenotype. However, the issue of cellularity in nodule and cord specimens is important. As stated by Luck,<sup>12</sup> the nodule is hypercellular, whereas the cord is hypocellular. Thus, although fibroblasts display similar force generation in an experimental model, if this is translated across to Dupuytren's tissue as a whole, the nodule appears to remain as the motor in the contraction process by virtue of its larger number of fibroblasts.

#### **Response to mechanical stimuli**

Tensional homeostasis in dermal fibroblasts also suggests that if external forces are applied on the matrix, the fibroblasts within it will react to return the tension toward the preferred plateau. Most striking was the mechanical response observed in Dupuytren's fibroblasts after a rapid uniaxial increase in external force. The fibroblasts contracted in response to a loading stimulus. This response could only be elucidated using a real-time experimental setup such as the culture force monitor. It is interesting that underloading also results in contraction, which demonstrates that Dupuytren's fibroblasts contract regardless of the mechanical stimulus applied. This further alteration of tensional homeostasis is especially interesting if extended to the clinical setting. Because it is a disease that affects the palmar fascia, fingers that are constantly flexing and extending will invariably transmit rapid changes in mechanical load to Dupuytren's tissue. An altered level of tensional homeostasis could lead to fibroblasts continuing to contract unchecked, resulting in tissue shortening



**FIGURE 7:** Histogram demonstrating the mean postoverload gradients for TGF- $\beta$ 1-stimulated fibroblasts derived from carpal ligament (n = 4), Dupuytren's nodule (n = 8), and Dupuytren's cord (n = 8). Note the trend for positive overload gradients in all cell types after TGF- $\beta$ 1 stimulation, after the first to third overload (p < .001 for carpal ligament; p < .01 for Dupuytren's nodule and cord).

in both passive loading and unloading conditions. Furthermore, an abnormal and opposite response to increased load, as encountered in the overloading experiments here, will exacerbate this. Indeed, there have been anecdotal reports of patients who continually attempt to overcome the condition by stretching their fingers, and develop particularly severe disease.<sup>30,31</sup> More recently, when external splints or fixation devices have been used to stretch and straighten contracted digits forcibly, contractures recur aggressively if the device is removed without surgical excision.<sup>32</sup> In this experimental model, Dupuytren's fibroblasts continue to contract regardless of the type of mechanical stimulus applied, whether overloading or underloading. This fundamental finding may help explain reports showing rapid recurrence of Dupuytren's disease after simple fasciotomy, with recurrence of contracture.<sup>33–35</sup> However, the human digit is subjected to many other forces that also may influence cellular behavior. This experimental model is able to observe only the reaction to uniaxial load, which may limit interpretation of these results.

#### Effects of TGF- $\beta$ 1 stimulation

In an attempt to understand contractile behavior further, we studied the effects of TGF- $\beta$ 1 stimulation. This growth factor is relevant in Dupuytren's disease, in which it has been found to increase both cellular contraction and fibrosis.<sup>18,22,27</sup> In this experiment not only did overall force generation in response to TGF- $\beta$ 1 increase for all cell types, but the rate of contraction also was altered, particularly in the early 2-hour stage. We postulate that TGF- $\beta$ 1 has a role in the early stages of fibroblast attachment and contraction. These findings correspond with a number of studies demonstrating increased contractility of dermal fibroblasts in response to TGF- $\beta$ 1.<sup>8,36–38</sup>

Not only did TGF- $\beta$ 1 alter the early phase of contraction, it also increased overall force generation in Dupuytren's fibroblasts and exacerbated the contractile response of these cells to tensional overloading. In this work, we did not explore the exact intracellular mechanisms by which TGF- $\beta$ 1 stimulation of fibroblasts may increase sensitivity to external mechanical load. TGF- $\beta$ 1 stimulation may result from upregulation of cell–matrix interaction molecules<sup>39</sup> or via a direct effect on the cytoskeletal motor.<sup>24</sup> Alternatively, TGF- $\beta$ 1 may upregulate any of the aspects of intracellular transduction pathways that are thought to be involved in the way cells recognize and respond to external forces.<sup>40</sup>

Transforming growth factor- $\beta$ 1 has been shown to enhance the contractile behavior of Dupuytren's fibroblasts that may underlie the fascial tissue shortening that leads to clinical contracture. By locally blocking the effects of TGF- $\beta$ 1, the enhanced contraction may be abrogated.

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