

The Contractile Properties and Responses to Tensional Loading of Dupuytren's Disease-Derived Fibroblasts Are Altered: A Cause of the Contracture?

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Dupuytren's disease causes disability because of the development of finger flexion deformities, with distinct nodule and cord formation. This results in physical shortening of the diseased fascial tissue through a combination of cell-mediated contraction and matrix remodeling. It is this fixed tissue fabric shortening that prevents finger extension. In this experimental study, the relative contractile properties of Dupuytren nodule- and cord-derived fibroblasts were quantified in a culture force monitor model, in comparison with normal carpal ligament fibroblasts. Nine nodule, 10 cord, and four carpal ligament fibroblast cell lines were studied; each cell line was derived from a separate patient. The contractile forces generated by nodule and cord fibroblasts were significantly greater than the force generated by carpal ligament fibroblasts. There were also significant differences between nodule- and cord-derived fibroblasts, with the nodule cells demonstrating the greatest contractile force generation. The contraction profiles of both cord and nodule Dupuytren fibroblasts demonstrated delays in the attainment of tensional homeostasis, with an absence of a plateau phase by 20 hours. After the contraction phase, cell-seeded constructs were subjected to a series of four uniaxial mechanical overloads and cellular responses were monitored during each subsequent 30-minute period. Dupuytren nodule and cord fibroblast responses were significantly altered, compared with carpal ligament fibroblasts, exhibiting an increased and opposite response. Dupuytren fibroblasts, particularly nodule fibroblasts, exhibited increased force generation and a delay in reaching tensional homeostasis. The data suggest that these cells have an inherently higher basal tension and contractile ability. This results in increased shortening of the matrix, and the delay in reaching tensional homeostasis might exacerbate this response. These results represent a theoretical framework regarding the fundamental processes involved in the pathogenesis

and progression of clinical flexion deformities in Dupuytren disease. (*Plast. Reconstr. Surg.* 113: 611, 2004.)

Dupuytren's disease is a fibroproliferative condition affecting the hands and fingers. It can cause significant disability, with the development of flexion deformities of the digits. The diseased tissue originates from normal fascial bands and demonstrates thickening and excess collagen deposition,¹ an increase in the ratio of type III collagen to type I collagen,²⁻⁴ and associated changes in other aspects of the extracellular matrix. Clinically, two forms of the disease, with nodules or cords, are encountered. Many authors have proposed that the disease progresses from nodules, which are active and are termed proliferative,⁵ through an involutinal phase to quiescent cords.^{6,7} Current theories suggest the cause of flexion deformities to be a combination of cell contraction and simultaneous matrix remodeling.⁴ Together, these cause gradual stepwise reductions in the length of the matrix. This fixed physical shortening of diseased fascia causes flexion deformities, by preventing digital extension. Since the report by Gabbiani et al.⁸ in 1971 and the subsequent demonstration of myofibroblasts in Dupuytren tissue,⁹ these specialized cells have been implicated in tissue

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contraction and the contractures observed in Dupuytren's disease. The clinical appearance of Dupuytren's disease as either nodules or cords has led many authors to examine the prevalence of the myofibroblast phenotype in these two discrete regions.¹⁰⁻¹² Nodules are rich in myofibroblasts; however, cords contain few or none of these specialized cells. We demonstrated that these differences are maintained for up to five passages from primary cell cultures derived from these specific regions (Bisson et al., unpublished data). In view of this skewed distribution and the presumed role of myofibroblasts, many authors have suggested that nodules are the source of active cell contraction in Dupuytren's disease.^{5,13}

Cell behavior in three-dimensional matrices is part of normal fibroblast activity, and attachment to, locomotion through, and force generation within such substrates are of key importance in the physiological setting. In fibrotic conditions such as Dupuytren's disease, abnormalities of this behavior may provide the key to understanding disease triggering or progression. Models for analysis of such behavior have been developed, initially for elucidation of the role of fibroblasts in wound healing and granulation tissue contraction but with equal applicability to fibrocontractive disorders such as Dupuytren's disease.^{14,15}

Elsdale and Bard¹⁶ first proposed the use of hydrated collagen lattices for the study of cellular behavior in three dimensions. It was subsequently observed that fibroblasts, when seeded into three-dimensional collagen lattices, formed a tissue-like substance¹⁷ with cells attaching to the matrix and causing contraction, which could be measured. Several tissue contraction models based on this phenomenon have been produced, many using circular gels set in multiwell plates, where a reduction in diameter provides an indication of cell contraction.^{14,18,19} Depending on the model used, different mechanisms have been proposed. In free-floating gels, a gradual contraction is observed, which is thought to be attributable to fibroblast attachment and locomotion.²⁰ In contrast, in fixed gels, significant isometric force builds and can be measured as the rapid contraction of the tethered collagen gels when they are released. Some authors have attempted to quantify the force generated within collagen lattices with strain gauges or force transducers.²¹⁻²³ Eastwood et al.²⁴ developed the culture force monitor, which is able to

measure actual forces within a fibroblast-populated collagen lattice with a sensitive calibrated force transducer. By studying dermal fibroblasts, those authors were able to define a typical contraction profile and divide it into three stages.²⁵ The same apparatus was subsequently used to develop the theory of tensional homeostasis,²⁶ on the basis of the responses of dermal fibroblasts to mechanical stimuli. Fibroblasts in three-dimensional matrices seem to exist at a preferred level of tension and contract or relax the matrix, in response to external forces, to maintain this tensional equilibrium.

We hypothesized that Dupuytren's disease fibroblasts might possess different contractile properties and exhibit altered responses to external forces, compared with nondiseased fibroblasts. This could underlie the tendency of the fibrotic fascial tissue to progressively shorten.

In this study, we used the culture force monitor to investigate the basic contractile properties of Dupuytren's disease cells derived specifically from nodules or cords, with carpal ligament fibroblasts for comparison. We then measured the responses of these cells to rapid increases in force with mechanical loading of the collagen lattices.

MATERIALS AND METHODS

Cell Culture

With local ethics committee approval, primary fibroblast explant cultures were obtained from Dupuytren nodules or cords or carpal ligament tissue. Cell cultures were bathed in normal growth medium, which was Dulbecco's modified Eagle's medium (Gibco, Paisley, Scotland) supplemented with 10% fetal calf serum (Gibco), 100 units/ml penicillin/100 μ g/ml streptomycin (Gibco), and 2 mM L-glutamine (Gibco) and buffered with 1 M *N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid solution (3.5 ml). Cells were routinely passaged at confluence and were used for experiments at passage 5 or less. Five million fibroblasts were used for each collagen lattice; generally, two T225 tissue culture flasks were required, with cells that were just subconfluent.

Culture Force Monitor

With the technique described by Eastwood et al.,²⁴ fibroblasts were trypsinized, counted, and suspended in normal growth medium at a con-

centration of 10^6 viable cells/100 μ l. Collagen gels were prepared with type I rat tail collagen (2.3 mg/ml; First Link UK, West Midlands, United Kingdom) mixed with $10\times$ minimal essential medium at a ratio of 10:1 (by volume). The resulting solution was neutralized with sodium hydroxide (5 M and then 1 M). Then, 500 μ l of fibroblast suspension was added to 4.5 ml of the gel solution and thoroughly mixed, producing a 5-ml gel suspension containing 5×10^6 cells. The solution was poured into a previously prepared and sterilized rectangular mold between floatation bars, which was placed in an incubator at 37°C to set. After gelation, the collagen lattice was floated by filling the mold with growth medium and gently detaching the gel from the sides and base of the mold with a sterile needle. The lattice was then mounted on the culture force monitor base stage (Fig. 1), with one floatation bar attached to a fixed point by its wire A-frames and the other attached to the force transducer (calibrated in dynes). Connection to a desktop computer allowed the force within the system to be measured every 1 second, in real time. Experiments were then left to continue for 20 hours.

At the end of that period, a series of uniaxial loading forces were applied to the gels, for investigation of the responses of the fibroblasts to increases in force. The overloading was achieved by rapidly moving the mounting stage a set distance (30 μ m) from the force transducer. After each overload, the cellular responses were monitored for 30 minutes before

an additional overload was applied. In total, four overloads were performed for each gel. Four carpal ligament, nine Dupuytren nodule, and 10 Dupuytren cord cell lines were investigated. Each carpal ligament cell line was derived from a separate patient and processed independently. The Dupuytren's disease tissue was split into defined regions of either nodule or cord; each nodule cell line was derived from a separate patient, as was each cord cell line. Each line was processed and tested independently. Cell viability within the collagen lattices, even for the poorly contracting cell lines, was confirmed at 24 hours in parallel experiments in which cells were recovered from the gels with collagenase D digestion and stained with trypan blue.²⁷

Mean contraction profiles were generated for each fibroblast type, and various parameters related to the profiles and responses to overloading were calculated and compared. Statistical analyses were performed with SigmaStat software (Jandel Scientific Software, San Rafael, Calif.), with unpaired *t* tests being applied to all sets of data being compared. Tests for normality of the data sets were passed in all cases, except for the comparison of the mean 20-hour nodule and carpal ligament gradients. In that case, a Mann-Whitney rank sum test was used.

RESULTS

The mean contraction profiles for the three cell types investigated are presented in Figure 2. Carpal ligament cells ($n = 4$) generated very

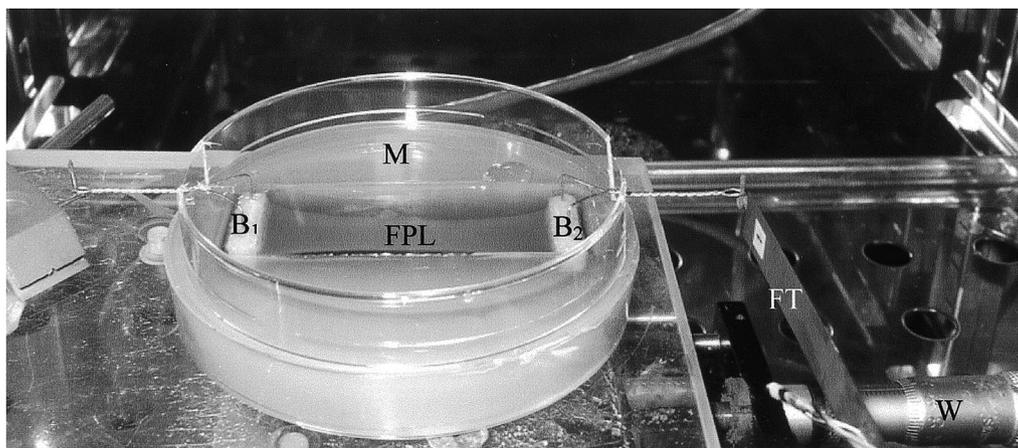


FIG. 1. Culture force monitor apparatus. A silicon polymer mold (*M*) fits within a Petri dish to form a well containing medium. Within this, a fibroblast-populated collagen lattice (*FPL*) is suspended between two floatation bars, one (*B*₁) attached to a fixed point and one (*B*₂) attached to the force transducer (*FT*). The mounting stage is movable in a uniaxial direction, toward or away from the transducer, with a micrometer-calibrated wheel (*W*).

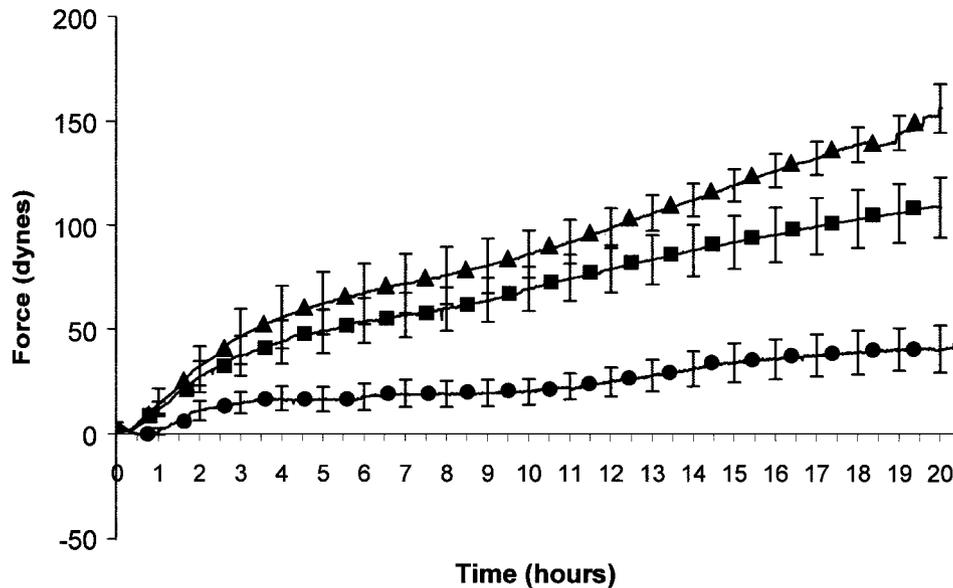


FIG. 2. Mean contraction profiles of nodule-derived cells ($n = 9$) (▲), cord-derived cells ($n = 10$) (■), and carpal ligament-derived cells ($n = 4$) (●). Error bars, standard errors. Nodule cells demonstrated a significantly higher contraction profile, compared with cord and carpal ligament cells. They also exhibited a steeper gradient at 20 hours.

little force during the course of the experiment, whereas nodule-derived fibroblasts ($n = 9$) demonstrated steady continued contraction for the entire 20-hour period. The mean contraction profile for cord fibroblasts ($n = 10$) was between those two. The peak force generated at 20 hours was determined for each cell line, and the means are presented in Figure 3. Nodule cell lines generated a mean \pm standard error of 145.1 ± 7.9 dynes, whereas cord cells generated 109.3 ± 14.2 dynes ($p < 0.05$). Carpal ligament fibroblasts generated a mean peak force of 39.9 ± 13.1 dynes, which was significantly less than the values for nodules and cords ($p < 0.001$ and $p < 0.05$, respectively).

As can be seen from Figure 2, there was continuing contraction in the Dupuytren cell lines at 20 hours. We quantified the rate of this continuing contraction for each cell type by calculating the mean rate of change of force production at 20 hours, in dynes per minute (force in dynes at 20.5 hours minus force at 19.5 hours divided by 60) (Fig. 4). It is clear that nodule cells were contracting at a greater rate at that point (mean \pm standard error, 0.09 ± 0.02 dynes/minute) than were cord cells (0.044 ± 0.009 dynes/minute), although the difference failed to reach statistical significance ($p = 0.068$); carpal ligament cells demonstrated a plateau. Both nodule and cord gradients at that time point were significantly greater than the carpal ligament gradient ($p <$

0.05). The failure of Dupuytren's disease-derived fibroblast contraction profiles to plateau was not noted when dermal fibroblasts were studied,^{25,26} which suggests that the former cells are delayed in achieving the equilibrium of tensional homeostasis. This has implications regarding how Dupuytren cells behave in a three-dimensional matrix and may explain why fibroblasts continue to contract.

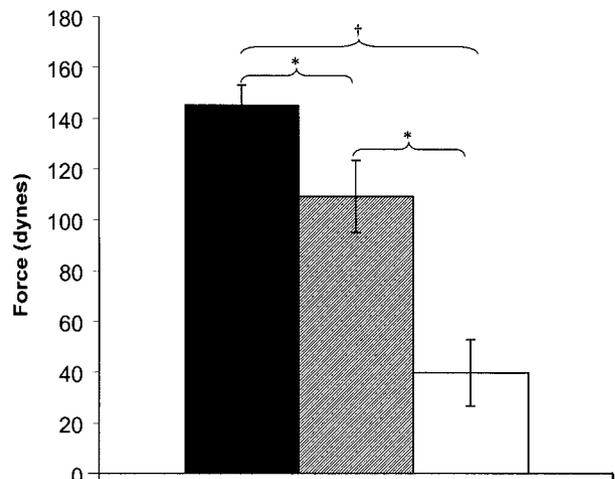


FIG. 3. Mean forces generated by nodule-derived cells ($n = 9$) (■), cord-derived cells ($n = 10$) (▨), and carpal ligament-derived cells ($n = 4$) (□) at 20 hours with the culture force monitor. Error bars, standard errors. Nodule fibroblasts generated significantly more force at 20 hours than did cord or carpal ligament cells. Cord cells generated significantly greater force than did carpal ligament cells ($*p < 0.05$, $†p < 0.001$).

The effects of applying a series of mechanical stimuli to the collagen lattices were then observed. The response of a blank control gel, containing no cells, to the four overloads is presented in Figure 5, *above*. After each overload, there was a gradual reduction in the force in the system during the subsequent 30 minutes. This was the result of the elastic properties of the collagen gel. Equilibrium seemed to be reestablished after approximately 30 minutes, as illustrated after the fourth overload in Figure 5, *above*. When cells were seeded into the lattice and similar uniaxial overloads were applied, altered patterns were observed for many cell lines in the first postoverload period, representing the cellular responses to the mechanical stimuli. Figure 5, *below*, illustrates a typical nodule cell line in which there was an increase in force in the system; the resident fibroblasts seemed to contract in response to the first overload. This was an unexpected finding, given the current theories regarding tensional homeostasis, but seemed to occur only after the first overload. As demonstrated in Figure 6, this abnormal response occurred mostly in nodule cell lines, in which the mean gradient of the first postoverload period was $+0.10$ dynes/minute; cord cell lines demonstrated a similar but lesser response, at $+0.05$ dynes/minute. Both differences were significant, compared with control collagen gels without cells (-0.23 dynes/minute) or carpal liga-

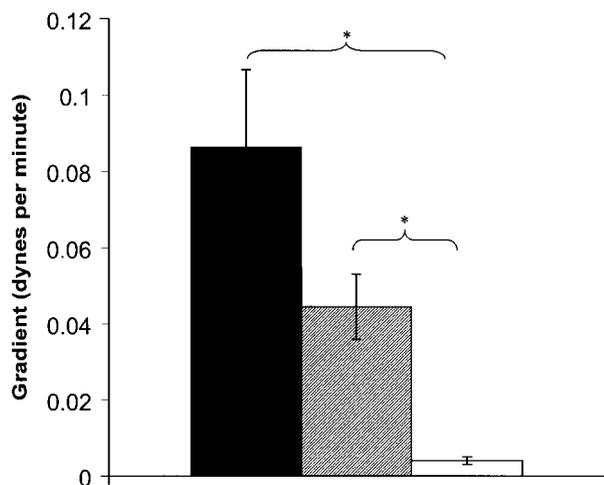


FIG. 4. Mean gradients of contraction profiles at 20 hours for nodule-derived cells ($n = 9$) (■), cord-derived cells ($n = 10$) (▨), and carpal ligament-derived cells ($n = 4$) (□). Error bars, standard errors. Nodule fibroblasts were contracting at a greater rate at that point than cord cells, which in turn were contracting at a greater rate than carpal ligament cells, which exhibited a plateau ($*p < 0.05$).

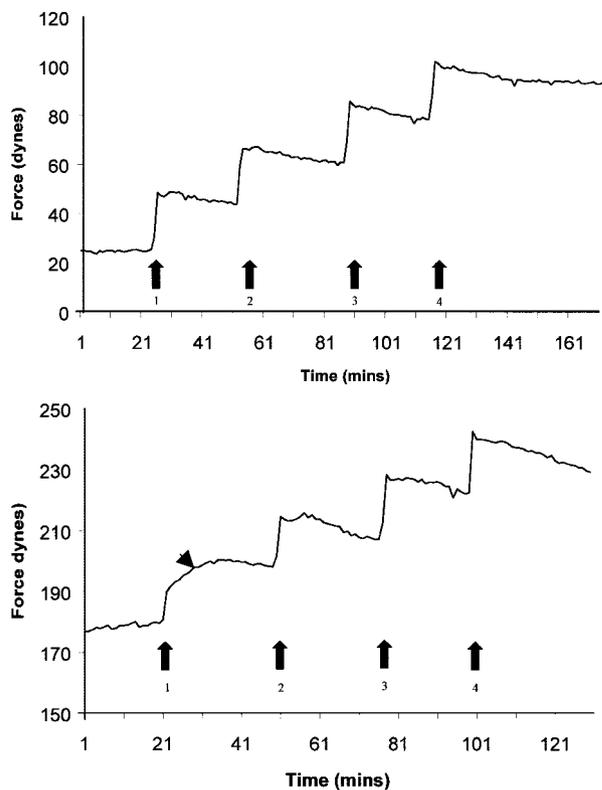


FIG. 5. Typical patterns of responses to each of four uniaxial overloads (arrows) in a control acellular gel (*above*) and in a gel seeded with 5×10^6 nodule-derived fibroblasts (*below*). The difference in the contraction profile in the 30 minutes after overload 1 for the nodule fibroblast-populated lattice (arrowhead) should be noted.

ment cell lines (-0.16 dynes/minute) ($p < 0.01$). Carpal ligament cells did not demonstrate a response to the overload and behaved in the same manner as control acellular collagen gels throughout the overloading cycles. In other words, the carpal ligament cells were “mechanoinsensitive.” The postoverload gradients for subsequent periods returned to control values for all cell types, and there were no significant differences among Dupuytren fibroblasts, carpal ligament fibroblasts, and control gels.

DISCUSSION

Using the culture force monitor model, we were able to determine the effects on collagen lattice contraction of fibroblasts derived specifically from Dupuytren nodule and cord regions. We compared these effects with those of fibroblasts derived from a similar, nondiseased, fascial tissue (carpal ligament). Many authors have used the same cells for comparisons in Dupuytren’s disease studies.^{4,15,19,28} The major-

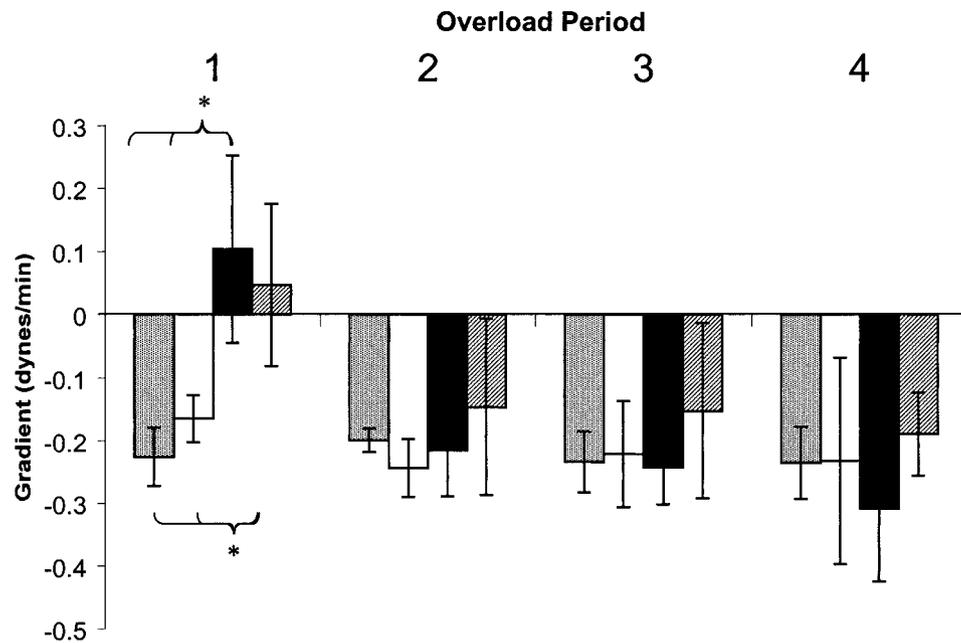


FIG. 6. Mean gradients in each of the four 30-minute postoverload periods for collagen gels seeded with no cells ($n = 3$) (▨), nodule-derived cells ($n = 9$) (■), cord-derived cells ($n = 10$) (▧), or carpal ligament-derived cells ($n = 4$) (□). A negative value represents a decrease in force during the 30-minute period, whereas a positive value indicates a contractile response from the cells. There were significant differences between both nodule and cord cell responses and acellular and carpal ligament cell responses in the first postoverload period ($*p < 0.01$). There were no significant differences in subsequent periods.

ity of previous work with the culture force monitor focused on dermal fibroblasts,²⁴⁻²⁶ for which a typical contraction profile has been described.²⁵

Contraction has been divided into three phases. Phase 1 is thought to be attributable to cellular attachment and locomotion through the matrix and is characterized by an early rapid contraction until approximately 8 hours. There is a plateau of force generation in phase 2, and by 15 hours a steady state, with balanced forces of cell contraction versus matrix tension, becomes established. In our study with Dupuytren's disease-derived fibroblasts, there was an identifiable first phase in which more rapid contraction was evident (Fig. 2). After that phase, there was continued steady contraction in both nodule and cord cell lines until the conclusion of the experiments. Carpal ligament profiles produced very little force (only 40 dynes, compared with approximately 125 to 200 dynes reported for dermal fibroblasts²⁴⁻²⁶) and, although there was a slow sustained contraction, a plateau occurred by 20 hours, in contrast to the Dupuytren's disease-derived fibroblasts.

Grinnell²⁰ described two main models of cell-

mediated collagen lattice contraction, namely, free-floating gels, which contract slowly with time and in which cell locomotion and attachment are thought to be the mechanisms, and the stressed released model, in which physical cell contraction is proposed to be the mechanism when a tethered mature gel is released from its attachments and undergoes rapid contraction. This contraction is related to the amount of isometric force the cytoskeleton has developed. This type of contraction has been linked to myofibroblasts and protomyofibroblasts,²⁹ with contractile force being developed through the actin cytoskeleton. Several investigators correlated contraction with myofibroblast numbers or α -smooth muscle actin contents.^{15,30}

In our model, the culture force monitor demonstrates some characteristics of both mechanisms, with the early phase of contraction being analogous to a free-floating gel as cells attach and move through the matrix²⁵ and later force being essentially in a tethered system, with the gel restrained between the force transducer and a fixed point. The overall force generated at 20 hours may reflect a combination of these two mechanisms. Nodule-derived

cells seem to have increased capabilities in both respects, compared with cord-derived cells, with a rapid early rate of contraction and greater continued contraction at 20 hours. Therefore, the overall force generated at 20 hours is significantly greater in nodule-derived fibroblasts than in cord-derived fibroblasts (Fig. 2).

In a recent study, Moyer et al.⁷ noted that nodule fibroblasts were more contractile than cord fibroblasts in a free-floating model, which corresponds to our findings. They also demonstrated that this increased contraction disappeared in late passages, with aged nodule fibroblasts acting like cord cells, which supports the theory of nodule-to-cord progression. The free-floating model they used, however, was not able to measure the actual contractile forces that developed or record the responses of cells to mechanical loading, as our model did. Other authors demonstrated enhanced contractility of Dupuytren nodule fibroblasts, compared with carpal ligament fibroblasts, but only when the cells were subdivided into high- and low- α -smooth muscle actin expression groups.¹⁵ In contrast to our results, those authors initially observed equivalent levels of collagen lattice contraction by carpal ligament fibroblasts in a stressed released model.¹⁹ Their lattices were left for 5 days before release, compared with our 24-hour experiments.

Neither of those models (free-floating or stressed released collagen gels) allows cell-mediated contraction to be observed in a setting analogous to that occurring *in vivo*, in which cells are never released from surrounding matrix attachments. The culture force monitor configuration does permit such observations, with the collagen gel transmitting forces to the transducer while remaining tethered at all times; therefore, it provides a more accurate model for study. Interestingly, with a free-floating collagen lattice contraction assay, others demonstrated that Dupuytren nodule fibroblasts were less contractile than dermal fibroblasts, although all tissue was obtained from patients with advanced disease.¹⁴ Our Dupuytren's disease specimens were all obtained from primary operative procedures.

In comparing our results with published culture force monitor values for dermal fibroblasts, we observed equivalent force generations by nodule fibroblasts in 20 hours. The lack of force generation by carpal ligament fibroblasts was unexpected, because most cell

lines investigated with this model developed significant contraction. Rayan and Tomasek¹⁹ reported comparable contraction by nodule and carpal ligament fibroblasts. Rabbit tendon fibroblasts displayed late contraction, however, developing only 9 dynes/ 10^6 cells at a similar time point.²⁵ This force generation is analogous to the findings we noted with carpal ligament and may reflect the behavior or environment of these cells *in vivo*, where perhaps they are stress-shielded³¹ by dense surrounding matrix.

Brown et al.²⁶ proposed the theory of tensional homeostasis, demonstrating that dermal fibroblasts develop a steady state tension in which forces are balanced. If there is a subsequent increase in the external load, then resident cells rapidly respond by relaxing, to remove this tension and return to their preferred level. The converse is also true, with decreased external loads stimulating cell contraction. On the basis of our studies, we suggest that the continued contraction of Dupuytren fibroblasts for 20 hours represents a delay in reaching the equilibrium of tensional homeostasis.

Although the actual force generated at that time point was similar to that developed by dermal fibroblasts, the eventual level may be much greater, depending on when equilibrium is reached. This may signify an inherent alteration in Dupuytren fibroblasts, for which the preferred level of tension is greater. In diseased tissue, resident fibroblasts could steadily contract in concert to achieve this. The abnormal contraction combined with matrix remodeling, which seems to be increased in Dupuytren's disease when tension increases,^{32,33} may well be sufficient to overcome extension forces in affected digits, leading to clinical flexion deformities.

The results of our overloading studies support the theory that tensional regulation is altered in Dupuytren cells. A contractile response to increased externally applied force, as observed in the first overload periods, does not follow the theory of tensional homeostasis. Nodule fibroblasts demonstrated this most clearly, with cord fibroblasts exhibiting a lesser abnormal response. In general, the increase in force of each overload was approximately 20 to 30 percent of the total developed force, which should have been sufficient to elicit a typical cell-mediated homeostatic response.²⁶ The opposite was observed for Dupuytren cells in the first postoverload period.

It is unclear why this contraction disappeared with subsequent overloads. It could be linked to the continued increase in the contraction profile (i.e., because the cells had not yet reached tensional homeostasis). There was, for example, some correlation when the 20-hour gradient was compared with the first post-overload period gradient (data not shown). Theoretically, if the cells had not yet reached their preferred level of tension despite an externally applied load, there would be a continued contractile response. In our experiments, this homeostatic equilibrium might have been attained only after a second overload force and an additional 20 percent increase in the total tension in the system. Alternatively, this contraction in response to applied loads could be an inherent abnormal response of Dupuytren cells and could act as a positive feedback loop, causing further cell-mediated contraction. The disappearance of this response in subsequent postoverload periods might be attributable to increased matrix stiffness in this second situation.

Recent results from our laboratory suggested that increased loading of acellular collagen gels caused increased matrix stiffness. Matrix stiffness, for example as a result of increased collagen concentration, is known to affect fibroblast contraction^{17,34} and can also be responsible for stress-shielding of cells within a three-dimensional environment,³¹ such that they perceive less of the overall forces applied to the matrix. Overloads after the first one may increase matrix stiffness such that Dupuytren fibroblasts are no longer able to perceive the change in load and become mechanoinensitive.

In any case, an abnormal response to tensional loading by Dupuytren fibroblasts is a significant finding. A clinical situation in which Dupuytren cells are continually striving to reach tensional homeostasis but failing to attain it could be envisioned; further external forces would cause additional cellular contraction, as observed here after the first overload. This positive feedback would exacerbate the condition and, in combination with matrix remodeling, could cause unchecked disease progression.

This may also explain the observation that thick aponeurotic cords develop, with rapid clinical contractures, among patients who continually manually stretch their fingers in an attempt to overcome the disease.^{35,36} The regular extension would provide an external load-

ing of the Dupuytren cells, causing not only additional collagen deposition but also further cell contraction. Similarly, among patients who have undergone continuous elongation techniques³⁷ to extend severely contracted digits before surgical treatment, it is noted that the contractures recur very quickly if the operation is delayed after the traction is removed.^{32,37} In this situation, the external stretching or overloading force applied to the resident cells could be providing strong positive feedback and, as soon as the external fixation is removed, there is an aggressive cell-mediated contraction combined with the high level of matrix remodeling that has been demonstrated to exist during this procedure.³² Our *in vitro* results certainly suggest that there is little value of splintage alone as therapy for Dupuytren's disease. More controversially, we raise questions regarding the use of postoperative splinting. Although in general the continuity of tight tissue bands is disrupted after operative procedures, does splinting cause loading of residual Dupuytren's disease fibroblasts, thus stimulating further contraction?

In summarizing our findings from these studies of Dupuytren fibroblast-mediated matrix contraction, we have attempted to combine the findings with current knowledge of Dupuytren's disease, to formulate an overall hypothesis of clinical contracture development. This is illustrated in Figure 7, with numbers indicating where our findings fit. We propose that the Dupuytren nodules are responsible for a greater proportion of tissue contraction than are cords, although both may be involved as a contractile unit. We support the view that there may be a natural history of disease progression from nodule to cord tissue, as first outlined by Luck.⁵ Resident cells, especially those within nodules, would apply force to the surrounding matrix in an attempt to achieve a higher level of tensional homeostasis, as evidenced by the failure of culture force monitor-measured contraction to plateau (Fig. 2). Cell contraction could be exacerbated by positive feedback from external forces, as observed in our overloading studies (Figs. 5 and 6). Factors such as rapid continuous matrix remodeling could have dual effects. First, remodeling could cause constantly changing cell matrix interactions, stopping the generation of balanced forces by fibroblasts and preventing the acquisition of homeostasis such that cells continue to contract. Second, as the matrix

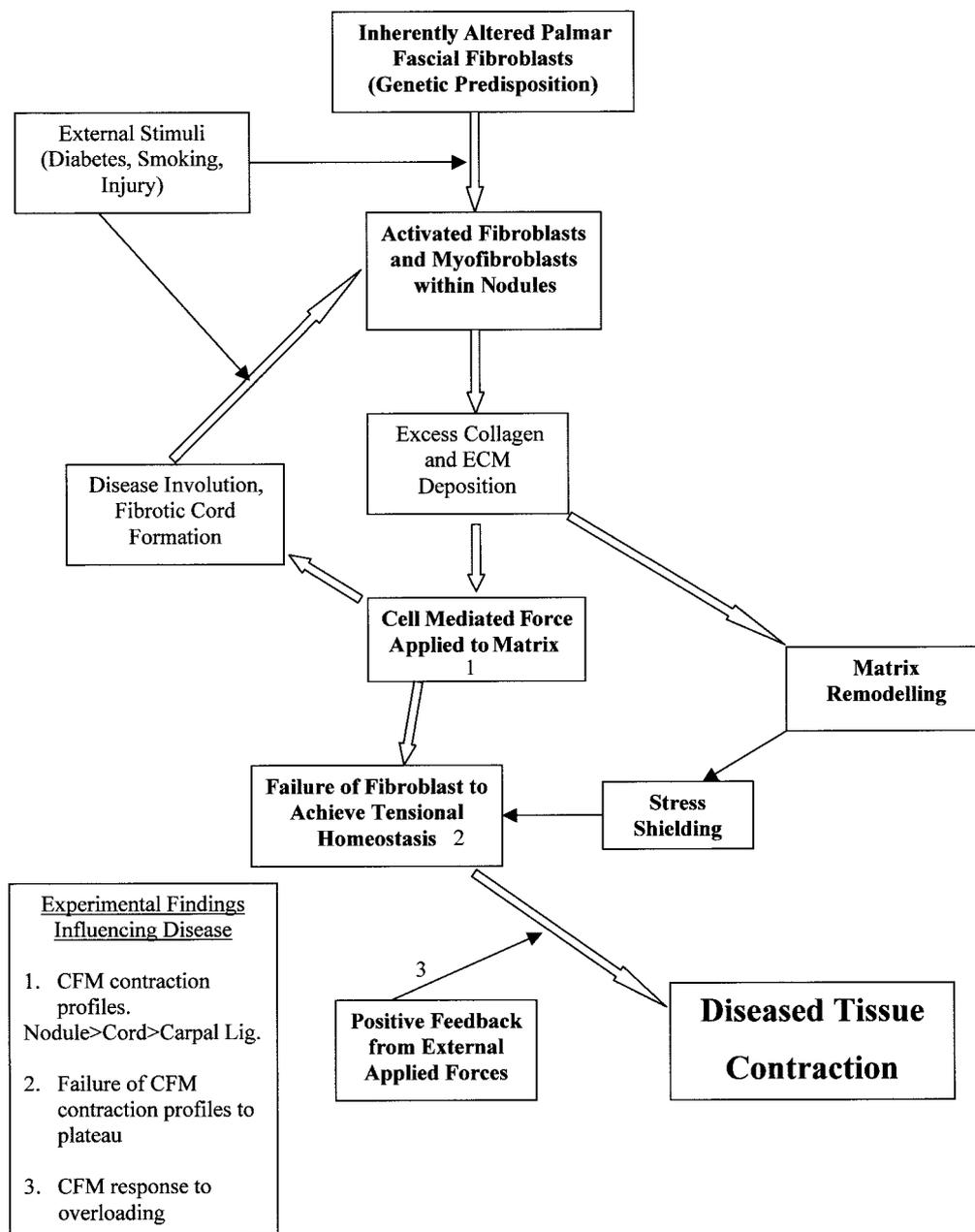


FIG. 7. Flow diagram depicting a theoretical framework for the development of clinical Dupuytren's contractures, incorporating findings from our investigation of nodule-derived and cord-derived fibroblasts. *CFM*, culture force monitor; *ECM*, extracellular matrix; *Lig.*, ligament.

contracts, remodeling could cause physical tissue shortening. The two processes together might lead to unchecked fascial contraction, resulting in clinical digital flexion deformities.

Using *in vitro* models, we have examined the specific properties of nodule- and cord-derived fibroblasts, to correlate them with the natural history of Dupuytren's contracture. Studies of the contractile properties of Dupuytren's disease cells and their response to tensional stim-

uli have provided us with insights into how cells within the diseased tissue matrix might behave to cause contracture development and progression. Further work now needs to be performed to identify the interactions between these factors and the process of matrix remodeling. From such studies could emerge a clear understanding of why Dupuytren's disease occurs and progresses. Future therapies for the condition could target the process of cellular con-

traction or even the more basic function of cell-matrix interactions, to halt contracture advancement or prevent recurrence.

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Discussion

The Contractile Properties and Responses to Tensional Loading of Dupuytren's Disease-Derived Fibroblasts Are Altered: A Cause of the Contracture?

Discussion by Boris Hinz, Ph.D.

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In this article, Bisson et al. demonstrate that fibroblasts derived from Dupuytren nodules and cords exhibit greater contractile activity and respond differently to mechanical stimulation, compared with control fibroblasts from carpal ligament. The authors attached fibroblast-populated collagen gels to an isometric force transducer in a culture force monitor, which is particularly useful for recording adaptations of fibroblast contractile activity to changes in the external mechanical load. The most interesting finding of this work was that Dupuytren fibroblasts, unlike control cells, did not reach a constant level of tensile force development during the 20-hour culture period and reacted to a first mechanical overloading with increased force development, instead of the expected relaxation. This failure to reach tensional homeostasis may be one explanation for the dysregulation of fibroblast contractile activity and the development of contractures *in vivo*. The authors propose that Dupuytren fibroblasts favor a higher baseline level of matrix stress for tensional homeostasis, suggesting correct stress perception. However, it is conceivable that these cells are simply unable to appropriately detect and compute the level of extracellular load, leading to cellular underestimation of the actual stress and consequently to a contractile overreaction.

How do fibroblasts sense mechanical stimuli? In the past few years, it has become evident that a functional cytoskeleton of contractile actin filament bundles (stress fibers) is crucial for the perception of extracellular stress and the development of tensile force.^{1,2} The trac-

tional forces developed by migrating and expanding fibroblasts reorganize the loose collagen matrix during the initial culture force monitor growth phase (approximately 9 hours) and increase matrix stiffness,³ which leads to development of the protomyofibroblast phenotype (i.e., the formation of stress fibers and prominent matrix contacts) (Fig. 1).⁴⁻⁶ Cell-matrix adhesions seem to be the primary sensors for mechanical tension,^{5,7} and Dupuytren fibroblasts have been demonstrated to develop specialized fibronexus adhesions.^{8,9} In addition to perceiving stress, cell-matrix adhesions are responsible for transmitting tensile forces to the substrate, and it was recently suggested that subsequent phases of tractional force generation in the culture force monitor model are correlated with the expression of different cell-matrix receptors.¹⁰ Therefore, alterations in the molecular composition and signaling properties of cell-matrix adhesions may be key elements in the development of pathological contracture, and a thorough investigation of these structures with different mechanical loads may facilitate elucidation of their role in Dupuytren's disease.

Another possibility for the inherent alteration in Dupuytren fibroblasts is their capacity to develop greater net forces, compared with control cells, leading to a defect in the "fine-tuning" of their contractile responses to extracellular factors. In contrast to normal fibroblastic cells, fibroblasts in Dupuytren nodules have been demonstrated to express high levels of α -smooth muscle actin,¹¹ which is the actin isoform typically expressed by smooth muscle

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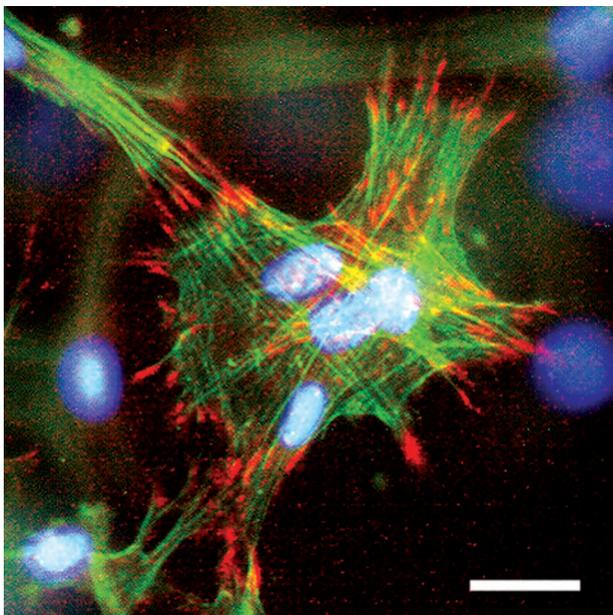


FIG. 1. Rat lung fibroblasts grown for 5 days in three-dimensional, attached (stressed) collagen gels, resembling the late growth phase in the culture force monitor model. Cells were fixed and immunostained for α -smooth muscle actin (green), localized in stress fibers, and vinculin (red), a component of cell-matrix adhesions. Bar, 50 μ m.

cells, and to modulate into differentiated myofibroblasts (Fig. 2).^{2,6} It is increasingly accepted that expression of α -smooth muscle actin alone is sufficient to enhance fibroblast contractile activity,⁶ suggesting that the same external stimulus can lead to greater tension development when stress fibers contain α -smooth mus-

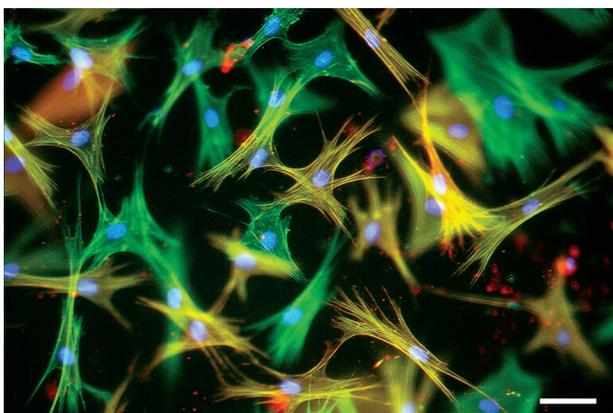


FIG. 2. Rat lung fibroblasts grown in attached collagen gels. With increasing matrix stress attributable to cell-derived tensile forces, fibroblasts first developed cytoplasmic β -actin-containing stress fibers (green) and gradually increased expression of α -smooth muscle actin (red). It should be noted that, after 3 days of culture, only a subpopulation of cells (approximately 50 percent) coexpressed α -smooth muscle actin and cytoplasmic β -actin in stress fibers (resulting in yellow staining); this fraction increased to approximately 90 percent after 5 days. Bar, 50 μ m.

cle actin, compared with stress fibers containing only cytoplasmic actins. Loss of stress, such as caused by the isolation of fibroblasts from tissue or the trypsinization of cells from culture dishes to initiate culture force monitor culture, leads to rapid disassembly of stress fibers and degradation of α -smooth muscle actin.¹² Polymerization of cytoplasmic actin precedes the expression and incorporation of α -smooth muscle actin in stress fibers¹³ (Fig. 2), which depends on multiple factors, such as transforming growth factor- β ,¹⁴ ED-A fibronectin,¹⁵ and high levels of extracellular stress.^{12,16} The continuously increasing tensile force of Dupuytren fibroblasts in the culture force monitor may reflect the longer time required to produce and to orchestrate these factors. It would be interesting to study whether Dupuytren fibroblasts are able to reach tensional homeostasis after longer culture times; to prevent effects from increasing cell numbers, such experiments would need to be performed in the presence of inhibitors of cell proliferation. To conclude, this article presents a nice in vitro model of how clinical Dupuytren's contractures may develop, and it will be of great interest to use this model to correlate contractile cell responses with cytoskeletal properties.

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