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Microfilament system in the microvascular endothelium of the palmar fascia affected by mechanical stress applied from outside

Investigation of effects of TEC

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Abstract The effect of externally applied mechanical stress was investigated by thin section electron microscopy of the microvessels in the unaffected palmar fascia in the carpal tunnel syndrome and in patients with Dupuytren's contracture before and after application of a continuous elongation device. In the unaffected palmar fascia the microfilaments of the endothelial cells were connected to a few adherens junctions and focal contacts; stress fibres were absent. In the cord of Dupuytren's disease the microfilaments were increased in quantity. The length ratios of the connections with the lateral and basal cell membrane were significantly higher than in the control group and increased to an even greater extent in the continuously extended fascia. Stress fibres appeared in the endothelial cells of postcapillary venules in the non-extended cord and in the endothelium of both arterioles and venules after extension elongation. The numerous intermediate filaments and the rare microtubules remained unchanged in the endothelial cells of all palmar fasciae analysed. In the endothelial cells of the microvessels the mechanical stress applied from outside mainly affected the contractile component of the cytoskeleton.

Key words Stress fibres · Adherens junctions · Focal contacts · Intermediate filaments · Dupuytren's contracture

Introduction

The cytoskeleton of vascular endothelial cells has important functions. It connects the cells to each other and to the subendothelial extracellular matrix, thus stabilizing the cell layer against the blood flow (reviewed in [17]).

Many investigations have been performed to demonstrate the influence of shear stress on the endothelial cells. However, in *in vitro* studies, these cells lose their physiological environment and, therefore, their reactions cannot be easily translated to the *in situ* situation [28]. *In vivo* studies analysing the endogenous haemodynamic effects on the endothelium have been restricted to the great arteries (such as the aorta or carotid artery) of different species [13, 18, 20, 35, 36]. A detailed analysis of the endothelium in microvessels (terminal arterioles, capillaries, postcapillary venules) has been carried out in a few organs using the laser confocal microscope [32] or by thin section electron microscopy [10].

Calculation of the wall shear rate indicates that this is higher in the small arteries than in the large ones [15, 22, 30, 33]. This observation can explain the presence of stress fibres in the endothelium of the small arteries and arterioles [21, 32]. In capillaries and postcapillary venules the diffuse distribution of microfilaments suggests a low shear stress [32].

To elucidate whether exogenous mechanical factors might influence the endothelial cells of the vessel wall, we investigated the vessels inside the human palmar fascia undergoing three different stress situations due to tension: continuous changes in mechanical forces as occurring in the palmar fascia during movements of the healthy hand; predominance of active extending forces which, during attempts to move the finger, are opposed to the progressive flexion contraction in patients with Dupuytren's disease; continuous passive extension with constant force applied to the contracted fascia of patients with Dupuytren's contracture treated with a continuous extension device. In thin section electron microscopy remarkable differences between the three groups were found in the quantity of microfilaments and in the extent of their connections with the plasma membrane in the vascular endothelium. Moreover, the appearance of the stress fibres in the endothelial cells of arterioles and postcapillary venules in the continuously elongated cord suggests a correlation between external stress acting on the palmar fascia and organization of the cytoskeleton.

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Table 1 Patients data for the quantitative analysis

Patient group	Age (years)	Sex	Hand providing the tissue
Unaffected palmar fascia	53	Female	Right
	60	Female	Right
	63	Female	Left
	64	Female	Left
Cord	56	Male	Right
	61	Male	Left
	63	Male	Right
	64	Female	Left
Extended cord	58	Male	Right
	60	Male	Right
	61	Male	Left
	63	Female	Right

Materials and methods

The following specimens were analysed: unaffected palmar fascia from five patients (female; age 53–74 years; three right hands, two left hands) with carpal tunnel syndrome, no history of Dupuytren's disease, and normal mobility of the hand; the cord in the excised palmar fascia of 20 Dupuytren's patients (17 male, 3 female; age 33–69 years; 11 right hands, 9 left hands); the palmar fascia from four patients (three male, one female; age about 60 years; three right hands, one left hand) which was continuously extended for 3 weeks prior to surgery, as described previously [26]. In brief, two pins inserted through the fourth and fifth metacarpal bones supported the continuous extension device, which was connected by a Kirschner wire to the phalanx of the contracted finger. A screw enabled the retracted finger to be extended 2 mm per day. The treatment allows, in cases of severe contracture, the removal of the diseased palmar fascia with a reduced risk of complications [27].

All specimens were fixed immediately after surgery in 2.5% glutaraldehyde (Polysciences, Warrington, Pa., USA) in 0.1 M sodium cacodylate-hydrochloric acid (Schuchardt, Hohenbrunn, Germany) buffer, pH 7.3 for 6 h. After rinsing in the same buffer, the tissue was postfixed in 1% osmium tetroxide and 1.5% potassium ferrocyanide in water for 1.5 h [19], followed by dehydration in graded alcohols and embedding in Epon. Sections 1 µm thick were stained with 1% toluidine blue (Merck, Darmstadt, Germany) dissolved in 1% sodium tetraborate (Riedel-de Häen, Seelze, Germany) in water, and analysed with a light microscope. Larger arteries and veins in the surroundings of the palmar fascia could not be analysed in comparison, since they were not surgically removed. In the palmar fascia only microvessels were present. Selected regions were chosen for thin sections that were examined with a Siemens Elmiskop 101 electron microscope after staining with uranyl acetate and lead citrate.

For quantitative analysis the specimens of four patients in each group were chosen. They were age-matched, but could not be sex-matched. The subject details were listed in Table 1. In each patient, complete cross sections of ten arterioles, ten capillaries and ten venules were documented in electron micrographs at a final magnification of ×16,000 and analysed with a Videoplan image analyser (Kontron-Elektronik, Munich, Germany). Without knowledge of the group from which the microvessels had been taken, the number of adherens junctions and focal contacts in each vascular endothelium entirely contained within the field was determined. The length of the adherens junctions (as indicated in Fig. 2) and that of the lateral plasma membrane of the endothelial cells were traced on a digitizing tablet and their ratio was calculated [34]. In the same way, the length of the focal contacts (Fig. 2) and that of the endothelial plasma membrane in contact with the limiting basement membrane were measured and their ratio was evaluated. The mean values and the standard deviation in each group are listed in Tables 2–5 and considered for statistical analysis. Using the

statistic package of the Videoplan image analysis system, the Gauss distribution was confirmed by the χ^2 -approximation test in all groups [8]. Significant ($P < 0.05$) and highly significant ($P < 0.001$) differences between the three groups of patients were determined by the approximate *t*-test and the F-test [8].

Results

Morphological analysis

The unaffected palmar fascia was characterized by a lattice of bundles of collagen fibres. In the septa separating these bundles, single terminal arterioles and postcapillary venules were distributed. Capillaries were only present inside the bundles. The endothelium of the microvessels exhibited a cytoskeleton consisting of a few microfilaments, numerous intermediate filaments and rare microtubules (Fig. 1). The microfilaments abutted on to small adherens junctions or formed isolated focal contacts with the abluminal plasma membrane (Fig. 1).

In Dupuytren's disease, only the cords were compared. It is well known that stationary and proliferating cells differ in the extent and distribution of their microfilaments [6]. Therefore, the nodules, which have a high proliferation rate, were not examined.

In the cord of the contracted palmar fascia, thick bundles of collagen fibrils were irregularly distributed and separated by thin septa with microvessels. The microvascular endothelium was filled with many intermediate filaments, but microtubules were scanty. Microfilaments, more frequently seen than in the unaffected palmar fascia, were connected to enlarged adherens junctions and focal contacts (Fig. 2). In the postcapillary venules stress fibres, characterized morphologically by intercalated cytoplasmic dense bodies (reviewed in [7]), were bound to the basal plasma membrane (Fig. 2).

In the cord of the extended palmar fascia the microvessels were arranged in the septa parallel to the collagen bundles, which had the same orientation as the externally applied passive mechanical stress generated by the continuous extension device (Fig. 3). The endothelium of the microvessels had a pronounced texture of intermediate filaments, which was comparable to that of



Fig. 1 Unaffected palmar fascia. The endothelium of a postcapillary venule contains numerous intermediate filaments (*arrowheads*) and some microfilaments connected with isolated focal contacts (*arrow*). The lumen of the vessel (*L*) and the nucleus of an endothelial cell (*N*) are identified. $\times 80,000$; bar $0.5 \mu\text{m}$

the unaffected palmar fascia and the non-extended Dupuytren's specimens; microtubules were rare. In comparison with the other groups, microfilaments were most numerous in the extended fascia; they were connected at the enlarged adherens junctions with neighbouring endothelial cells and at extended focal contacts with the subendothelial extracellular matrix. In addition, **remarkably large stress fibres appeared in the endothelial cells of terminal arterioles and postcapillary venules** (Fig. 4a,b). They frequently showed dense bodies and were bound to the basal plasma membrane at extended focal contacts (Fig. 4b).

Quantitative analysis

To verify the remarkable changes in the microfilament system of the vascular endothelium between the three groups of patients, quantitative analysis was performed on the number and size of adherens junctions and focal contacts, both structures representing the sites where the microfilaments are connected to the plasma membrane.

Whereas in capillaries and postcapillary venules of the cord the number of adherens junctions per endothelial profile increased highly significantly in comparison with the same vessels of the unaffected palmar fascia, no

differences were found between the contracted and the extended palmar fascia (Table 2). The arterioles retained their density at the adherens junctions in all groups of patients (Table 2). To compare the different size of these junctions, their length was related to that of the endothelial lateral plasma membrane [34]. Highly significant differences in the ratio were demonstrated in all types of microvessels analysed (Table 3). The size of the adherens junctions increased in the contracted cord when compared with the unaffected palmar fascia and had the highest values in the extended cord (Table 3). In terminal arterioles and postcapillary venules the increase (both in the contracted cord compared to the unaffected palmar fascia and in the extended cord compared to the contracted palmar fascia) amounted to 30%–31%, in the capillaries the increase was 19%–20%.

The number of focal contacts per vascular endothelial profile increased significantly in the contracted cord compared with the unaffected palmar fascia (Table 4). However, in arterioles and postcapillary venules of the extended fascia, the focal contacts had a reduced, but not significantly altered density and in capillaries they were unchanged when compared with the non-extended cord (Table 4). In contrast, the ratio of the length of the focal contacts related to the plasma membrane in contact with the basement membrane changed in a highly significant manner. The microfilaments were bound to 10%–21% of the basal plasma membrane in the unaffected palmar fascia, to 35%–48% in the contracted cord and to 56%–85% in the extended cord; the highest values were consistently found in the arterioles and the lowest in the capillaries (Table 5). Therefore, in each



Fig. 2 Dupuytren's disease, contracted cord. Inside the endothelium of a postcapillary venule the microfilaments and the stress fibre (arrow) are connected with an adherens junction (A) and a focal contact (F). The length of both junctions measured in the quantitative analysis are indicated with bars. $\times 80,000$; bar $0.5 \mu\text{m}$

type of microvessel the increase in size was comparable; related to the unaffected palmar fascia it amounted to 25%–27% in the contracted cord and to 46%–64% in the extended cord.

Discussion

We have made a morphological and quantitative analysis of the cytoskeletal alterations in the endothelial cells of the microvessels in the unaffected palmar fascia and in the palmar fascia of patients affected by Dupuytren's disease. In the unaffected palmar fascia, the endothelial cells of the microvessels display few microfilaments compared with the diseased palmar fascia. Several cytokines have been found in the active stage of Dupuytren's disease [1, 2, 4, 16, 31]. Some of these stimulate the ex-

pression of alpha smooth actin in fibroblasts and myofibroblasts [9, 29] and may have an effect on the aggregation of actin in endothelial cells [17]. Because of this the active stage of the disease was excluded from the present study, although it cannot be ruled out that the effects caused by these cytokines persist in the cord. **Since the quantity of microfilaments is higher in the vascular endothelium of both Dupuytren's groups than in the unaffected palmar fascia, it is possible that this effect is caused by the disease itself.**

However, the arrangement of the microfilaments in bundles is different in the two groups of Dupuytren's patients and it is thus conceivable that this may be determined or influenced by other factors. **There are no stress fibres in the unaffected palmar fascia, they are present only in the postcapillary venules in the contracted palmar fascia, and in both terminal arterioles and postcapillary venules in the extended palmar fascia.** Stress fibres contain several actin-associated proteins necessary for a sliding mechanism, and the contractility of the fibres has been demonstrated in different in vitro systems (for review see [17]). In cultured cells the stress fibres are

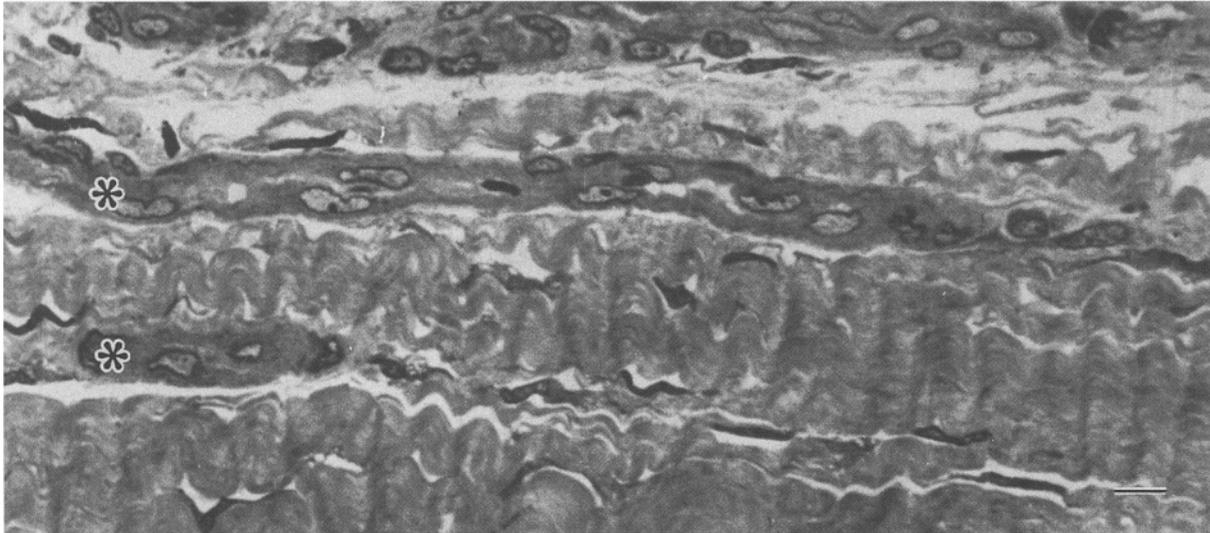


Fig. 3 Dupuytren's disease, palmar fascia after continuous elongation. Microvessels (asterisks) are arranged parallel to the collagen bundles. $\times 717$; bar 10 μm

Table 2 Number of adherens junctions per vascular endothelial profile (n number of endothelial cells in each group and each type vessel counted)

* $P < 0.001$ compared to the unaffected palmar fascia. There is no significance between the two cord groups

	Unaffected palmar fascia (mean \pm standard derivation)	Cord	Extended cord
Arterioles	3.9 \pm 1.5 ($n=85$)	3.7 \pm 1.4 ($n=79$)	3.6 \pm 0.8 ($n=82$)
Capillaries	2.7 \pm 0.9 ($n=42$)	4.0 \pm 1.7 * ($n=43$)	3.7 \pm 1.3 * ($n=45$)
Venules	3.0 \pm 1.2 ($n=130$)	4.3 \pm 1.2 * ($n=124$)	4.6 \pm 1.2 * ($n=125$)

Table 3 Ratio of the lengths of adherens junctions and the lateral cell membrane in the vascular endothelium

* $P < 0.001$ compared to the unaffected palmar fascia
** $P < 0.001$ between the cord groups

	Unaffected palmar fascia	Cord	Extended cord
Arterioles	0.21 \pm 0.11 ($n=85$)	0.51 \pm 0.16 * ($n=79$)	0.82 \pm 0.18 *,** ($n=82$)
Capillaries	0.18 \pm 0.13 ($n=42$)	0.37 \pm 0.15 * ($n=43$)	0.57 \pm 0.23 *,** ($n=45$)
Venules	0.13 \pm 0.10 ($n=130$)	0.43 \pm 0.16 * ($n=124$)	0.74 \pm 0.22 *,** ($n=125$)

Table 4 Number of focal contacts per vascular endothelial profile

* $P < 0.05$ compared to the unaffected palmar fascia
** $P < 0.001$ compared to the unaffected palmar fascia
*** $P < 0.05$ comparing cord groups

	Unaffected palmar fascia	Cord	Extended cord
Arterioles	2.5 \pm 1.0 ($n=85$)	3.7 \pm 2.0 * ($n=79$)	3.0 \pm 1.7 ($n=82$)
Capillaries	2.4 \pm 1.2 ($n=42$)	3.7 \pm 1.8 * ($n=43$)	3.7 \pm 1.5 ** ($n=45$)
Venules	2.4 \pm 1.6 ($n=130$)	3.9 \pm 1.5 ** ($n=124$)	2.8 \pm 1.0 *** ($n=125$)

Table 5 Ratio of the lengths of focal contacts and the basal cell membrane in the vascular endothelium

* $P < 0.001$ compared to the unaffected palmar fascia
** $P < 0.001$ between the cord groups

	Unaffected palmar fascia	Cord	Extended cord
Arterioles	0.21 \pm 0.10 ($n=85$)	0.48 \pm 0.15 * ($n=79$)	0.85 \pm 0.15 *,** ($n=82$)
Capillaries	0.10 \pm 0.08 ($n=42$)	0.35 \pm 0.15 * ($n=43$)	0.56 \pm 0.19 *,** ($n=45$)
Venules	0.15 \pm 0.10 ($n=130$)	0.42 \pm 0.17 * ($n=124$)	0.73 \pm 0.16 *,** ($n=125$)

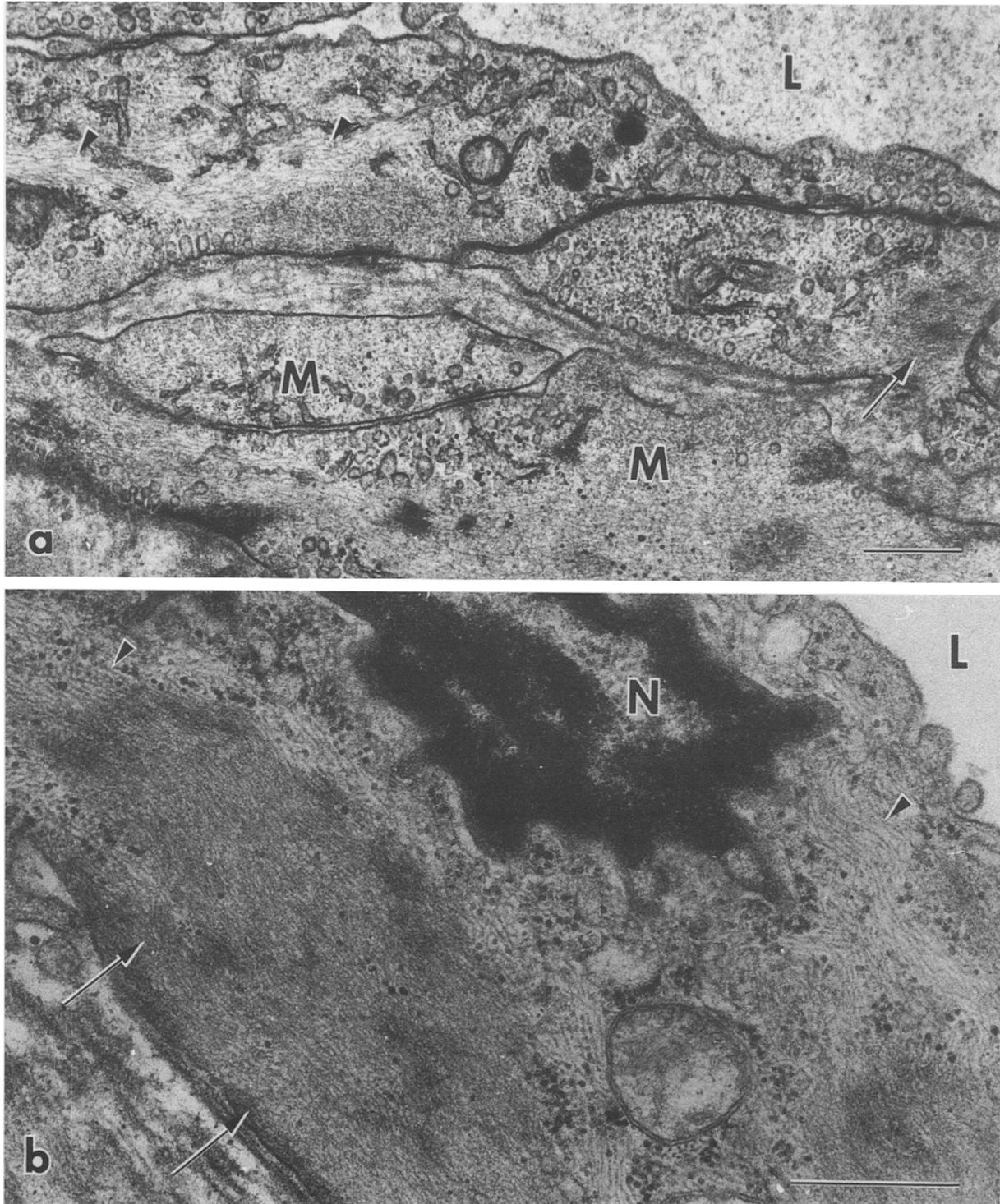


Fig. 4a, b Dupuytren's disease, palmar fascia after continuous elongation. The endothelial cells of an arteriole (**a**) and of a post-capillary venule (**b**) show stress fibres connected with the basal cell surface (*arrows*), microfilament bundles and intermediate filaments (*arrowheads*). Smooth muscle cells (*M*) can also be seen. **a** $\times 32,000$, **b** $\times 56,000$; bar $0.5 \mu\text{m}$

probably organized to restrain mechanical tension [12]. In the vascular system the high shear forces correlate with the expression of the stress fibres in the endothelial cells (see Introduction). Therefore, these fibres protect the cells from increased mechanical stress and connect them tightly to the subendothelial matrix.

To find out how tightly the endothelial cells are connected to each other and to the subendothelial extracellular matrix, a quantitative analysis of the number and size of adherens junctions and focal contacts was per-

formed (Tables 2–5). In Dupuytren's patients the number of the junctions was not highly significantly altered compared to the unaffected palmar fascia or between the two cord groups, but the increase in the ratio of the length was always highly significant. The highest values were found in all types of microvessels of the extended cord, the lowest in the unaffected palmar fascia; intermediate values were obtained for the contracted cord. Therefore, the differences in the connections in the vascular endothelium are correlated with the appearance of stress fibres.

Since comparable changes in the connections were present in all microvessel types of the palmar fascia and stress fibres were also present in postcapillary venules, haemodynamic causes can be ruled out. But different levels of externally applied stretching forces are present in the three groups of patients. In the normal palmar fascia, during movement of the hand varying tensile forces are interrupted by phases of relaxation. In Dupuytren's disease both hands are involved in more than 50% of the patients [3, 25] and the persistent flexion contraction of their finger requires an increase of the extending forces and a reduction of the relaxation time. The extension device used produces constant high mechanical stress, continuously elongating the diseased fascia until the finger is completely extended. Therefore, both the presence of stress fibres and the extent of adherens junctions and focal contacts in the endothelial cells are correlated with the extending forces inside the palmar fascia. This is in agreement with an adaptation of the cells to various levels of mechanical stress.

The signalling pathway of the mechanical stress from outside into the endothelium has to be elucidated. In vitro, it has been demonstrated that shear stress modulates endothelial cell morphology and F-actin organization through regulation of proteins associated with focal contacts [14]. The integrins transmit the signal and cause the phosphorylation of different signalling proteins, which change, for example, the assembling mode of actin [11, 23, 24]. Subsequently, the cell function adapts as demonstrated in the extended palmar fascia. The stress applied from outside changes the cell shape, the orientation of the cytoskeleton in the myofibroblasts and the composition and arrangement of the surrounding extracellular matrix, which has been degraded and newly synthesized by these cells [5].

In conclusion, endothelial cells respond to mechanical stress applied from outside by producing a remarkable and highly ordered microfilament system. This is connected at extended adherens junctions and is bound to the larger focal contacts with the extracellular matrix, suggesting that the cells protect themselves from mechanically induced injury in this way.

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