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Occurrence of Myofibroblasts in the Different Phases of Morbus Dupuytren (Dupuytren's Contracture)

Über das Vorkommen von Myofibroblasten in den verschiedenen Erkrankungsphasen des Morbus Dupuytren

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Summary

Twenty-one surgically removed specimens of Morbus Dupuytren (M. D.) were studied by light and electron microscopy. The cell type observed in the *proliferative phase* shows the basic ultrastructural features of fibroblasts, while the majority of the cells in the *involutional phase* resemble myofibroblasts. Myofibroblasts exhibit ultrastructural characteristics of both smooth muscle cells and fibroblasts and are said to behave functionally like smooth muscle cells. In the *residual phase*, typical fibrocytes of connective tissue are found. These findings confirm the concept that fibroblasts are capable of converting into myofibroblasts and demonstrate that myofibroblasts represent an intermediate cell type of transitional cellular differentiation. The significance of myofibroblasts for the development of the contracture in M. D. is discussed.

As the result of clinical and histological studies Luck (1959) identified three phases in Morbus Dupuytren (M. D.). He delimited an initial *proliferative phase*, an *involutional* and a *residual phase*. Gabbiani and Majno (1972), who studied the cells composing the nodules of M. D. by light and electron microscopy, demonstrated fine structural characteristics in these cells, similar to those of smooth muscle cells. They suggested that the cells under consideration were fibroblasts that had differentiated into contractile cells, which they called myofibroblasts.

As Gabbiani and Majno (1972) did not consider the different histologic phases of M. D., it was the aim of the present examination to study the occurrence of the myofibroblast in the different phases of M. D.

Material and Methods

Surgically removed specimens of M. D. from 21 patients (20 male and 1 female, aged 37–66 years) were studied. All tissues were fixed immediately after removal. Spe-

cimens for electron microscopy were cut into 1 mm cubes, fixed at room temperature for two hours by immersion in buffered 6.25% glutaraldehyde, rinsed in 0.2 M saccharose, postfixed in 2% buffered osmium tetroxide and embedded in epon. Sections approximately 1 µm thick were cut with glass knives and stained with Azure II-methylene blue (Richardson et al., 1960). The sections were studied by light microscopy and the phases were classified according to Luck (1959).

Solitary and multiple nodules as well as different phases of M. D. were found in the same surgical specimen. 10 nodules were classified as proliferative, 6 as involutional and 12 as residual. Two nodules were classified as transitional from proliferative to involutional and one nodule as transitional from involutional to residual.

At least six representative areas of each phase from various patients were selected for electron microscopic examination, cut with diamond knives, stained with uranyl acetate and lead citrate, and examined in a Philips electron microscope EM 300.

Results

On light microscopic examination the *proliferative phase* of M. D. is characterized by multiple foci of hypercellular areas forming nodules of various sizes. Sometimes small foci coalesce, forming conglomerate nodules. The nodules are composed of plump cells with oval, pale nuclei and prominent nucleoli (Fig. 1 a). The cytoplasm is large and the cell boundaries are indistinct. In general the nuclear contour is smooth; occasionally small folds are noted. The cells display no particular orientation. In the interstitium varying amounts of collagen bundles arranged in loose, interweaving bands are found. The foci are frequently rather vascular with small irregularly distributed capillaries. The walls of the capillaries seem to blend without sharp demarcation with the proliferating fibrous tissue. In general, the margins of the proliferating nodules are indistinct; some nodules appear to be invading the collagen component of the fascia without encapsulation.

On electron microscopy, the cells display a well-developed rough endoplasmic reticulum with many prominent and dilated cisternae filled with electron-lucent material. Moreover, a few mitochondria and free ribosomes can be seen. Sporadically fields of irregularly arranged microfilaments are found (Fig. 2 a, 2 b). Furthermore, in some cells intracellular deposits of "fibrous long spacing" collagen occur (Gokel and Hübner, 1977). In the interstitial space irregularly arranged collageneous fibrils are found, which are apparently produced by the fibroblasts.

In the *involutional phase* of M. D. the nodules are smaller and the number of the cells is markedly decreased (Fig. 1 b). In contrast to the proliferative phase, the cells are now smaller, arranged parallel and separated by broad bands of collageneous fibres. They are spindle-shaped and terminate in long, thin cytoplasmic processes. They display dense nuclei and

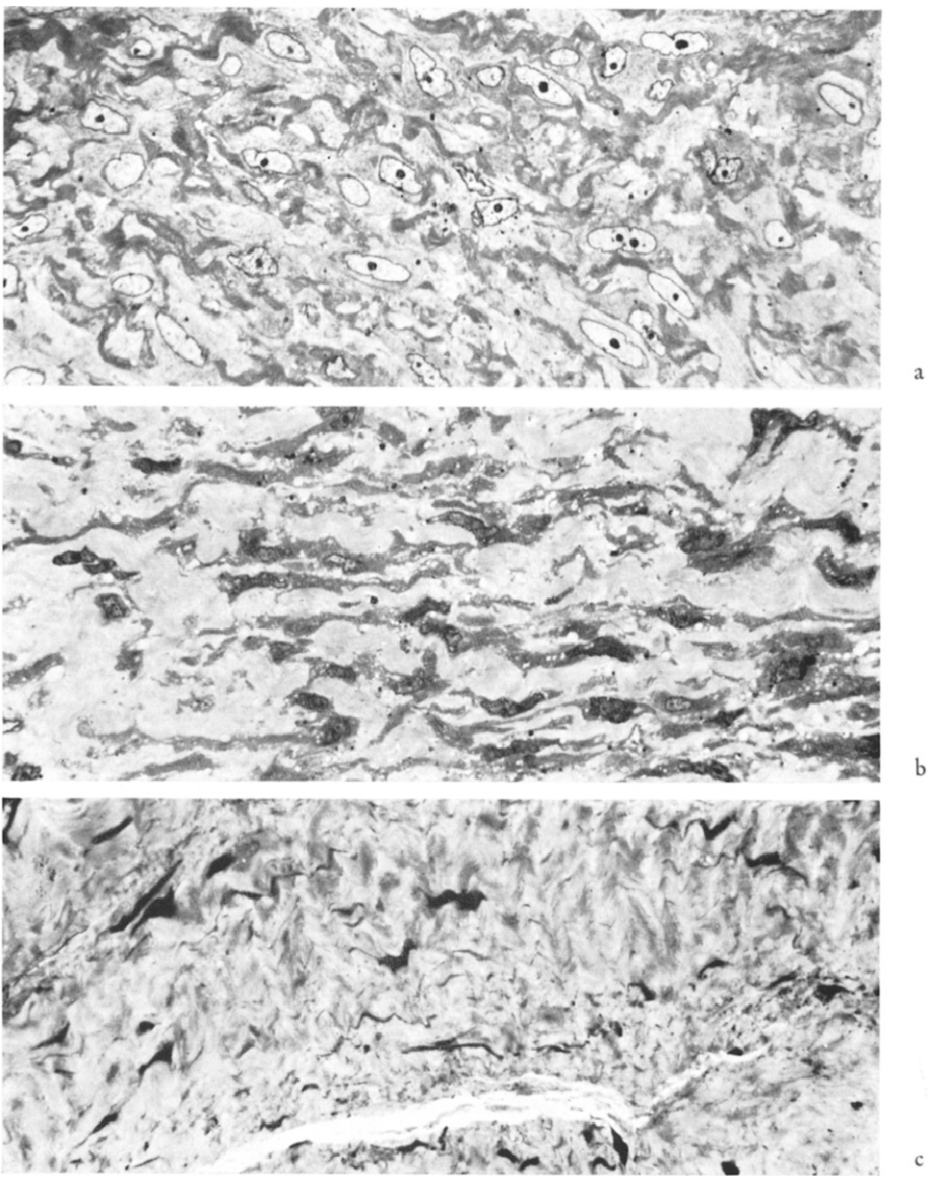
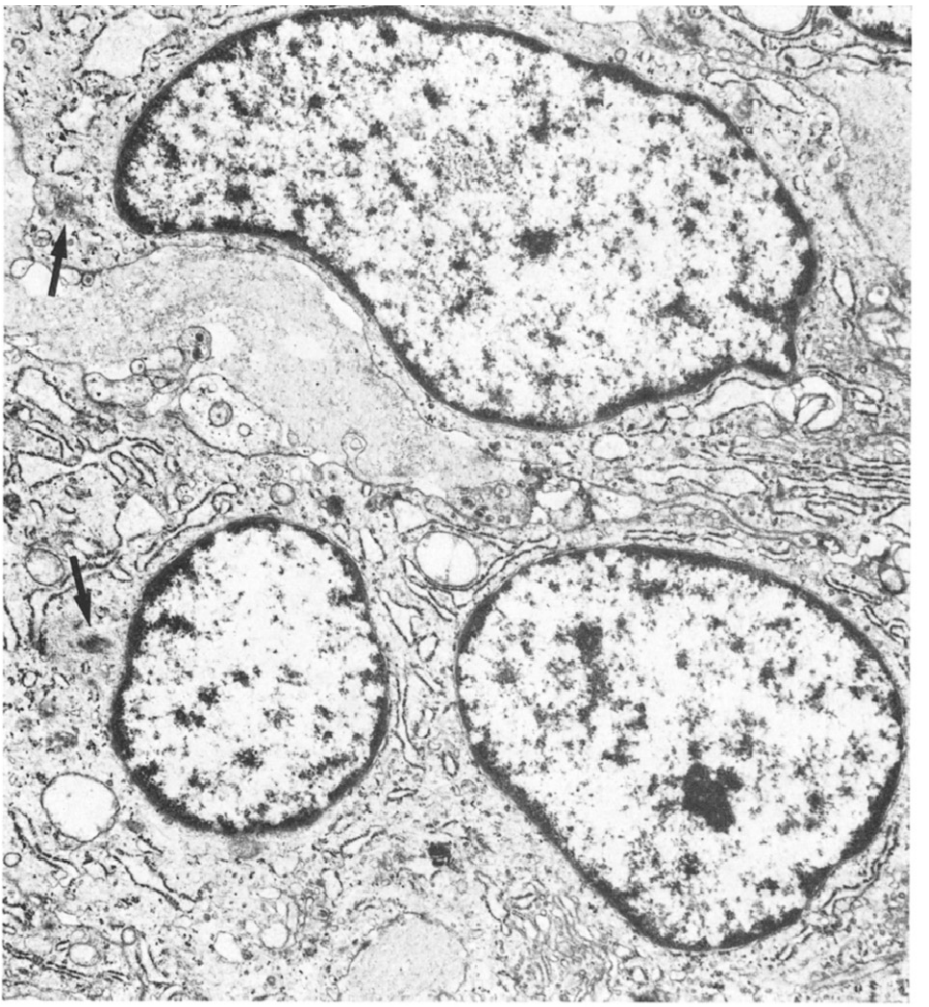
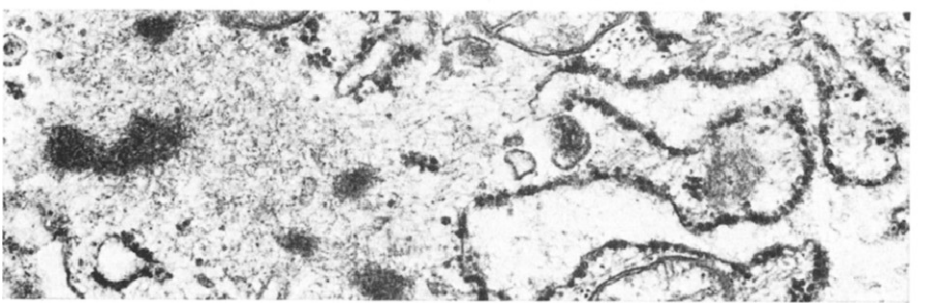


Fig. 1. Light microscopy of different phases of Morbus Dupuytren. a) proliferative phase, b) involutional phase, c) residual phase. Arch. No. EM 1516, 1720, 1956. × 530.



a



b

Fig. 2 a. Proliferative phase of Morbus Dupuytren. Plump cells with oval nuclei, prominent nucleoli and well developed ergastoplasm. Note occasional bundles of microfilaments (arrows). Arch. No. 9865. × 11,000.

Fig. 2 b. High power view of irregularly arranged microfilaments. Arch. No. 9868. ×43,000.

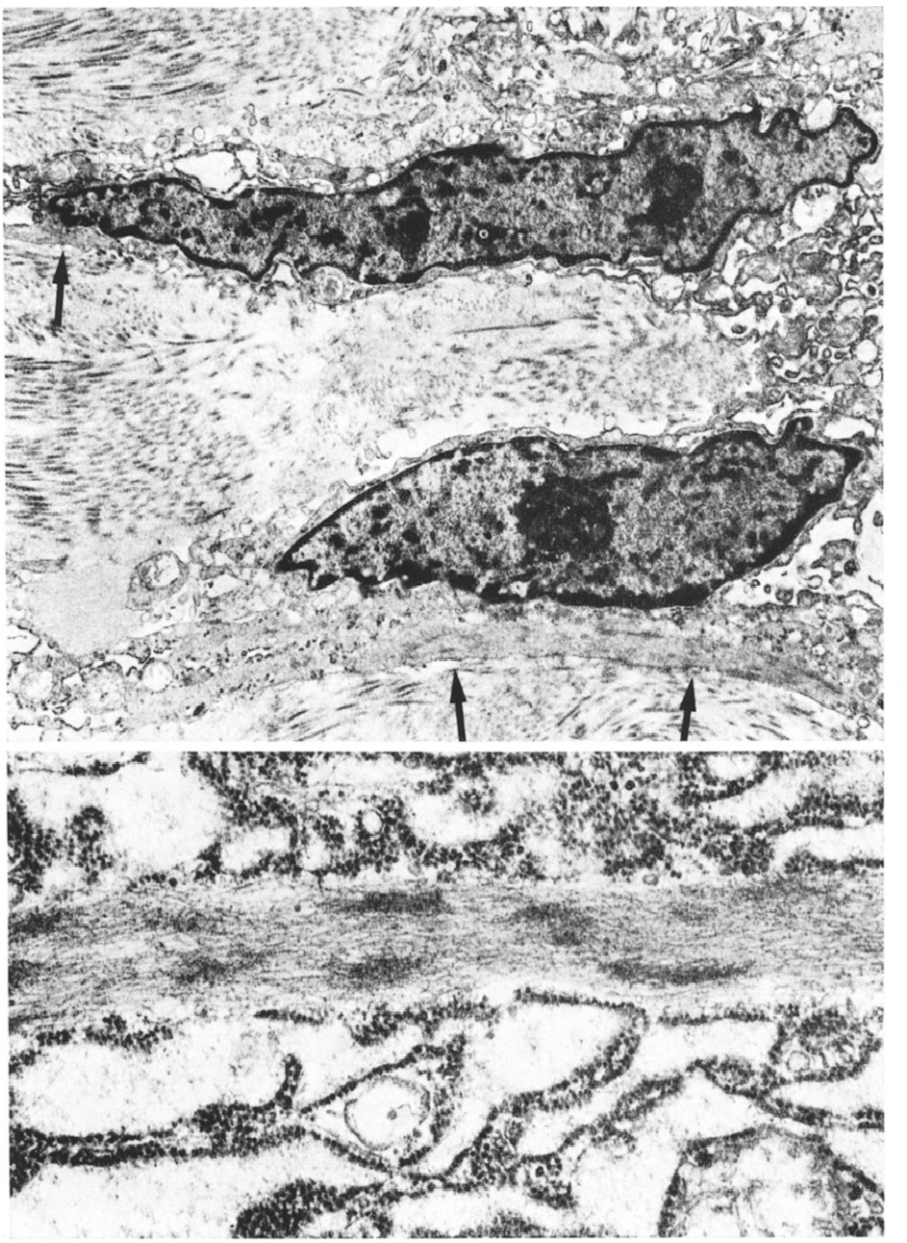


Fig. 3 a. Involutional phase of Morbus Dupuytren. Myofibroblasts with nuclear indentations and bundles of myofilaments beneath the cell membrane (arrows). Arch. No. 12004. × 7,900.

Fig. 3 b. High power view of myofilaments with electron-dense areas. Arch. No. 9885. × 43,000.

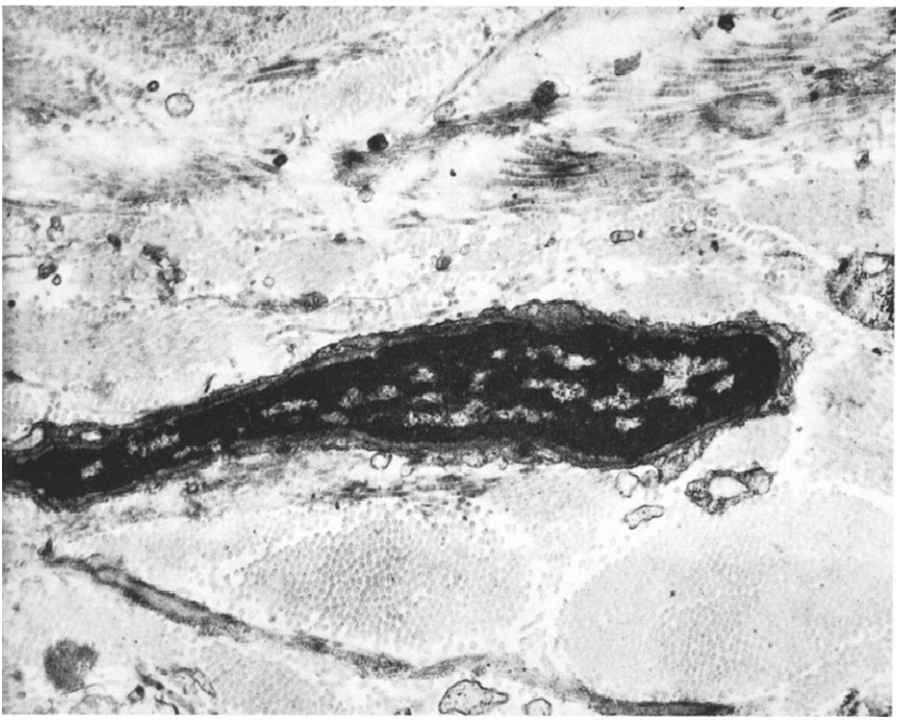


Fig. 4. Residual phase of Morbus Dupuytren showing a fibrocyte with small cytoplasmic rim. Abundant collagen bundles in the interstitium. Arch. No. 11992. × 16,000.

nucleoli and a small cytoplasmic rim. When cut longitudinally the nuclear contour appears wrinkled.

On electron microscopic examination, the cells of the *involutional phase* display elongated nuclei with irregularly distributed dense chromatin and prominent nucleoli. The nuclei show wrinkles and indentations (Fig. 3 a). In the cytoplasm, bundles of packed microfilaments are noted which run parallel to the long axis of a cell, usually beneath the cell membrane (Fig. 3 b). Individual microfilaments measure approximately 50–80 Å in diameter and show irregularly interspersed electron dense areas. Besides the microfilaments, the remaining cytoplasm is packed with cisternae of a well-developed rough endoplasmic reticulum characteristic of fibroblasts. Sometimes a few pinocytotic vesicles are present in the cytoplasm subjacent to the plasma membranes; parts of the cell surface are covered by a material with the structural features of basal lamina. Cell to cell junctions are not observed. The extracellular tissue consists of large bundles of collageneous fibres.

Tissue from the *residual phase* of M. D. resemble mature scar tissue. The collageneous fibres are thick and closely packed in parallel bundles resulting in a lamellar pattern. A few elongated cells queezed between collageneous bundles are noted (Fig. 1 c). The nuclei stain intensively and the cytoplasmic rim is small. On electron microscopy, the cells present electron-dense nuclei with clumped chromatin. The cytoplasm displays only a few cell organelles; a rough endoplasmic reticulum and myofibrils are absent (Fig. 4).

Discussion

Transitional cell forms between fibroblasts and smooth muscle cells were first described by O'Shea (1970) in the rat ovary and by Moss and Benditt (1970) in the chicken aorta. Similar cells forms were also observed by Majno et al. (1971) and Gabbiani et al. (1972) in experimental granulation tissue. They called these cells "myofibroblasts" and regarded them as a distinct cellular type characterized by fine structural features typical of fibroblasts and smooth muscle cells (Gabbiani et al., 1973). Since their first description, myofibroblasts have also been found in human granulation tissue under various conditions (Ryan et al., 1974). The similarity between myofibroblasts and smooth muscle cells was also documented by immunfluorescence studies with antismooth muscle serum (Hirschel et al., 1971) and by high contents of chemically extractable actomyosin (Majno et al., 1971).

On the basis of histological findings, we could distinguish three phases in the course of M. D., which conforms with standard descriptions (Luck, 1959; Mackenzie, 1970). The cell type found in the *proliferative phase,* which is to be interpreted as the initial phase of the disease, resembles the typical fibroblast of immature connective tissue. The predominant cell type in the *involutional phase* of M. D. is, in many respects, similar to myofibroblasts as they were first described in M. D. by Gabbiani and Majno (1972). The cells observed in the *residual phase* correspond to fibrocyts typically found in mature connective tissue.

Our results support the concept that fibroblast can change into myofibroblasts. They furthermore strongly suggest that the myofibroblasts in the *involutional phase* represent an intermediate cell form with a transitional cellular differentiation. A similar transitional myoblastic cellular differentiation was also observed in granuloma pouches by immunfluorescence method (Gabbiani et al., 1972).

Despite many studies, the mechanism of contracture in M. D. is still the subject of some debate. Two different working hypotheses exist. In the

first hypothesis, the beginning of the pathogenesis of contracture is seen in changes of the collagen fibres, followed later in the disease by a collagen shrinkage (Millesi, 1965). The cellular reaction is regarded as a secondary and reactive phenomenon. The second hypothesis favours the concept that the disease starts with a cellular proliferation and that the contracture is due to active contraction by myofibroblasts (Gabbiani and Majno, 1972).

Several ultrastructural studies failed to find any significant structural abnormalities of the collagen in M. D. (Jahnke, 1960; Patel, 1961; Dahmen, 1968). On the other hand, Nemetschek et al. (1976) recently demonstrated that a shrinkage of fascial fibres need not necessarily be accompanied by structural changes visible in the electron microscope.

The theory that myofibroblasts are responsible for shrinkage of connective tissue in wound healing or granulation tissue was impressively sustained by pharmacological studies. Strips from granulation tissue could be made to contract or relax by several drugs which are known to stimulate smooth muscle cells (Gabbiani et al., 1972; Ryan et al., 1974). In contrast to these results, analogous studies with tissue strips from M. D. showed no reaction to any of the drugs used with granulation tissue (Gabbiani et al., 1973). It remains however, uncertain from the experiments done by Gabbiani et al. (1973), from which phase of M. D. the strips were taken. As demonstrated by our study, a significant contraction should only be expected in the *involutional phase,* when myofibroblasts constitute the predominating cell type. On the other hand, the negative results could also be explained by the dense collageneous bundles present in the nodules of M. D., which might not allow any significant shortening of the tissue strips in vitro (Gabbiani et al., 1973). From our results as well as from the results of earlier studies (Ryan et al., 1974), it is evident that the formation of myofilaments and the deposition of collagen is a concurrent process. Thus any tissue contraction caused by myofibroblasts might simultaneously be fixed by the deposition of collagen bundles. This twofold activity of myofibroblasts can explain the stepwise shrinkage of the palmar fascia and the gradual progression of M. D. Finally the concept that the tissue contraction is due to active contraction of myofibroblasts agrees with studies by Luck (1959), who noted frequent tissue contraction in the *involutional phase,* and by Meister et al. (1976, 1977), who found a high incidence of tissue contraction in the *proliferative* as well as in the *involutional phase.*

The conditions and the stimuli for the modulation of fibroblasts into myofibroblasts and finally into fibrocytes are not yet understood. Recently myofibroblasts have also been described in nodular fasciitis (Wirman, 1976) and circumscribed fibromatosis (Feiner and Kaye, 1976). Even in

tissue cultures of fibroblasts myofilaments have been observed (Goldberg and Green, 1964). The observation that proliferating cells in the above noted and unrelated conditions can acquire myofibroblastic features indicates that different factors may influence this process. It was therefore suggested by Montandon et al. (1973), that proliferating cells may develop myofilaments as a general adaptive response to microenvironmental changes. Additional studies are necessary to eluciate the circumstances of the cellular origin, metabolic function and metamorphosis of myofibroblasts, in both normal and pathological conditions.

Zusammenfassung

Operationspräparate von 21 Patienten mit Morbus Dupuytren (M. D.) wurden licht- und elektronenmikroskopisch untersucht. Danach handelt es sich bei den Zellen in der *Proliferationsphase* um Fibroblasten. Die in der *Involutionsphase* vorherrschenden Zellen entsprechen Myofibroblasten. Myofibroblasten zeigen feinstrukturell Kriterien von Fibroblasten wie auch von glatten Muskelzellen und sollen kontraktile Eigenschaften besitzen. Die Zellen in der *Residualphase* gleichen Fibrozyten, wie sie in reifem kollagenem Bindegewebe beobachtet werden. Die Ergebnisse unterstützen die Vorstellung einer Differenzierung von Fibroblasten in Myofibroblasten. Sie machen gleichzeitig deutlich, daß die Myofibroblasten eine Zellform mit vorübergehender Differenzierung darstellen, die zwischen dem Fibroblasten und dem Fibrozyten steht. Die Bedeutung der Myofibroblasten für die Entwicklung der Kontraktur beim M. D. wird diskutiert.

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