Postnatal Development of Structure and Arrangement of Tendon Cells
A Scanning and Transmission Electron Microscope Study
in the Rat Calcaneal Tendon

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Summary: The postnatal development of the three-dimensional structure and arrangement of the tendon cells in the calcaneal tendon of rats from 5 to 90 days of age were examined under the scanning and the transmission electron microscope (SEM and TEM).

Exposed tendon cells were seen as winged bricks with a stellate profile in a cross section. Their slender perikarya were stacked successively in rows along the long axis of the tendon. The plate-like cytoplasmic processes, which were oriented along the perikaryal long axis, extended radially and joined those of the neighboring cells in rows.

Therefore, the tubular channels for collagen fascicles were lined with the chained perikarya and their processes of the tendon cells. The cell rows were usually composed of many cells in developing stages, while short rows of one or two cells were observed in the fully developed stage.

The cytoplasmic processes were the primary processes in the lateral extension, and their fine branches formed the secondary processes. The secondary processes were numerous at younger stages, showing a fine meshwork due to their mutual joining in the cross sections. In advanced stages, the meshes were coarse and the secondary processes were also perforated either with grouped fenestrations or large pores. In the fully developed stage, the secondary processes were fragmented on the perikarya, while the primary processes extended in the thin delicate sheets with large perforations.

The findings in the present study suggest that the tendon cells are arranged in a three-dimensional network by their mutual joining. The tendon cells of the rat calcaneal tendon may not proliferate very much after birth, but do expand their nursing area in line with normal growth by an elongation of the main primary processes and a reduction of the secondary processes and perikaryal mass. The interfascicular clefts, which were caused by an intervention of either the processes at any developmental stages or the fragmented processes at a certain level of the tendon, may also play a role in the passage of tissue fluid.

Tendon cells, the only cell elements of the tendon, have been named “winged cells” due to their characteristic structures (Bargmann, 1977; Krstic, 1978). Many studies have been made on the structural relation between the tendon cells and the fibrillar fascicles in the adult tendon (Elliott, 1965; Parry, 1978; Squier and Bausch, 1984), and in the developing tendon (Trelstad and Hayashi, 1979; Scott, Oxford and Hughes, 1981; Squier and Magnes, 1983).

Krstic (1978) presented a three-dimensional model of the tendon cell in order to propose that the plate-like processes of a similar length to that of tendon cells extend radially and contain collagen fascicles either in the furrows or tubular channels between the processes. The fine structural examination in the rat tail tendon (Squier et al., 1983, 1984) showed the presence of thin secondary ordered processes, which surround the subdivided collagen fascicles. Furthermore, developmental studies have also reported that the cell processes increase in number even at the postnatal stage in the rabbit calcaneal tendon (Ippolito, Natali and Postacchini, 1980), whereas they decrease in the rat tail tendon (Squier and Magnes, 1983).

To elucidate the surface structure of tendon cells, Squier and Bausch (1984) made reconstruction models by the TEM observation of serial sections. Their models were mainly concerned with perikarya, and not with either fine processes or the
connections of processes. Therefore, a clear image of the tendon cell has not yet been previously presented as far as we know. The present study was undertaken to carry out the visualization of tendon cells after the removal of the intercellular substances, especially the tendon fibers, by using the method of enzymatic digestion (Evans et al., 1976) in developing and fully developed tendons. The findings were discussed from the viewpoint of the three-dimensional arrangement of the cells.

Materials and Methods

The calcaneal tendons of Wistar rats at 5 to 90 days of age were examined. The tendons were exposed in animals anesthetized by an intraperitoneal injection of pentobarbital to be dripped with the fixative in situ for about 10 min, cut transversely and longitudinally into slices of about 0.5 mm thick, and then immersed in the fixative for a day at room temperature. The fixative used here was 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4).

For the SEM study, the glutaraldehyde-fixed tissues were washed with 0.1 M phosphate buffer (pH 7.4), placed in 6N HCl for 10 min at 60°C, rinsed by several changes of the same buffer mentioned above, and then placed in the buffered collagenase solution (Sigma, type II) (pH 6.8) at a concentration of 1 mg/ml for 12 hrs at 37°C. After rinsing, the tissue specimens were immersed in 2% tannic acid for several hours, postfixed with 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.4), and then dehydrated with a series of graded acetone and t-butyl alcohol. The specimens were dried by the freeze-drying method of t-butyl alcohol (Inoue and Osatake, 1988), coated with gold in an ion sputter coater and observed in either a Hitachi LB-450 or an S-900 SEM.

For the TEM study, the glutaraldehyde-fixed tissues were postfixed with the osmium tetroxide fixative prepared in a similar formula mentioned in the section on specimen preparation for SEM. The specimens were then dehydrated with a series of graded alcohol and propylene oxide, embedded in Epon resin, cut into thin sections, and then stained doubly with uranium and lead. The observation was conducted on a Hitachi H-500F TEM.

Results

At the young stage (5-day-old), the tendon cells were arranged in a fine network, filling up the space encircled with the peritendineum (Fig. 1), as figured in the collagen tendon and the rat tail (Goerttler, 1969). The cytoplasmic processes with some branches radially extended from the perikarya to join those of either the neighboring cells or the same cell. Therefore, fine meshes for collagen fascicles (Figs. 2, 3) were delineated with those processes.

Tendon cells at this stage were stacked in long rows, parallel to the long axis of the tendon (Figs. 7, 8). On the lateral surface of the cells in rows, the plate-like processes in various sizes were oriented along the cell rows, which separated either the longitudinal channels or the furrows. These processes were also successively joined to those of the neighboring cells in rows to form long walls (Fig. 7).

At the advanced stage (30-day-old), the channels for the collagen fascicles greatly expanded laterally. The primary processes from the perikarya were conspicuous by their long and thick size as compared with the delicate branches of the secondary processes (Figs. 4, 8). The secondary processes decreased in number and were quite thin or fragmented, compared with those at a younger age. Some of them extended obliquely to form funnel-shaped spaces (Figs. 5, 6), which may correspond to the anchoring sites of the collagen fibrils (Greenlee and Ross, 1967; Trestad and Hayashi, 1979; Squier and Bausch, 1984). Furthermore, the cytoplasmic plates of the secondary processes were perforated with either grouped fenestrations or large pores (Figs. 4, 6, 8). The fragmented processes were observed as either the cytoplasmic strands crossing over intercellular spaces, or the only longitudinal

Explanation of Figures

Plate I

Fig. 1. A SEM survey view of a cross section of a 5-day-old rat tendon after collagenase digestion. The cut surface is filled up with a network of tendon cells. F: fat tissue, PE: external peritendineum, PI: internal peritendineum. ×300

Fig. 2. A close-up of the tendon cells in a 5-day-old rat. The cells show a stellate profile, being formed with perikaryon (P), radiating the primary processes (arrows) and their branches as the secondary processes (arrowheads). The processes are joined with those from the neighboring processes to form fine meshes. PE: external peritendineum. ×2,000
crests on cell bodies (Figs. 4, 8).

The adjoining structure of the neighboring perikarya was represented by the thin streaks which extend either transversely or obliquely on the lateral surface of the cell rows (Fig. 8). The cellular adjoining to the long processes over the intervening cells in the same row in the rat tail tendon (Squier and Bausch, 1984) was not observed in the present study.

At a fully developed stage (90-day-old), the channels for the collagen fascicles greatly expanded transversely. The perikarya of most cells were also much more slender. The primary processes were also perforated with large pores (Figs. 5, 9). Therefore, large defects were observed on the walls separating the neighboring fascicular channels (Figs. 5, 9). The cell rows were sometimes short chains of two or three cells (Fig. 9).

Cytoplasms of tendon cells at all stages were filled with the granular endoplasmic reticulum with expanded cisterns containing amorphous material, and also were associated with well developed Golgi apparatus and some mitochondria (Figs. 3, 11, 12a). The surface of the cells was fully corrugated (Figs.

Plate II

Fig. 3. A TEM graph of a 5-day-old rat tendon. The tendon cell perikarya (P) with radiating processes are evenly arranged. ×2,500

Plate III

Fig. 4. The tendon cells in a cross section of a 30-day-old rat. The connections of the thick primary processes (arrows) are conspicuous between those of the neighboring cells. Most of the secondary processes (arrowheads) are delicate and fragmented on the primary processes and the perikarya (P). ×2,000

Fig. 5. A transverse section of a tendon in a 90-day-old rat. The thick processes from the perikarya (P) are also perforated with large pores (bidirectional arrows), with falciformed rims and communication neighboring channel spaces. ×2,000
Three Dimensional Arrangement of Developing Tendon Cells

Plate III
9, 10) by impressions of fibrils in a parallel arrangement (Figs. 11, 12b). Furthermore, the cell surface was often coated with a thin layer of amorphous substance, which extended further into the interfascicular clefts (Fig. 12a).

Discussion

The present study succeeded in the visualization of three-dimensional surface structures of tendon cells and their arrangement under the scanning electron microscope after the removal of the intercellular fibrous elements. The cellular arrangement in a three-dimensional network is considered to be effective for the unification of development and the maintenance of physical condition in such solid tendon tissue as would be expected for bone cells in Harversian systems (Bargman, 1977; Ham and Cormack, 1987).

A reduction in the number of processes in growth contradicts the observation of Ippolito and his co-workers (1980) on the rabbit tendon. This may be due to the differences of the species and the

Plate IV

Fig. 6. A stereo-view of a part of tendon cells in a tendon of a 30-day-old rat. The pores and their rims (arrows) are seen on cytoplasmic processes. x1,200

Plate V

Fig. 7. A longitudinally cut surface of a 7-day-old rat tendon. The lamellar processes separate the longitudinal channel spaces. Arrows point to cytoplasmic processes. The transverse streaks (arrowheads) represent the cell boundaries between the adjoining cells in rows. x5,500

Fig. 8. Longitudinally stacked tendon cells in rows in a 30-day-old rat. The cell boundaries (arrowheads) are represented by transverse streaks. The arrows indicate the processes, aligned in longitudinal wings along the cell rows, on which large pores (asterisks) are also seen. x4,000

Fig. 9. A longitudinal section of a 90-day-old rat tendon. The cell rows of stacked tendon cells are short and connected with the strand-like processes to the neighboring cell rows. Large pores (asterisks) are numerous on processes. x4,000

Fig. 10. A high magnification of the tendon cell surface in a 30-day-old rat. The cell surface is rough with similarly-sized furrows of fibril impressions over the cell boundary (arrows) of cells. x21,000
voluminous increase of collagen fascicles, because at the neonatal stage, the rabbit tendon cells are still proliferating (Ippolito et al., 1980), whereas the cell division in rats was not observed in the present study and the fibroblast frequency per unit area in the cut surface of developing tendons is rapidly re-
duced after birth and reaches a constant value at 10 days of age. The thickening of the primary processes in contrast to their elongation at a young stage (30-day-old) may be ascribed to the mobilization of cytoplasm to the primary processes by reducing the numbers of the secondary processes to keep a mutual joining between the neighboring cells, though some growth of the cytoplasm is expected. The cytoplasmic mobilization seems to take place by the terminal stage of the tendon development. However, at the fully developed stage, the large perforations of the primary processes lead to the partial defects of septal walls between the channels for the collagen fascicles.

The grouping of fibrils corresponds most closely to the description of Elliott (1965), in which the bundles of collagen fibrils to be encircled by the anastomosing processes of tendon cells, especially in the early stage of development, were classified as primary tendon fascicles. However, the fascicles are not necessarily encircled with the processes, but mostly with clefts (Squier and Bausch, 1984), which

Plate VI

Fig. 11. A TEM graph of a longitudinal section of the tendon cell rows which are interspersed among collagen fascicles (CF). The cytoplasm is filled with the granular endoplasmic reticulum. Some mitochondria and Golgi apparatus (G) are also seen. The adjoining of the neighboring cells demonstrates streaks (arrows) containing a flocculent material which can also be seen on the cell surface. N: nucleus. ×5,000
contained fragmental thin processes in either the adjacent sections or the amorphous substance continued to the coating layer of the cell. This indicates that the clefts are not an artifact. It is suggested that the clefts are due to the presence of processes at any levels of the tendon, or vestigial spaces of the retracted processes. Therefore, the cleft spaces in labyrinthic channels may well play a role in the passage of tissue fluid for its thorough permeation in the tendon.

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Plate VII
Fig. 12. TEM graphs of crossly sectioned tendon cells (TC) and collagen fascicles (CF) in a 90-day-old rat. (a): The streaks of dense and flocculent material extend from the cell surface into the interfascicular clefts (arrows). (b): A high magnification of a framed portion in (a). The cell membrane is scalloped with shallow furrows impressed by the fibrils (F). The connecting filaments (arrows) can be seen between fibrils. (a): ×10,000, (b): ×27,000


