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Scanning Electron Microscopic Observation of Ossification and Calcification of the Ligamentum Flavum

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Abstract

According to SEM observation of the ligamentum flavum with no other ossifications in roentgenograms, elastic fibers formed a dense and regular pattern with interconnecting micro-fibrils.

Observation of the ossification of the thoracic ligamentum flavum showed degenerative changes of fibers, followed by the appearance of numerous osteocyte lacunae with granular substance. And finally there appeared osteocytes, resulting in the enchondral ossification.

Observation of the calcification of the cervical ligamentum flavum showed a punched-out region composed of different shapes of crystals, determined CPPD by X-ray diffraction study.

The calcification and ossification of the ligamentum flavum are completely different conditions.

I. Introduction

Although the exact cause of pathological ossification and calcification of the spinal ligaments is still obscure, they are assumed to be based upon the same condition, and in addition to that, local factors, such as anatomical and biological singularity, trauma, inflammatory and degenerative changes in nature, may play a part to produce the different conditions of the ossification and calcification.

As for the ossification of the posterior longitudinal ligament which often compresses the spinal cord, since TSUKIMOTO (1960) had reported the first case in the cervical spine in autopsy, many reports concerning the pathohistology and etiology of the ossification have been published and the causes of the ossification are being investigated enthusiastically.

On the other hand, as for the ossification of the ligamentum flavum which is often to be a posterior factor causing spinal cord compression at the level of the thoracic spine, HIRAOKA

Key words: Scanning electron microscopy, Ligamentum flavum, Ossification, Calcification, Calcium pyrophosphate dihydrate (CPPD).

索引語：走査電子顕微鏡、黄色靭帯、骨化、石灰化、ビロリン酸カルカウム。
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(1955) found the ossifications in cadavers and Yamaguchi (1960) reported his experience of surgical treatment for the disorder. Nevertheless, there had been few reports for some time after those reports because of technical difficulty of pointing out the ossification in roentgenograms and ignorance of the condition itself. Since the detailed descriptions by Kirita (1960), Yanagi (1972), Moriwhaki (1973) and Tsuk (1981) were reported, the clinical features due to ossification of the ligamentum flavum have received much attention in the past several years. But there still has been no exact answer for the pathogenesis and mechanism of the ossification of the ligamentum flavum.

Although some investigations have been performed by means of light microscopy or transmission electron microscopy (TEM), an appropriate explanation on the pathogenesis and mechanism of the ossification and calcification of the ligamentum flavum has not been obtained because of their methodological limitation; in these observations, the specimens must be flat and thin in slices, and can only be observed in two-dimensional field. In recent years, the increasing availability of scanning electron microscopy (SEM) has made it possible to analyze three-dimensional observation. Relatively large specimens can be observed by means of SEM and the majority of the exposed, external surfaces can be studied directly. The range of useful magnifications available in SEM is large, extending from those achievable with light microscope to those with TEM. The depth of field is greatest among these methods. The SEM is therefore very useful for observing the surface of the specimens as a whole. Nevertheless there has been no detail description on observation of the ossification or calcification of the ligamentum flavum by means of SEM so far. The purpose of this paper is to clarify the pathogenesis and mechanism of the ossification and calcification of the ligamentum flavum by means of SEM, compared with the findings of light microscopy.

II. Materials and Method

Specimens were obtained from eight cases of the ossification of the thoracic ligamentum flavum and two cases of the calcification of the cervical ligamentum flavum. Among them three cases were accompanied with the ossification of the posterior longitudinal ligament at the cervical and lumbar region. The age ranged from 38 to 66 years, at the average of 51.8 yrs. (male: 53 yrs., female: 46 yrs.) As a control group, nine specimens of the ligamentum flavum were obtained from the cases of spinal canal stenosis or spinal tumor when laminectomized. No ossifications were observed among them in roentgenograms. The age ranged from 16 to 72 years, at the average of 47 yrs. (male: 46.6 yrs., female: 47.5 yrs.).

For light microscopy the sectioned specimens were stained with hematoxylin-eosin and Van Gieson and von Kossa after decalcification. The specimens were examined by ordinary light microscopy.

For SEM observation, specimens were fixed with 2% glutaraldehyde and examined by a soft X-ray instrument (softex 'MB-2 type) and prepared by two procedures as follows: half of each sample was immersed in 1% osmium tetroxide and the other half was decalcified and fixed. After dehydration in graded series of ethanol, specimens were cracked by ethanol cracking method.
and dried by the critical point drying method using liquid CO₂, followed by evaporation of gold-palladium. The samples were then examined by SEM (25-S III, type. JEOL Co.) (Fig. 1).

III. Results

A. Control Group

According to the SEM observation of the interlaminar region of the thoracic ligamentum flavum on the control group, elastic fibers, 1.0 μ-6.0 μ in diameter at the average of 3 μ, were parallel in one direction with little extracellular ground substance and entered the surface of the bone obliquely (Fig. 2). Although most of them formed a dense and regular pattern, some of

![Fig. 2. Elastic fibers were parallel and oriented in one direction and entered the surface of the bone obliquely. (Field width: 1100 μ)](image-url)
them formed a wavy pattern and some of them were divided into a few branches with interconnections between each fiber. Many interconnecting fibers between elastic fibers were observed in the dense extracellular ground substance. Under high power view, the little fibrillar connection, which were micro-fibrils, 200 Å-2 μ in diameter at the average of 5 μ, formed a dense and regular pattern. The surface of the fibers became smooth and there was less tendency of branching of micro-fibrils (Fig. 3).

At the attachments of the bone, elastic fibers were inserted into the surface of bone and their pattern of attachment distinguished two major classes; one in which elastic fibers entered directly, and the other which formed woven bundles, 20 μ-30 μ in diameter, with obscure demarcation. A few blood vessels were observed along the surface of the bone (Fig. 4).

Observation of the laminar region showed the lamellar arrangement of the collagen sheet on the surface of the lamina bone (Fig. 5). A detailed observation revealed that each lamella was composed of dense fine fibrils which crossed each other among the lamellar sheets. And all fibers formed in two direction. one parallel with the surface of the bone and the other parallel with elastic fibers entering the surface of the bone. In decalcified samples, this lamellar arrangement of the collagen fibers was clearly observed (Fig. 6).

Fig. 3. Most of elastic fibers formed a dense and regular pattern. (Field width: 315 μ)
High power view (above left) showed interconnecting micro-fibrils between elastic fibers.
(Field width: 15 μ)
Fig. 4. Elastic fibers were inserted into the surface of the bone, most of them formed woven bundles with obscure demarcation. (blood vessels) (Field width: 154 μ)

According to the observation of the attachments of the bone with age changes, osteocyte lacunae, probably corresponding to chondrocytes or osteoblasts, were recognized to be adjacent

Fig. 5. The lamellar arrangement of the collagen sheet on the surface of the bone (Field width: 154 μ)
Fig. 6. In decalcified samples, lamellar arrangement was clearly observed. (Field width: 37 μ)

Fig. 7. Osteocyte lacunae (→) were recognized adjacent to the surface of the bone. (Field width: 110 μ)
to the surface of the bone (Fig. 7).

Under high power view, Figure 8 shows half-formed osteocyte lacuna with random orientation of fine fibrils in the back wall of the lacuna. The surface at below right is a mineralizing front with short segments of collagen fiber bundles. In the surroundings of the osteocyte lacuna fibers form a regular pattern and parallel with the surface of the bone, which is indicated in above left part in Fig. 7 and fibers form an irregular, random pattern which is indicated in below right in Fig. 7. The level reached by the mineralization plane is oblique to the surface and perpendicular to the long axis of the elastic fibers which are inserted into the surface of the bone.

Short Summary:

According to the SEM observation of the interlaminar region of the thoracic ligamentum flavum on the control group elastic fibers formed a dense and regular pattern, some of them formed a wavy pattern and some of them were divided into a few branches with interconnections between fibers.

Elastic fibers, which formed woven bundles with obscure demarcation closer to the attachments, were inserted into the surface of the bone.

According to the observation of the attachments with age changes, a not unusual observation
was of osteocyte lacunae with random orientation of fine fibrils in the back of the lacuna. A mineralization front represented a series of short segments of collagen fiber bundles and the level reached by the mineralization plane was oblique to the surface and perpendicular to the long axis of the elastic fibers which were inserted into the surface of the bone.

B. Ossification of the Thoracic Ligamentum Flavum

Soft X-ray examination of samples showed a protrusion of the ossification from the lamina into the spinal canal at the side of attachments (Fig. 9).

On the other hand, the sectioned specimens were examined by light microscopy of hematoxyline-eosin stain. The structure of the ossification region was similar to that of normal attachment of the ligament or tendon to the bone surface (Fig. 10). At the high magnification, chondrocytes and hyalinization were observed (Fig. 11).

SEM observations of the interlaminar structure showed degenerative changes as follows: Each bundle of elastic fibers about 8 μ in diameter formed a large and wavy pattern, some of which were separated into a few branches. However, most bundles were arranged parallel to each other. Collagen fibers with wavy patterns connected adjacent bundles (Fig. 12).

At the surroundings of the ossification, woven fibers which consisted of micro-fibrils about 1 μ in diameter formed a thick and irregular pattern instead of elastic fibers. In the ground
The structure of the ossification of the ligament was similar to that of normal attachment of the ligament or tendon to the bone surface. (H. & E. ×40)

Substance, there appeared to be oval and rosen-shaped granular particles which measured 1200 Å in diameter (Fig. 13).

Closer to the ossification, various degenerative changes were observed such as an irregular network composed of microfibrils which were 0.8 μ–1.0 μ in diameter. There appeared to be a

Fig. 10. The structure of the ossification of the ligament was similar to that of normal attachment of the ligament or tendon to the bone surface. (H. & E. ×40)

Fig. 11. Chondrocytes and hyalinization (H. & E. ×200)
wide space between the fibrils and the extra fibrillar substances which were recognized as granular particles of 2000 Å in diameter (Fig. 14).

For closer observation, a number of large, round or oval shaped, developing osteocyte lacunae 0.4 μ–2.5 μ in diameter were found on all the matrix surfaces; many lacunae were conjoined.

Fig. 12. Degenerative changes of the elastic fibers (Field width: 30 μ)
meaning that two, three, or four cells were encapsulated in the matrix to form large, complex lacunae. The matrix surface was smooth owing to the high proportion of mucopolysaccharide
ground substance it probably contained (Fig. 15).

Further in, there appeared to be a proliferation of osteocytes with small vessels in the center, which was clearly recognized in decalcified specimens (Fig. 16).

Short Summary:

According to the SEM observation of the interlaminar region of the ossification of the thoracic ligamentum flavum, elastic fibers represented degenerative changes such as forming a separated and wavy pattern and forming an irregular network composed of micro-fibrils. In the ground substance, there appeared to be oval and rosen-shaped granular particles, 1200 Å in diameter.

Closer to the ossification, the structure of the region was similar to that of normal attachment of the ligament or tendon to the bone surface. SEM observation showed a number of large, round or oval shaped, forming osteocyte lacunae which were probably corresponding to chondrocytes or osteoblasts. The matrix surface was smooth owing to the high proportion of mucopolysaccharide ground substance it probably contained.

In the late stage of the ossification, there appeared to be a proliferation of osteocytes with small vessels in the center.

Calcification of the Cervical Ligamentum Flavum

Soft X-ray examination of samples showed 3 radiopaque nodular lesions, located in the para-

![Fig. 15. A number of large, round or oval shaped, developing osteocyte lacunae with smooth matrix (Field width: 15.2 µ)](image)
median portion of ligamentum flavum in C5 and C6 (Fig. 17).

On the other hand, the sectioned specimens were examined by light microscopy. By hematoxylin-eosin stain, the ligamentum flavum was characterized by a particularly large deposit of

Fig. 16. In decalcified specimens, a proliferation of osteocytes with small vessels in the center (Field width: 169 μ)
crystals which appeared as a punched-out region in the ligament completely filled with crystals. There appeared to be an inflammatory reaction with a proliferation of fibrocytes and varying degrees of infiltration with round cells and foreign-body giant cells (Fig. 18). Surrounding the microcrystalline deposit, a few cells with bright perinuclear similar to that of chondrocytes were found in the relatively less degenerative ligament in or surrounding the deposit (Fig. 19).

By von Kossa’s stain, the nodules were positive for calcium and spread into the spinal canal.

Fig. 18. An inflammatory reaction surrounding the calcium deposit (H. & E. ×40)

Fig. 19. A few cells with bright perinuclear that were found in the relatively less degenerative ligaments in or surrounding the deposit. (H. & E. ×200)
Fig. 20. The nodules were positive for calcium and spread out into the spinal canal. (von Kossa's stain × 1.5)

Although some of them represented degenerative change such as irregularity of fibers, there was no evidence of connection with calcium deposit (Fig. 20).

By Van Gieson's stain, the elastic fibers which stained black and collagen fibers which stained red decreased or disappeared compared to the surrounding normal ligament (Fig. 21).

SEM observation was divided into three parts as follows; at the center, border, and surround-

Fig. 21. The elastic fibers which stained black and collagen fibers which stained red decreased or disappeared compared to the surrounding normal ligament. (Van Gieson's stain × 1.5)
ings of the deposit.
At the center of the deposit, crystal deposition in the ligamentum flavum showed accumulation of the layer where there was a clear line of demarcation between the collagen framework and crystals (Fig. 22). In high power view, the crystals were pin-like, rod-like, granular or rectangular in shape, and 0.6 μ-4.0 μ by 0.2 μ-1.4 μ in size (Fig. 23).
At the border of the deposit, a clear line of demarcation between the collagen framework and crystals, measured 4.2 μ in length. Chondrocyte-like cells were not recognized (Fig. 24).
At the surroundings of the deposit, various degenerative changes were observed and the elastic fibers formed an irregular, branching, and wavy pattern. Crystals were not recognized.
In the outer layer of the degenerative fibers, there appeared to be normal regular fibers (Fig. 25).
By X-ray diffraction study, the crystal was determined as calcium pyrophosphate dihydrate (CPPD: Ca₃(PO₄)₂·2H₂O) and was distinctively different from that of hydroxyapatite or sodium urate.

Short Summary:
According to the SEM observation of the calcification of the cervical ligamentum flavum, crystal deposition represented accumulation of the layer where there was a clear line of demarcation between the collagen framework and crystals. And chondrocyte-like cells were not recognized that were observed in the light microscopy. By X-ray diffraction study, the crystal was determined as calcium pyrophosphate dihydrate (CPPD).
In the surroundings of the deposit, various degenerative elastic fibers were observed. And in the outer layer of the degenerative fibers, there appeared to be normal regular fibers.

IV. Discussion

The ligamentum flavum, composed predominantly of yellow elastic tissue, joins the laminae and articular processes as a continuous structure formed by a medial thicker half uniting the laminae and a lateral thinner portion surrounding the articular joints and blending with their capsules. The ligamentum flavum is broad, protecting the posterior part of the spinal canal. Since the detailed descriptions by Kirita (1970), Yanagi (1972), Moriwaki (1973), Tsue (1981), et al. \cite{5,8,41} were reported about the ossification of the ligamentum flavum, many reports especially in treatment of myelopathy due to the ossification have appeared \cite{5,12,22,26}. However, as for the patho-histological views, there has been no consistent opinion. Rupturing of elastic fibers, the bleeding tissue, the proliferation of connective tissue, the granulation of tissue, the appearance of chondrocytes, calcification and so on have been investigated for this condition by means of light microscopy. Through the light microscopic observations, Tomita \cite{34} reported that the structure at the attachments of the bone was complicated and various cells such as osteo-
Fig. 24. At the border of the deposit, a clear line of demarcation between the collagen framework and crystals (Field width: 611 μ).

Fig. 25. At the surroundings of the deposit, a clear line of demarcation between degenerative fibers and normal regular fibers (Field width: 376 μ).
cytes, chondrocytes and any other cells were observed, and that this might play an important role in the ossification of ligamentum flavum. However, there have been no descriptions by means of SEM so far.

Lateral views of the plain roentgenogram of the thoracic spine sometimes shows the presence of distinct thorn-shaped ossification between the two adjacent laminae at the anatomical location of the ligamentum flavum. HIRAOKA reported that distinct ossification of the ligamentum flavum was recognized mostly in the lower thoracic spine and the upper lumber spines in 30 percent of 128 cadavers, especially at the level of Th8-Th9 to L1-L2. And the ossification of the ligamentum flavum was recognized from the age of 20 years and frequently seen with age changes. On the basis of these findings SAKO mentioned that the ossification of the ligamentum flavum was caused not only by aging biological changes but also by degenerative changes of vertebral body.

The present observation revealed that at the attachment portion, osteocyte lacunae were recognized at the age of 16 years even in the cases with no ossification in roentgenograms and that with age changes, there also appeared to be various changes, such as decrease of the numbers of the elastic fibers, hyalinization, and the appearance of chondrocytes and osteoblasts. Although these findings are the same as the previous studies, the remarkable observation in this paper is that, at the attachments where the elastic fibers enter the surface obliquely and the level reached by the mineralization plane was also oblique to the surface, being perpendicular to the long axis of the elastic fibers. And this suggests that tension stress in normal mobility on the fibers affects the mineralization process in some way.

At the standpoint of view for the pathogenesis and mechanism of the ossification of the spinal ligaments, the changes in the ossification of the posterior longitudinal ligament represented not only enchondral ossification but also intramembranous ossification, as SASAKI mentioned. However, the changes in the ossification of the ligamentum flavum seemed to represent enchondral ossification mostly according to the previous studies.

Then how does the tension stress contributes to osteogenesis. The present report should be compared to those series previously reported.

According to URIST, the fragments of the tibia were not immobilized, and no attempt was made to control the activity of the animals: consequently callus formation, including formation of cartilage, may be assumed to have been at a maximum.

SARMINENTO also reported that cartilage formation occurred in both of no-cast group and cast group of rats with experimental fractures of one femur, but there was more cartilage in the rats that used extremity than in those that did not.

In our daily clinic, it is widely accepted that only a little callus is observed in strongly fixed fracture by compression osteosynthesis. Therefore, it might be a common knowledge that the tension stress—mobility—contribute to osteogenesis. And it seems that callus formation results against mobility.

Although ligaments exist in every part of the body, how comes the fact that the ossification of the ligamentum flavum tends to occur in the spinal ligaments, especially at the level from the
lower thoracic spine to the upper lumbar spine. There must be another factor to contribute to osteogenesis.

The second factor is the condition of relatively low oxygen. Fujimura reported that the spinal canal of the thoracic region is narrow and the spinal cord in this part is considered as "vascular critical zone" because of its minimal blood supply. Ikata had pointed out the importance of the ischemic condition of the spinal cord due to the changes of communicating vessels of the thoracic region with age changes.

Then how does the combination of mobility and low oxygen contribute to osteogenesis. Bassett reported that in the tissue-culture experiments, mesenchymal cells had produced bone tissue with high oxygen and compaction, and cartilage with low oxygen and compaction. On the other hand, they had produced fibrous tissue with high oxygen and tension.

The predominance of unidirectional tensional forces on connective tissues induces the production of a dense fibrous tissue, such as in tendons and ligaments. On the other hand, where the combination of compression and rotation are operating, the result in each instance has been a change from the connective tissue to chondrocytes and osteocytes, as Scapinelli mentioned.

Repeated minor trauma to the spine, especially at the transitional region (thoraco-lumbar region) of the biological curve of the spine, produces architectural imbalance, resulting in increase of lordosis and instability.

According to the present observation of the part surrounding the ossification, elastic fibers represented degenerative changes such as forming a separated wavy pattern and forming an irregular network composed of micro-fibrils as if they were confronting the multiple tensions by promoting chondrogenesis. Further in, there were a number of large, round or oval shaped, forming osteocyte lacunae which were probably corresponding to chondrocytes or osteoblasts. And there was no invasion of blood vessels. Consequently, it is assumed that the changes represent enchondral ossification.

Azuma mentioned through the investigation of the mechanism of the ossification of the posterior longitudinal ligament that there appeared to be granules, corresponding to acid mucopolysaccharide, among the degenerative fibers by means of TEM and these granules might play an important role to the mechanism of the ossification.

The present observation showed that there appeared to be oval and rosen-shaped granular particles in the surroundings of the ossification and that the matrix surrounding osteocyte lacunae had a smooth surface owing to the high proportion of mucopolysaccharide ground substance. These granules are difficult to be identified by means of SEM because of its technical limitation and must be compared with the findings of TEM. Therefore, further investigations about granular particles may be important to elucidate the etiology of the ossification of the ligamentum flavum.

At the standpoint of considering the etiology of the ossification of the ligamentum flavum, we could not deny the fact that other ossification of the spinal ligaments were frequently found to coexist in the same patients and they were observed in many parts throughout the spinal column. Yanagi, et al. had already pointed out such a tendency and recommended a genetic name
"Ossification of spinal ligaments". Therefore, this present study has been divided into two parts: one is the ossification found only at the thoracic ligamentum flavum and the other is ossification accompanied with ossification of the spinal ligaments such as the ossification of the posterior longitudinal ligament. However, there was no significant difference between them.

Although CPPD deposition in the cartilage has been known as pseudo-gout syndrome and many cases have been reported [20, 31] with CPPD deposition in the fibrocartilagenous areas, such as menisci, discus articularis of the wrist, and symphysis pubis, deposition in the ligament has been reported in only a few cases causing cervical myelopathy [13, 14, 23].

According to the present observation, the crystal deposition was obviously isolated from the fibrous tissue and detached from the lamina. And chondrocytes or osteocytes were not recognized. In the surroundings of the deposit, various degenerative elastic fibers were observed. In the outer layer of the degenerative fibers, there appeared to be normal regular fibers.

Consequently, it brings up an important consideration, that is, the distinction between the calcification of the ligamentum flavum and the ossification of it.

V. Conclusion

1. Scanning electron microscopic observation was performed to examine the pathogenesis and mechanism of the ossification and calcification of the ligamentum flavum. Specimens were obtained in operations from eight cases of the ossification of the thoracic ligamentum flavum and two cases of the calcification of the cervical ligamentum flavum. And as a control group, nine specimens of the ligamentum flavum were obtained from cases of spinal canal stenosis or spinal tumor when laminectomized.

2. According to the SEM observation of the interlaminar region of the thoracic ligamentum flavum on the control group, elastic fibers formed a dense and regular pattern, some of them formed a wavy pattern and some of them were divided into a few branches with interconnections between the fibers. Elastic fibers, which formed woven bundles with obscure demarcation closer to the attachments, were inserted into the surface of the bone. According to the observation of the attachments with age changes, a not unusual observation was of osteocyte lacunae, probably corresponding to chondrocytes or osteoblasts. The level reached by the mineralization plane of them was oblique to the surface, being perpendicular to the long axis of the elastic fibers. And this suggests that functional pull on the fibers affects the mineralization process in some way.

3. According to the SEM observation of the interlaminar region of the ossification of the thoracic ligamentum flavum with no other ossification in roentgenograms, elastic fibers represented degenerative changes such as forming a separated and wavy pattern and forming an irregular network composed of micro-fibrils. At the surroundings of the ossification, there appeared to be a number of large, round or oval shaped, forming osteocyte lacunae which were probably corresponding to chondrocytes or osteoblasts. In the late stage of the ossification, there appeared to be a proliferation of osteocytes with small vessels in the center. Consequently, it is assumed that the changes represented enchondral ossification.

4. According to the SEM observation of the calcification of the cervical ligamentum flavum.
crystal deposition represented accumulation of the layer where there was a clear line of demarcation between the collagen framework and crystals. The crystals were pin-like, rod-like, granular or rectangular in shape. By X-ray diffraction study, the crystal was determined as calcium pyrophosphate dihydrate (CPPD). And chondrocyte-like cells were not recognized. In the surroundings of the deposit, various degenerative elastic fibers were observed with inflammatory reaction in light microscopy.

5. The ossification and calcification of the ligamentum flavum are completely different conditions.

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黄色靱帯骨化および石灰化の走査電顕的研究

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黄色靱帯の骨化過程を解明する目的で、脊柱管狭窄症・脊髄腫瘍9例を対照として、胸椎黄色靱帯骨化症8例、頸椎黄色靱帯石灰化症3例について、手術時採取された黄色靱帯を走査電子顕微鏡を用いて観察した。

対照群としたX線所見上骨化を有しない胸椎黄色靱帯での観察によると、椎弓関節部では、一部に弾力線維の枝分れ・波状の走行を認めるも、走行は一定しておらず、各線維間も明瞭に区別される。椎弓付着部近傍においては、弾力線維は境界不明瞭となり線維束を形成し骨に付着する。骨付着部では、骨表面より靱帯内に軟骨細胞・骨芽細胞の存在を思わせるosteocyte lacunae を認め、石灰化は弾力線維に垂直な面で行なわれており、機能的な牵引力が石灰化に何らかの影響をおよぼしているのではなかろうかと推測される。

胸椎黄色靱帯骨化症での観察によると、骨化近傍になるにつれ、弾力線維は分裂数・蛇行micro-fibrilの網状構造などの変性所見を認める。骨化部においては、軟骨細胞・骨芽細胞の存在を思わせる多数のosteocyte lacunae が認め、さらに進行した骨化部では、毛細血管を中心とする層状構造の骨細胞の出現があり、骨新生を認める。以上の所見より、黄色靱帯の骨化機序は内転性骨化であると推測される。

頸椎黄色靱帯石灰化症での観察によると、石灰化部は線維性基質と明瞭に区別され、針状・棒状・四角柱状の結晶で構成されている。しかし、軟骨細胞は認められなかった。再びには、変性された弾力綱維が認められ、光顕にて、炎症所見が認められた。X線回析にて、石灰化部の結晶はCPPD (Ca2P2O7・2H2O)であることが判明した。

胸椎黄色靱帯骨化症と頸椎黄色靱帯石灰化症とは病態は全く異なるものである。