

## Original Article

# Scanning electron microscopy (SEM) study on filum terminale with human fetus

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**Abstract:** The filum terminale plays an important role in the pathophysiology of tethered cord syndrome (TCS). The study on ultrastructure of fetal filum can provide reference standard for diagnosis of TCS. Eleven fresh Chinese human aborted fetuses had their fila measured and removed. Transverse and longitudinal sections of the middle and distal segments of fila were submitted for scanning electron microscopy (SEM) analysis respectively. The bulk of the filum terminale is composed of 1  $\mu$ m to 5  $\mu$ m thick spring like longitudinal bundles of collagen separated by 5  $\mu$ m to 30  $\mu$ m layer intervals and 1  $\mu$ m to 5  $\mu$ m intervals in the layer, although a small quantity of capillaries and other components may be present. A delicate meshwork of collagen transverse fibers connects these bundles and fibers in every bundle. A delicate meshwork of collagen transverse fibers connects these bundles and fibers in every bundle. A complex tridimensional structure is evidenced on electron microscopy. In conclusion, a complex tridimensional structure composed by ordered arrangement of spring-like fibers and a small quantity of capillaries should elicit considerable elastic properties to the filum terminale. Its alternation of structure and component may be involved in TCS closely.

**Keywords:** Filum terminale, tethered cord syndrome, ultrastructure

## Introduction

The filum terminale is a fibrous band, composed of two distinct segments (intradural and extradural), that extends from the conus medullaris to the periosteum of the coccyx [1]. The proximal structure of the filum is similar to spinal cord, the middle and distal segments of which are fibrous tissue. In the first three months of the embryo, vertebral column grows the same as spinal cord, after that, the vertebrae exhibits a higher growth rate than does the spinal cord, resulting in an ascending movement of the conus medullaris relative to the vertebrae, pia mater on its surface is stretched into linear filum, attached to the coccyx. The caudal end of full term neonate spinal cord is close to adult, about L1-L2 vertebral level. The filum of adult is about 20 cm long (intradural, 15 cm; extradural, 5 cm), 1-2 mm thick. Atrophy of the caudal neural tube is responsible for the

formation of the filum terminale, the central canal and neural stem cell are noticed in the proximal section of the filum, as described in the filum of various species [2].

The clinical significance of the terminal filum lies in the tethered cord syndrome (TCS), which was first described by Garceau in 1953 [3]. Tethered cord syndrome is a stretch-induced functional disorder of the spinal cord. The mechanical cause of TCS is an inelastic structure, which includes filum terminale disease, (such as fatty filum, tumor, mineral deposition, etc.) myelomeningocele, lipomyelomeningocele, or scar formations adherent to the spinal cord or nerve roots, anchoring the caudal end of the spinal cord that prevents cephalad movement of the lumbosacral cord [4-9].

Stretching of the spinal cord occurs in patients either when the spinal column grows faster

**Table 1.** Specimen data

No.	GA (weeks)	Sex	CRL (mm)	FT length	FT initiate diameter (mm)	FT middle diameter (mm)	Conus
1	12-13	Male	161	28.5	1.10	0.1	L4
2	15-16	Female	182	35.3	1.18	0.15	L4
3	25-26	Female	252	41.4	1.22	0.18	L2-L3
4	29-30	Male	283	45.6	1.24	0.29	L1-L2
5	31-32	Female	302	55.6	1.29	0.28	L1-L2
6	28-29	Female	276	46.8	1.3	0.69	L2
7	30-31	Male	297	54.8	1.26	0.26	L2
8	13-14	Male	168	37.8	1.23	0.21	L4
9	34-35	Male	330	57.3	1.3	0.7	L2
10	33-34	Female	318	56.7	1.3	0.63	L1-L2
11	24-25	Female	249	40.6	1.22	0.36	L2-L3

GA = gestational age; CRL = Crown-rump length; FT = filum terminale.

than the spinal cord or when the spinal cord undergoes forcible flexion and extension. This syndrome is defined as a disorder manifested by motor and sensory deficits in lower limbs, incontinence, and musculoskeletal deformities.

Once diagnosed definitely, undoubtedly, surgical management of TCS is the best choice of all, furthermore, less complication and better effectiveness is attributed to application of new surgical technique [10], whereas the diagnosis of TCS is still difficult, especially when there is a normal level of the conus medullaris or normal thickness of the filum on radiological examination [11]. The terminal filum has received increasing attention on TCS pathogenesis with the deepening of study on TCS. The rat experiments conducted by Miklos and his colleagues have revealed that its basic components (central canal, gray matter, white matter) continued within the filum, uniform small size neurons and glial cells populated the gray matter; small nerve cell surrounding ependymal cell may be nerve stem cell (NSC) [12]. Or rather, NSCs arranged densely in the proximal segment while sparsely in the distal one, as we have previously reported [13, 14]. Tubbs et al. found previously undescribed smooth muscle cells within the filum terminale externum. Furthermore, histological analysis identified adipose, nerve, bone, and cartilage cells. Macroscopically, the filum thicker than 2 mm and its fatty change were distinguished easily on MR investigation or direct visualization during the surgical intervention, which is more

obvious under light microscopy [15]. The filum with normal appearance on MRI showed some hyalinization and dilated capillaries [16]. Fontes et al. made a more detailed study of 20 samples of normal adult fila by light and scanning electron microscopies [17], however, it has scarcely been reported about the SEM study on the structure of normal fetal fila has not been reported.

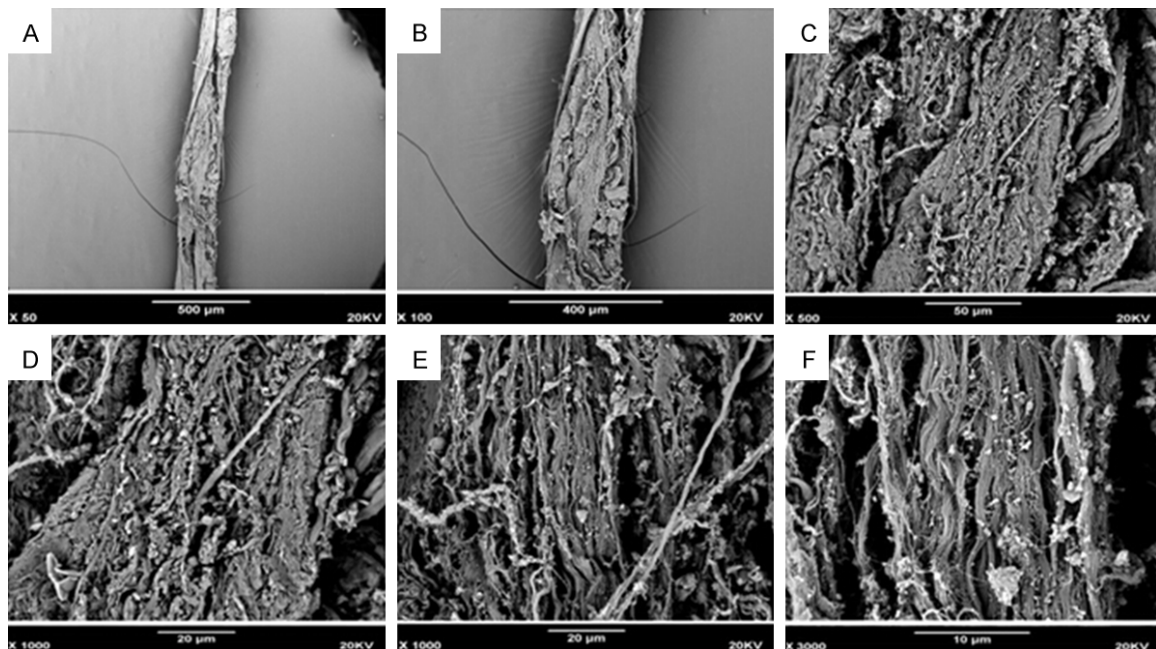
## Materials and methods

### Specimen origin

Eleven samples (five male, six female) were dissected 4 to 8 hours after abortion in second trimester pregnancy. (These had been investigated and validated by Ethical Review Committee, and informed consent forms were signed by their parents). The detailed data of the subjects are given in **Table 1**. There were no abnormalities on the skin of the spine region. None of these subjects involved orthopaedic, neurological, or urinary malformation. Dissection was initiated with a mediodorsal skin incision, followed by exposure of the spinous processes and laminae of the T11-S5 vertebrae. The initial point of filum was promptly identified as the junction of the terminal filum and the conus medullaris, separated downward until the end of coccyx. The 12th rib and 12th thoracic vertebra were taken as a reference point, length and thickness measurements were obtained with a vernier caliper after the filum was sectioned. No tension was applied to the fila for measurements. Each filum was divided into three sections equally, then, thoroughly washed in 0.1 mol/L phosphate buffered saline (PBS) before fixation.

### Scanning electron microscopy

Segments (1 cm) of the middle and distal segments of four fila were perfused with a fixative containing 2.5% glutaral, 4% paraform in 0.1 mol/L PBS for more than 2 h at 4°C. After washing in PBS, they were immersed in 12% dimethyl sulfoxide for two 30 min, 25% for two 30 min, and 50% for one 1.5 h, followed by freeze-frac-



**Figure 1.** Early stage, SEM picture of FT belonging to specimen no. 1 with male, 12~13 gestational weeks. The pia mantle of filum is opened, and internal structure is exposed (A, B); some fiber bundles arrange longitudinally, some fiber also connect obliquely from one bundle to another bundle (C, D); These collagen fibers arrange like spring at high magnification, and some intervals between fiber bundles exist (E, F).

turing in liquid nitrogen into cross section, sagittal plane, coronal plane; then, they were immersed in 50% dimethyl sulfoxide for 15 min and immersed in 0.1 mol/L PBS for 10 min. Succeedingly, they were dehydrated in an increasing alcohol series (30%, 10 min; 50%, 10 min; 70%, 10 min; 90%, two 10 min; 100%, three 10 min), replaced with amyl acetate for 1 min. Sections were critical-pointdried with liquid carbon, gold coated in a Balzers SCD-040 ion sputterer, and examined in a JEOL-JSM840 (Jeol, Tokyo, Japan) scanning electron microscope.

GA = gestational age; CRL = Crown-rump length; FT = filum terminale.

## Results

### *Macroscopic description*

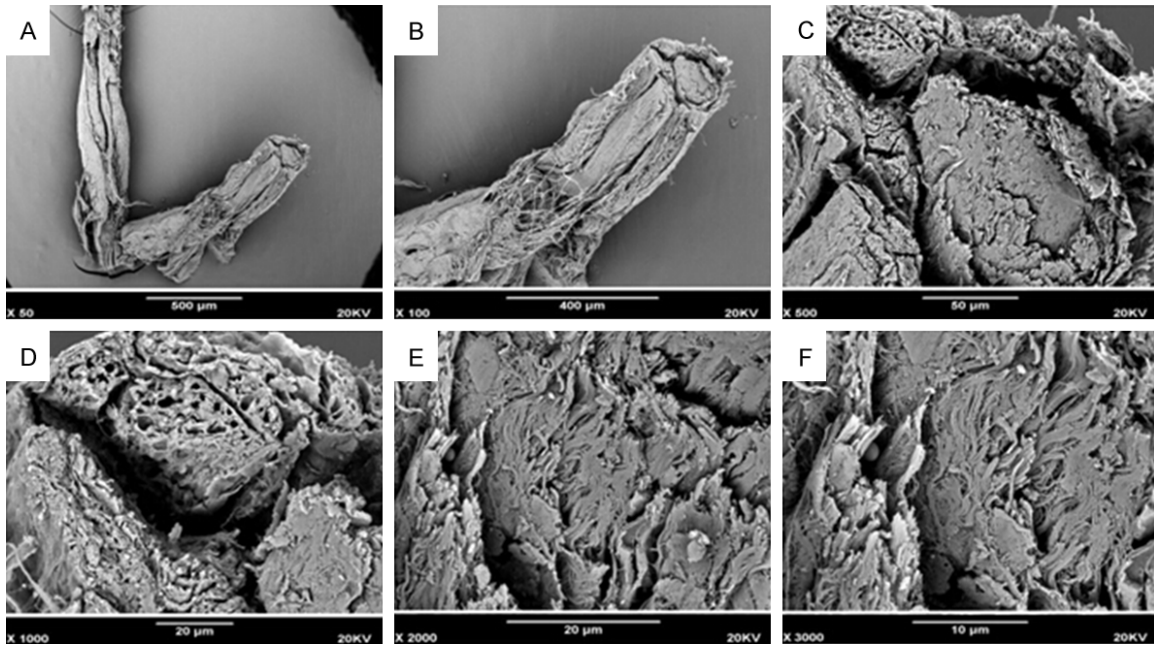
The conus medullaris was located at the lower end of the spinal cord. Originating at the base of the brain, this thick bundle of nerve tissue passes through the center of the spinal column, penetrating the vertebrae. At its terminal end, at the lumbar spine in the lower back, the spinal cord tapers into a cone shape and then into a narrow bundle of fibrous tissue called the

filum terminale, about 28.5-57.3 mm in length (mean value 45.5 mm). It consists of two parts: The upper part, or filum terminale internum, was about 21.2-43.1 mm long and reached as far as the lower border of S2. It was continuous above with the pia mater and contained within a tubular sheath of the dura mater. In addition, it was surrounded by the nerves forming the cauda equina, from which it can be easily recognized by its bluish-white color. The lower part, or filum terminale externum, closely adhered to the dura mater. It extended downward from the apex of the tubular sheath and was attached to the back of the first segment of the coccyx in a structure.

### *Electron microscopy ultrastructure*

A low power electron micrograph showed the appearance of filum on the low power microscope at early (**Figure 1A, 1B**), middle (**Figure 2A, 2B**), late stage (**Figure 3A, 3B**). The filum was surrounded with crapy pia mater (**Figure 3A, 3B**). Spring-like longitudinal collagen bundles were seen in the longitudinal section (**Figures 1C-F, 3A, 3D**). Some intervals ranged from 5  $\mu$ m to 30  $\mu$ m between layers were seen in the cross section (**Figures 2C-F, 3C**). The thickness of collagens ranged from about 1  $\mu$ m





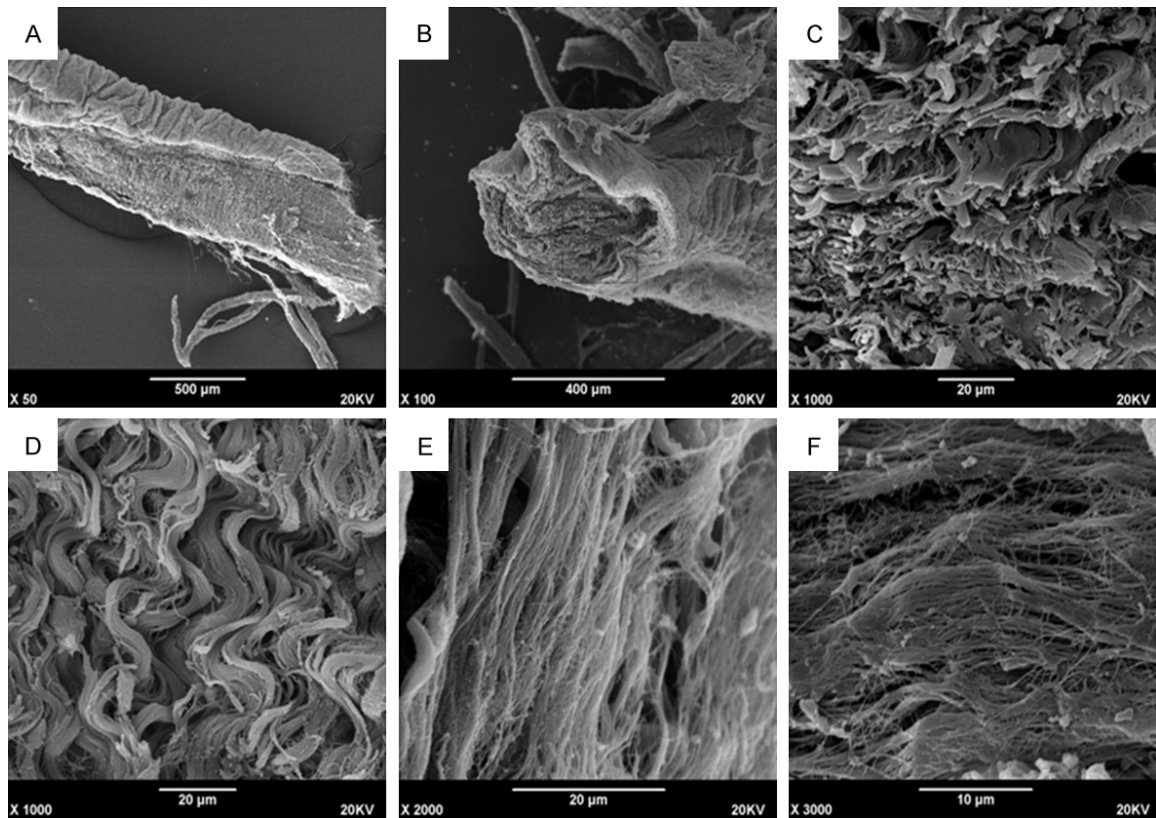
**Figure 2.** Middle stage, SEM picture of FT belonging to specimen no. 6 with female, 28~29 gestational weeks. The cross-sections of filum are presented in different multiples (A-F); That shows collagen fiber bundles were stratified distribution, some intervals exist between layers, another intervals exist between collagen bundles of the same lay (C-F).

to 5  $\mu\text{m}$  (Figures 2E, 2F, 3C, 3D). The collection of collagen bundles might be about 10  $\mu\text{m}$  wide zone in the same layer (Figures 2E, 2F, 3C). There were some intervals ranging from 1  $\mu\text{m}$  to 5  $\mu\text{m}$  between bundles in the same layer (Figures 2E, 2F, 3C, 3D). Collagen fiber also connected obliquely from one bundle to another bundle (Figures 1F, 3E). All layers and bundles from each other were linked with intricate meshwork of finer transverse fibers (Figure 3F). Blood capillaries were occasionally seen within these longitudinal bundles in the cross section (Figure 3C). High power scanning electron microscopy showed clearer structure.

### Discussion

Many scholars do have a deep understanding of that elasticity decrease of the filum is an important cause of TCS. Selcuki et al. observed the fila of normal adult corps via light microscopy; found that normal filum was composed of loose connective tissue, rarely fat cells, and a small quantity of capillaries [16]. This research on structure of normal fetal filum is similar to that of mentioned above. However, the difference is that fat cells not being found in our researches.

What is the difference of normal and abnormal filum that requires TCS concretely? Selcuki et al. observed normal and abnormal fila with symptomatic TCS, found that the former contained more connective tissue than normal, with dense collagen fibers, dilated capillaries with cell infiltration, random fat cells, and some areas of hyalinization; elevated numbers of collagen bundles, areas of hyalinization were frequently observed throughout the connective tissue in the latter [16]. The high amounts of dense collagen fibers cause the filum to lose its elastic properties, ultimately leading to conduction of a tethering effect to the conus medullaris [17]. Liu et al. found that the presence of abundant collagen bundles and adipocytes, sparse or invisible elastic and reticular fibers inside the FT [18]. Ozkan et al. compared pathological fila with normal fetal ones by light microscopy found adipose tissue, fibrosis, hyalinization, meningotheelial proliferation and elastic fibers in FT samples of TCS, none of these findings were observed in fetal samples [19]. Fontes et al. selected fila of 20 so-called normal adult corps, submitted to light and scanning electron microscopy, concluded that the bulk of the filum is composed of 5 to 20  $\mu\text{m}$  thick longitudinal bundles of Type 1 collagen



**Figure 3.** Late stage, SEM picture of FT belonging to specimen no. 10 with female, 33~34 gestational weeks. Crap pia mantle surrounding longitudinal spring-like collagen bundles and pia mantle surrounding collagen arranged like tree rings (A, B). Some intervals range from about 5  $\mu\text{m}$  to 30  $\mu\text{m}$  between layers, another intervals range from about 1  $\mu\text{m}$  to 5  $\mu\text{m}$  between collagen bundles of the same lay, the diameter of collagen bundle is about 1  $\mu\text{m}$ -5  $\mu\text{m}$ , the collection of collagen bundles become about 10  $\mu\text{m}$  wide zone (C), capillaries and red blood cell in the upper left corner of C. Longitudinal collagen bundles arrange like spring, about 1  $\mu\text{m}$ -5  $\mu\text{m}$  interval between bundles (**Figures 1D, 1E and 3D**). Collagen bundle are composed of many longitudinal fibers. Collagen fibers also connect obliquely from one bundle to another bundle (E). **Figures 1F and 3F** show clearer structure at high magnification.

separated 3 to 10  $\mu\text{m}$  intervals [17]. A delicate meshwork of predominantly Type 3 collagen transversal fibers connects these bundles, in addition to abundant longitudinally oriented elastic and elaunin fibers are found inside collagen bundles, and thought this elastic property played an important role in pathogeny, diagnosis and treatment of TCS, especially pathogeny explanation of TCS with normal conus position. Royo-Salvador et al. thought special neurology syndrome such as syringomyelia, scoliosis and Chiari malformation may occur secondary to elasticity decrease of the filum, but there was a controversy [20].

The main finding of the present study demonstrates the filum is not a simple longitudinal connective cord, but a complex tridimensional structure. Curving and longitudinal fiber bundles are in layers, some intervals between lay-

ers or bundles, but the latter is smaller than the former, in which sparse, transverse and delicate reticular fibers are found to connect longitudinal fibers or bundles, as well as some collagen connected between bundles and layers. High electron microscopy shows collagen fibers are linked with more transverse fibers. It has not been reported which involves collagen bundles are in layers, or intersect with each other between bundles and layers, and collagen fibers linked by more transverse fibers. This further indicates that the filum is a delicate, complicate and arranged orderly elastic structure, the decrease of which may affect its elasticity, resulting in TCS. That finer transverse fibers in this research maybe no other than type 3 collagen as Fontes et al. described, the diameter of type 1 or 3 collagen may be identical or close to that of elastic fibers. Under the scanning microscopy, we found spring-like structure of

longitudinal fiber bundles of human fetuses more distinct, more delicate, more explicit, by comparison with the fila of normal adult corps, which perhaps relates to freshness of specimens or promptness of their collection and fixation, or maybe because of obvious difference of the both, or abnormalities of their specimens proper (con of 4 samples be located below L2, the fila thickness of 2 samples more than 2 mm), this reflects a better elastic property.

This research found there was high concentration of elastic fibers, accompany with longitudinal collagen bundles. Few tissues (except the dermis, vascular wall, and lung, etc.) in the human body exhibit this amount of elastic structures; even fewer possess a single longitudinal alignment within parallel bundles. The flexibilities of elastic fibers could make the organization have certain deformation, the toughness of collagen fibers could make the organization keep certain shapes, and the concomitance of the both makes up of connective tissue, make the organization to keep certain shapes and certain adjustable elasticity. This aligned longitudinally and curving fibrous structure confirms important elastic property of the filum, and one of its important physical functions: as an elastic buffer which stops spinal cord stretch injury for the developing spinal column or an excessive flexion; conversely, elasticity decrease of the filum could injury spinal cord. In addition, transverse reticular fiber consists of type 3 collagen, and keeps longitudinal fibers bundles stable like mesh scaffolds. When the filum suffers to inflammation, or reshapes itself in the development, transverse reticular fibers will lose its function, make the arranged structure destroyed, that might also affect elasticity of the filum.

In summary, normal fetal filum is not a simple fibrous band, but a delicate, complex, tridimensional structure, containing longitudinally oriented elastic, collagen fibers and transverse reticular fibers, and a small quantity of blood vessels, short of adipose tissue, which reflects the filum of considerable elastic properties. Clinically, its alternation of structure and component may be involved in TCS closely, which has great significance especially for treatment of TCS with normal conus position.

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## Disclosure of conflict of interest

None.

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