Medical and Clinical Research: Open Access

Research Article

A Study About Development of The Reticular Fibers Under the Nail in Human Embryos

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Received: June 17, 2021; **Accepted:** July 10, 2021; **Published:** July 15, 2021

Citation: Kishi Y, Hashimoto N, A Study About Development of The Reticular Fibers Under the Nail in Human Embryos. Med Clin Res Open Access. 2021; 2(3):1-7.

ABSTRACT

This study demonstrates the formation of reticular fibers under fetus nail during the nail development. The seventy-one digits of human fetus had been kept in the buzan stein in the department of the second Anatomy since 1970. They were amputated to measure the length and checked the shape. Then paraffin fixation method was performed. While the nail was formed, we observed using H-E stein and PAM stein how the reticular fibers under the nail were produced. In the early stage of nail formation, the basal layer of the epidermis was positive in PAM stein from matrix and nail bed. Under the basal layer the reticular fibers also were dyed like an arched bridge from the tip of distal phalanx before the matrix primordium was formed. Within the period after the matrix proximordium was produced, the reticular fibers developed under the nail bed especially at the two parts. One part is between the distal phalanx and the matrix primordium. The other is between the tip of distal phalanx and the distal groove. These fibers became thick gradually. In our study, the nail bed and matrix were constantly positive in PAM stein during nail formation. It shows that the nail bed and matrix make the reticular fibers during the nail developmental stage. The ridged connection between the tip of the nail and the distal phalanx is made by the reticular fibers.

KEYWORDS

Nail Development, Fetus, Reticulation Fibers.

Introduction

Using 71 human fetal fingers preserved in the Department of Anatomy 40 years ago, the formation of subungual reticular fibers during nail formation was observed using H-E and PAM staining. Before the formation of the proximal nail fold, the basal layer of the epidermis from the matrix to the nail bed was positive for PAM staining, and the underlying layer of the epidermis up to the DIP joint in the area of the matrix and proximal nail fold was stained for reticulin fibers. In the period between the formation of the proximal nail fold and the formation of the nail, fibers were found not only in the basal layer and its lower layers, but also in the basal layer just below the matrix, especially in the proximal end of the proximal nail fold and the distal phalanx. There were two areas where fibers traveled between the distal end of the distal phalanx and the distal end of the matrix and the hyponychium. By the time the nail was completed, the Matrix and Nail Bed were stained with almost the same thickness, and the underlying layer was densely covered with reticulated fibers between the distal phalanx and the distal phalanx, in contrast to the palm side. During the formation period, the nail bed was always positive for PAM staining, indicating active formation of fine meshwork fibers.

There are two theories for the development of the nail: the three-layer, three-primary theory of Lewis [1], which states that the nail consists of the dorsal portion of the nail capsule base, the latent margin of the nail root, and the epidermis of the nail bed; and the one-layer, one-primary theory of Zaias [2], which states that the nail consists only of the nail matrix. According to Zaias [2], the human nail begins to develop at 9 weeks in utero and is complete

at 20 weeks. As for the tissue surrounding the nail, ossification of the terminal phalanx occurs at 14 weeks, muscle and tendon differentiate, and fiber bundles are formed in the dermal layer. In this study, we focused on the fiber bundles that exist between the nail bed and the terminal phalanx and observed the formation process using human fetal fingers.

Subjects and Methods

Human fetal fingers were used to observe the development of the normal nail and surrounding tissues. These 71 fingers were fixed in 10% formalin or Bouin's solution and stored in the Department of Anatomy, for more than 40 years. Since the age of the fetus was not known, the length of each finger was measured as a measure of development. Since the middle finger was the most common preserved finger and the middle finger had the best fixation condition, only the middle finger was cut at the MP joint and used as a specimen. The specimens were examined under a stereomicroscope, and some of them were dehydrated with ethanol, paraffin-embedded, and 4 μm thin sections were prepared in the sagittal direction. (H-E stain) and periodic acid methenamine silver stain (PAM stain), which is a method for detecting reticular fibers composed of collagen III that appear during wound healing and development. Some fingers were also subjected to PAM staining as 100 μm frozen sections in the sagittal or transverse direction.

Table 1: Number of fingers divided by the length of each finger

Length mm	thumb	Index finger	Middle finger	Ring finger	Little finger
1~2	0	1	9	1	1
2~3	0	1	16	1	2
3~4	2	1	8	0	0
4~5	0	0	7	0	3
5~6	1	1	1	2	1
6~7	1	2	1	2	1
7~8	1	1	0	1	1
8<	0	0	2	0	0

Results

Measurement of the Finger

The length of the finger was measured from the MP joint to the tip.

Nail Morphology of The Fetal Middle Finger

1: No nail was found in the fetal middle finger when it was less than 2 mm in length (Figure. 1a). At this stage, the nail bed had a distal groove called a distal groove.

2: When the length of the distal groove increased to 2 mm or more, the thickness of the distal edge of the finger increased and a ridge appeared. (Figure 1b).

3: When the length of the finger increased to more than 2.7 mm, a claw was formed at the tip, hyponychium was seen, and the claw was clearly separated from the tissue at the tip. (Figure 1c).

Observations in paraffin and thick frozen sections in sagittal direction (HE staining, PAM staining).

1: Differentiation of the nail bed into Distal Ridge, Nail Bed, and Matrix was observed even in HE-stained fingers at the stage where nail formation was not observed. However, the epidermal basal lamina is continuous with the palm side and the dorsal side, and a slightly thicker layer is formed from the Distal Groove to the Matrix (Figure 2a.b).

The cells under the basal layer of the epidermis from Matrix to Matrix Primordium were different from those in the fingertip region, and were regularly and densely intercalated with the distal phalanx.

PAM staining was applied to the fingers during this period and observations were made (Figure 3). As a result, even before the formation of the matrix primordium, a positive reaction was observed in the area corresponding to the basement membrane just below the basal layer of the epidermis from the matrix proximal to the Proximal Groove to the base of the nail bed. In the dermis of the posterior nail contour, a network of fine fibers with positive PAM staining was observed. However, there was no positive reaction in the subepidermis from the distal groove to the palm side. In addition, there were positive fiber reticulations around the cartilaginous primordium of the distal phalanx or the perichondrium surrounding the aggregation of mesenchymal cells, especially in the dorsal region.

In order to observe the travel of these fibers more clearly, PAM staining was applied to 100 μm thick sections (Figure 4a.b). Reticular fibers ran in the peripheral and central directions, bordering the area that should be the Primordium.

PAM staining showed positive images in the basal lamina from the Matrix to the Distal Ridge, with no positive reaction in the Matrix primordium and Proximal nail fold. The reticular formation of fibers under the matrix was more prominent and subepidermal to the DIP joint on the dorsal side. The reticular formation of fibers under the matrix became more prominent, and subepidermal reticular formation was observed dorsally up to the DIP joint. At this time, subepidermal reticular formation was also observed on the palm side, although to a lesser extent than on the dorsal side.

In particular, from the center of the nail bed to the periphery, the number of fibers under the basal layer of the epidermis decreased, but fibers running from just below the basal layer to the cartilage membrane at the tip of the terminal phalanx were formed.

2: The cells in the underlying dermis have larger nuclei than the cells in the nail bed, and in the Matrix Primordium, the nuclei run obliquely toward the terminal phalanx. (Figure5. a.b) The cells in the underlying dermal layer had large nuclei, which in the Matrix Primordium traveled obliquely toward the distal phalanx, and from the Matrix to the Nail Bed were aligned in a relatively vertical direction toward the bone. In the lower layer, there were cells with a small nucleoplasmic ratio and bright sporophytes.

3: PAM staining at the time of nail formation also showed no positive reaction in the proximal nail fold, but fine mesh fibers

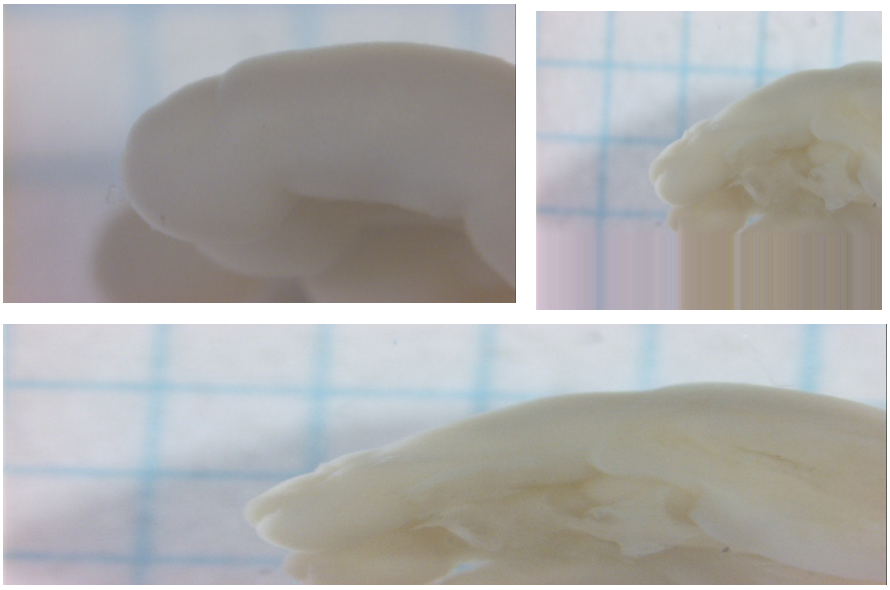


Figure 1a: When fingers are less than 2 mm long, nails have not yet formed.

1b: Ridge appearance at the tip of the finger when the length of the finger is between 2 mm and 2.6 mm.

1c: Nails are formed when fingers are at least 2.7 mm long.

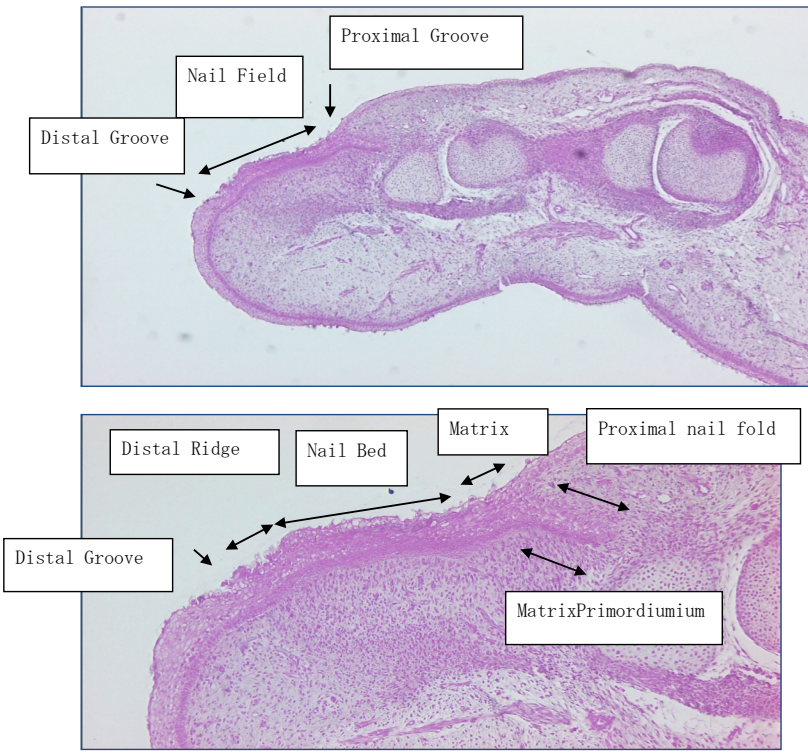


Figure 2: Differentiation of the nail bed into Distal Ridge, Nail Bed, and Matrix was observed even in HE-stained fingers at the stage where nail formation was not observed.

a: 4x

b: 7.5x

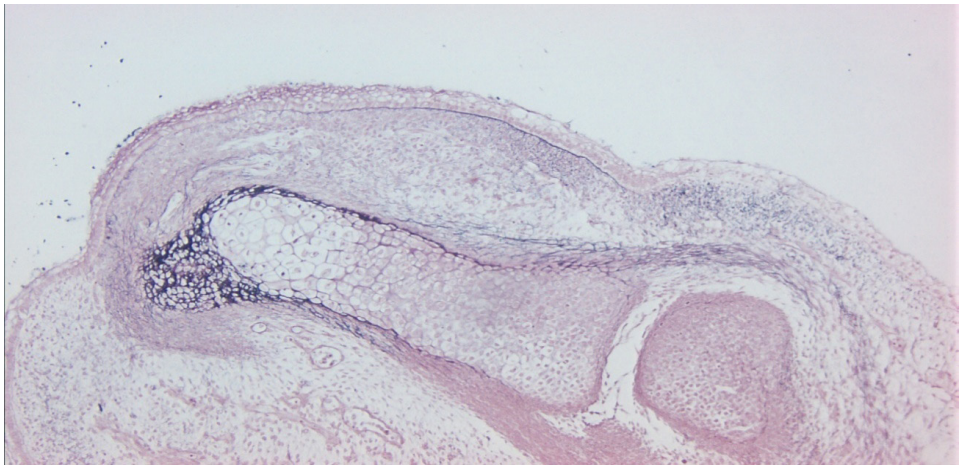
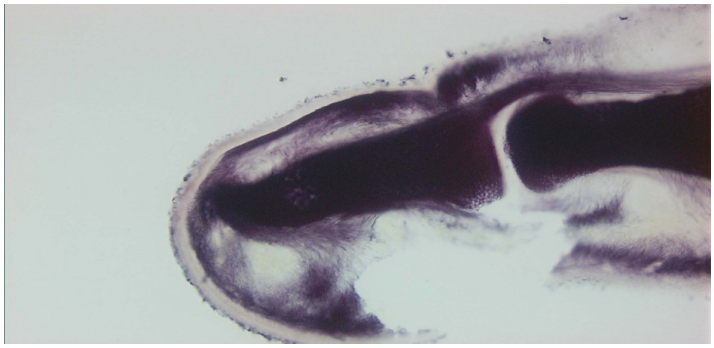


Figure 3: Even before the Matrix primordium was formed by PAM staining; positive reactions were observed in the area corresponding to the basement membrane just below the basal layer of the epidermis from the Matrix proximal to the Proximal Groove to the base of the Nail Bed.

X7.5



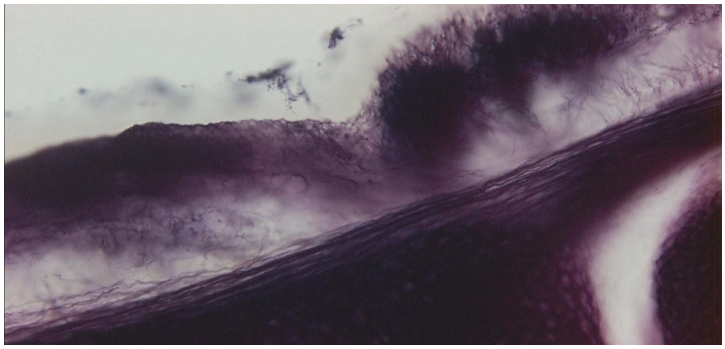


Figure 4: PAM staining of 100 m thick sections revealed dark staining in the basal layer of the epidermis and submatrix tissue of the Nail Bed, as well as in the Proximal Groove and DIP joint areas.

a:4x

b:7.5x

were stained between the distal phalanx and skin, including the area from the inferior part of the matrix to the upper part of the proximal nail fold (Figure 6a.b).

In thick sections stained with PAM at the time when the nail bed was divided into three parts and the keratin proximal to the Distal Ridge was thickened, the reticular fibers were still stained darkly in the nail bed, but their thickness decreased, and they formed a C-shaped run extending from the center of the distal end of the nail bed to the distal end of the terminal phalanx (Figure 7a.b).

A run of reticulation fibers was formed in the dermal layer just below the Distal Groove and at the tip of the distal phalanx (Figure 8 a.b).

4: In thick sections stained with PAM at the time of nail formation, the density of subungual reticular fibers was uniform from the matrix primordium to the distal end of the nail. However, between the subungual layer and the distal phalanx, there was a rather sparse but dense network of fibers running in a c-shaped pattern proximally and distally toward the distal phalanx, centered on the matrix. The nail was longer than the terminal phalanx and extended to the tip. Only at the tip of the finger, subepidermal reticular fibers were present in a block-like pattern toward the terminal phalanx, but the palmar side was very sparse compared to the dorsal side (Figure 9).

Discussion

Zaias [2] and Lewis [1] have proposed a one-layer, one-way theory and a three-layer, three-way theory, respectively, for the development of the nail. Zaias [2], using autoradiography, found that tritiated chrysin administered into the peritoneal cavity of monkeys migrated to the nail capsule only from the nail matrix, and not from the nail bed. In this study, they found that tritium chrysin administered intraperitoneally to monkeys migrated only from the nail matrix to the nail blade, and that tritium chrysin entering the nail bed did not migrate to the nail blade. In this study, the epidermis on the palmar side of the proximal nail cage was found to be a cuticle and did not migrate to the nail capsule. Suzuki [3] also reported from histological studies on the regeneration of the nail capsule after nail removal that the nail capsule consists only of the nail matrix and that the nail bed

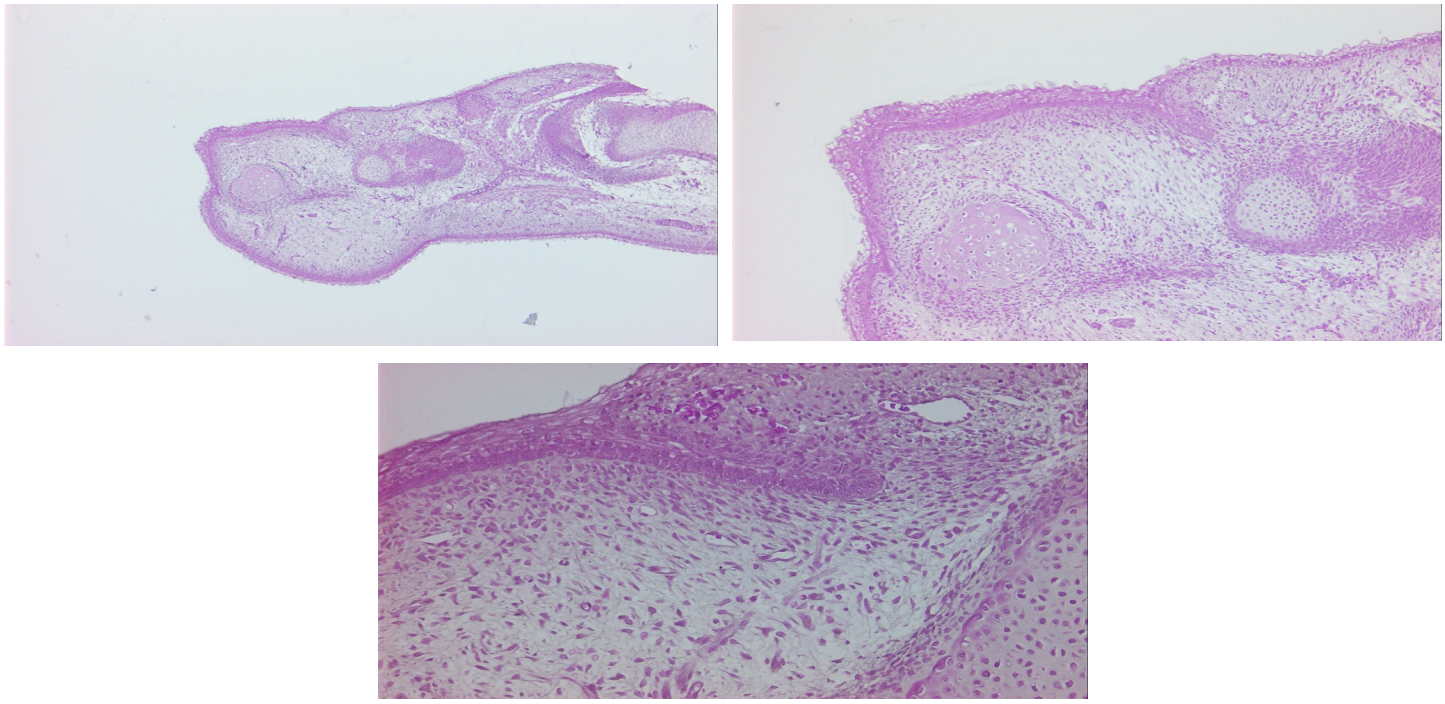


Figure 5: During the period when the tip of the nail is rounded and elevated above the distal groove, no nail formation is observed, but the Matrix cells are clearly separated from the Proximal Nail Fold layer, and cells with larger nuclei than those of the nail bed are aligned.

a: X4

b: X7.5

c:x

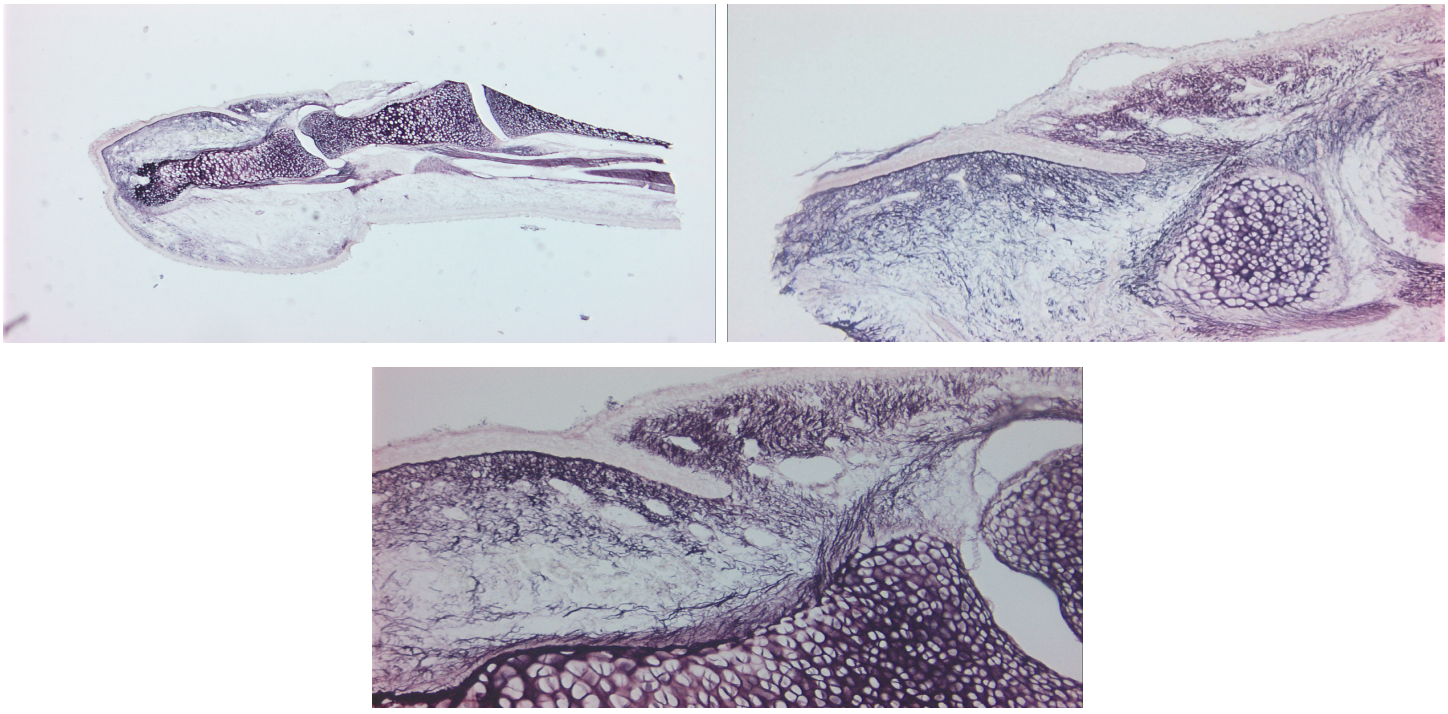


Figure 6: PAM staining showed a positive image in the basal layer from Matrix to Distal Ridge.

a: x4

b: x7.5

c

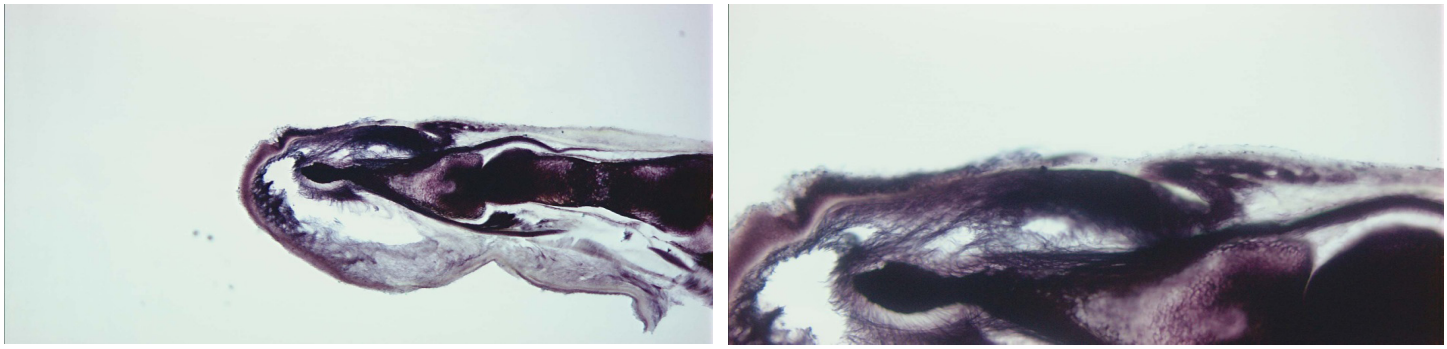


Figure 7: PAM staining of thick sections showed prominent reticular fiber formation in the central side of the matrix and nail bed and in the proximal nail fold.

a: x4

b: x7.5

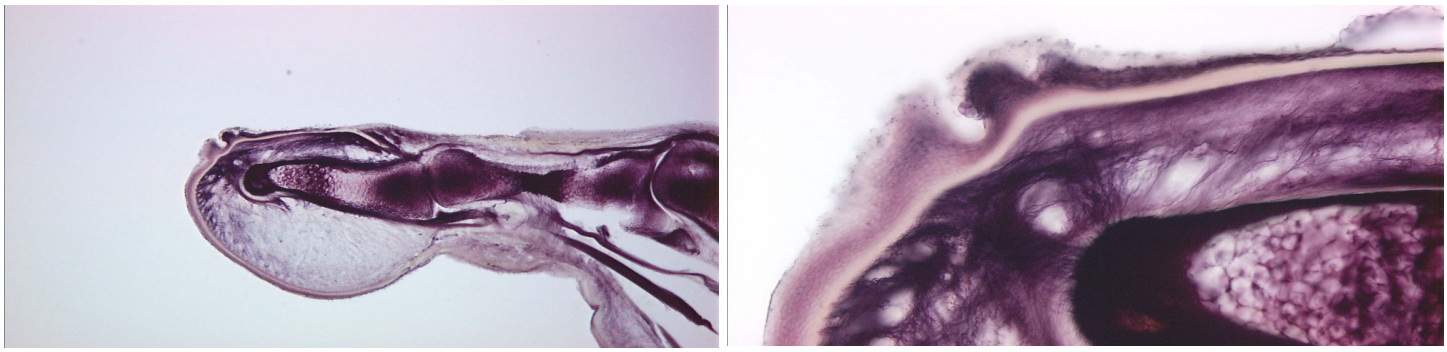


Figure 8: PAM staining at the time of nail formation did not show any positive reaction in the proximal nail fold, but it did show reticular fibers between the distal phalanx and the skin, including the area from the inferior part of the matrix to the upper part of the proximal nail fold.

8a: x4

8b: x7.5

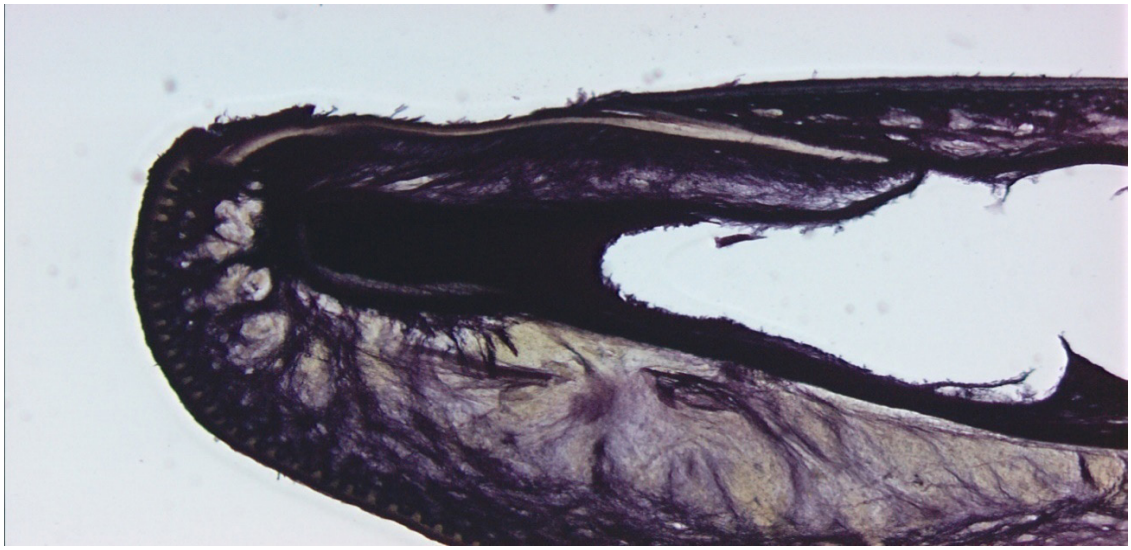


Figure 9: The density of subungual reticular fibers was uniform from the matrix primordium to the distal end of the nail, as observed in PAM-stained thick sections at the time of nail formation.

epidermis and the palmar epidermis of the proximal nail cage are not directly involved in the production of the nail capsule. Lewis [1] used silver-proteinate staining and divided the nails into three layers (dorsal, intermediate, and ventral layers) based on their staining properties.

The dorsal layer is formed from the epidermis of the palmar side of the proximal nail cage, the intermediate layer is formed from the latent edge of the nail root to the distal edge of the nail half-moon, and the ventral layer is formed from the epidermis of the nail bed from the distal edge of the nail half-moon to the hyponychium. The difference between the one-way and three-way theories is whether or not the nail capsule is formed from the nail bed. The nail bed before the formation of the nail consists of the basal layer, spinous layer, granular layer, and horny layer, and as the nail capsule is formed distally from the nail matrix and elongated, the granular layer and horny layer are pushed distally to form the hyponychium at the distal ridge. The cells of the nail bed beneath the nail capsule are stained basophilic by HE staining and are clearly different from those of the nail capsule, and it is said that the cells of the nail bed do not migrate to the nail capsule, but due to the difficulty of studying the developmental process of the human fetal nail, no conclusion has been reached yet. However, due to the difficulty of studying the developmental process of the human fetal nail, a conclusion has not yet been reached. In our specimen, there was no evidence of keratinization from the nail bed, nor was there any evidence of the fetal nail extending beyond the nail matrix, so we were unable to make a determination. As for the subungual tissues, Zaias [2] described the appearance of connective tissues that would later become tendons, muscles, and other mesenchymal tissues at 11 weeks of fetal life. Narisawa [4] also stated that mesenchymal cells cluster in the dermis of the proximal sulcus in the area that becomes the nail field at 11 weeks.

Lewis [1] reported that from 5 months onward, soft tissue invaginations extend proximally toward the DIP joint, suggesting a relationship between joint and nail diseases.

In the connective tissue, reticular fibers are thin fibers of about 20 nm in diameter made of type III collagen, especially found just below the basement membrane, and play a supporting role as part of the reticular plate. It is distributed in blood vessels, parenchymal organs, bone marrow, lymphoid tissue, smooth muscle, nerves, lungs, and fetal skin. In the skin, type III collagen is synthesized by fibroblasts in the basal layer and smooth muscle cells in the blood vessel walls. In the fetus, mRNA for type III collagen was identified in the fingers, developing blood vessel walls and periosteum, while type II was found in osteoclasts and cartilage around bones, and type II and III are not mixed together [5]. In our results, it was clear that the basal layer from the matrix to the distal ridge was positive for PAM staining and produced reticular fibers before the Matrix Primordium was formed. The formation of reticular fibers from the basal lamina of the palm side was later and less abundant than that of the dorsal side. On the dorsal side, the matrix primordium was dense in both peripheral and central directions, indicating the presence of a strong supportive tissue between the nail and the terminal phalanx.

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