

TEM Study of Cross Sectioned Baby Hair Shaft

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ABSTRACT

This study obtained the following results by observing the internal microstructure of cross-section of newborn baby hair using transmission electron microscopy (TEM). The cuticle cells in the layer of the newborn baby's hair cuticles were from 0.32 μm to 0.55 μm in diameter. The thickness of the innermost cuticle cells in contact with cortex in the cuticle layer was relatively thicker than that of the outer cuticle cells. The intercellular membrane in the cuticle layer was 0.03 μm thick and continued lengthways in a constant shape along the cell membrane's boundary.

The cortex of the newborn baby hair was filled with many small cortical cells. In the cortical cells' cytoplasm, macrofibrils surrounded by limited membranes were clearly observed, and there were melanin granules among them. The cortical cells also contained, irregularly shaped cell remnants with high electron density. In some cortical cells, small holes had formed among the macrofibrils and near the cortical membranes. The membrane complexes among the cortical cells were 0.02 μm thick, and the cortical cells were tightly joined to the boundary of these membrane complexes. The melanin granules had a shape similar to long-grain rice.

Keywords: Hair, Cortex, Cuticle layer, Melanin granule, TEM.

Introduction

The hair shaft's outer covering, the cuticle, surrounds the cortex and is the hair's protective outer layer. The undamaged cuticle layer in normal hair has a smooth surface, refracts light, and reduces friction force among the hairs. In normal hair, the cuticle layer is composed of approximately 4-10 cuticle cells and is elongated along the hair's long axis [1], although it varies depending on the hair's thickness.

The cuticle layer is composed of dead cells formed by the keratinization of cuticle cells in the hair follicle's hair root. Within these cells, the nucleus, mitochondria, and other cell organelles in the cytoplasm are decomposed and concentrated along the inner cell membrane of the cuticle cells to form endocuticles [2]. The endocuticle is a region formed by the dead cell organelles and is easily destroyed when the hair is damaged by physicochemical actions in the external environment [3-5].

The hair's cuticle layer is easily damaged by chemicals used for permanent dyeing, permanent waves, and bleaching, resulting in morphological changes [6]. The hair is also damaged by routine repeated cleaning and friction among the hairs themselves and combing. Oxidizers and reducers used in permanent hair dyes cause the hair's cuticle cells to swell. *Para*-phenylenediamine added to hair dyes and resorcinol that oxidizes these components can easily damage hair proteins [4]. In particular, these substances damage the cuticle layer that comes into direct contact with the hair, destroying the cuticle cells or separating them from each other.

The cortex gives the hair elasticity and strength, and elongated cortical cells are tightly joined to one another. Cortical cells are very close to each other, continuing lengthways in the same direction as the hair's axis. These cells are filled with macrofibrils approximately 0.5 μm in

diameter, and in the microfibrils, 2nm of microfibrils are arranged similar to a fingerprint on fingers [7]. Microfibrils arranged in the macrofibrils play an important role in the hair's elasticity and flexibility, and the amorphous matrix among these microfibrils supports the microfilament as sulfoprotein.

Melanin granules are spread by being scattered or concentrated among the macrofibrils of cortical cells. Melanin granules have a shape similar to long-grain rice and a diameter of approximately 0.3-0.5 μ m[8].

The medulla is located in the center of the hair fiber and along the fiber's axis. The medulla is filled with air inside an approximately 5-10 μ m hole formed by cell keratinization during the hair formation process [9]. The medulla is not continuously formed along hair's axis but is discontinuous; hence, there are regions where one hair has medulla and areas without medulla. In the scalp, the hair is mixed with hair with medulla and hair without medulla.

While many studies have been conducted on the microstructure of normal adult hair and the physicochemical changes caused by dyeing, perming, and bleaching [10-13], only Jung & Chang [14] have reported on the morphological characteristics of these hairs in newborn babies, and the microstructural studies of newborn babies' hair are unsatisfactory.

This study investigated the microstructural characteristics of the cross-sections of newborn baby scalp hair using transmission electron microscopy.

Materials & methods

Collection and treatment of experimental materials

The newborn baby hair was collected from infants within four weeks after childbirth. To collect the hair samples, we cut a hair shaft near the scalp using hair scissor and placed it in the plastic petri dish (90x15mm, SDL Life Science Co., Korea) with a filter paper (No. 7, 110mm, Toyo Roshi Kaisha, Ltd., Japan) to keep it from sticking to the foreign substances.

The collected hair samples were used after cutting them to a length of approximately 3mm using a sharp double-sided razor.

To prevent hair deformation due to the pressure applied to the blade surface during the hair cutting process, we held each razor with both hands and cut the sample placed in the center by pushing on both sides with the same pressure.

Observation of transmission electron microscopy

To observe the internal microstructure of the newborn baby hair, the experiment was conducted by placing a 3mm long sample into a 5 ml glass penicillin bottle. We placed approximately 3ml of 2.5% of paraformaldehyde-glutaraldehyde (0.4M phosphate buffer, pH 7.4) in a penicillin bottle containing the hair sample and prefixed it in a 4°C refrigerator for an hour. Next, we removed the prefixed solution, filled the bottle with approximately 3ml of phosphate buffer, and washed it twice before storing it in a 4°C refrigerator for 15 minutes.

The prefixed sample was post-fixed in a 4°C refrigerator with 1% osmium tetroxide (OsO₄) produced by diluting it with phosphate buffer (0.4M phosphate buffer, pH 7.4). After fixation, the material was washed twice with phosphate buffer for 15 minutes each and dehydrated at room temperature after diluting it in increasing concentrations of ethanol (Merck, German). The dehydration process was conducted once for 15 minutes in 50%, 60%, 70%, 80%, 90%, and 95%, ethanol solutions and twice for a 100% ethanol solution.

The dehydrated sample was subsequently replaced with propylene oxide and embedded in an Epon-Araldite mixture. During the embedding process, the propylene oxide and Epon-Araldite mixture was diluted to 2:1, 1:1 and 1:2 and processed for two hours each so that the propylene oxide that penetrated the hair was slowly replaced with the Epon-Araldite mixture. It was processed twice for an additional two hours with only the Epon-Araldite mixture.

A polymerized hair block was manufactured out of a 1 µm thick semi-thin section with ultramicrotome (LKB-2088, German) and then dyed on a hot plate (60°C) with 1% toluidine blue (1% borax) for 2 minutes.

After sufficiently cleaning the dyed hair section with distilled water, we identified the region to be observed with an optical microscope (CH30, Olympus, Japan).

To assess the microstructure of the hair cross-section, we double stained the sample with uranyl acetate and lead citrate for observation at 100 kV with the transmission electron microscope (H-7500, Hitachi, Japan) after ultra-thin sections were manufactured and attached to the copper grid.

Results & discussions

We observed the cross-section of the newborn baby hair with a transmission electron microscope. The cuticle layer was composed of six cuticle cells in the hair cross-section collected from the region close to the scalp (Fig. 1). In low-magnification transmission electron microscopic image, cuticle cells were observed to be tightly joined to each other, and the cortex was tightly joined inside the cuticle layer (Fig. 1). As shown in Fig. 2, the cross-sectional diameter of the cuticle layer composed of cuticle cells was 2.66µm. The uniaxial diameters of each cuticle cells were from 0.32µm to 0.55µm. The cuticle cells in the boundary that came into contact with cortical cell had relatively larger uniaxial diameters than the other cuticle cells. The innermost cuticle cells in the cuticle layer had diameters of 0.50µm, and the cuticle cells exposed to the outermost external environment werethe thinnest at 0.32µm (Fig. 2).

The uniaxial diameters of the cuticle cells that consist of cuticle layers of the newborn baby hair were smaller from the cortical boundary to the outside.

The cuticle cells consisted of intercellular membrane complex, layer A, endocuticles, and exocuticles[9,15].

As demonstrated by the high magnification transmission electron microscope, the cuticle cells were clearly separated into high electron densityexocuticlesand low electron density exocuticles. The endocuticles, exocuticles, and intercellular membrane complexes in the cuticle cell of the newborn baby hair were clearly distinguished, but layer A was not. The cuticle cells exposed to the hair's surface were shed and destroyed when damaged by artificially acting physicochemical stimuli[16].

The endocuticles contained less sulfur (S) and were less strong in the cell remnants than layer A or exocuticles, so this part was mainly destroyed by physicochemical stimuli[2].

In this study, the cuticle cells of the newborn baby hair were clearly distinguished from the exocuticles and endocuticles, and no destruction of the cuticle cells was observed. Artificially formed holes between the cytoplasm of adult hair cuticle cells and the cuticle cells' membranes were rarely found in the newborn baby hair cuticle layer. The exocuticles were evenly spread so that it had a homogeneous electron density since the fibrous keratinized protein, a precursor of the macrofibril in the cytoplasm, was evenly spread and crumpled into a ball with granules. The exocuticles were the region with lower electron density than the endocuticles and the strongest region of the cuticle cell.

In this study, in the boundary between the cortex and cuticle cells, the near membranes were closely connected to each other, forming an intercellular membrane complex. In the cuticle layer, the intercellular membrane complex continued lengthways in a constant shape along the cell membrane's boundary, and the membrane complex between the cortex and cortical cells also had a constant thickness. The intercellular membrane complex in the cuticle layer was 0.03 μm thick, and the membrane complex in the cortical cells was 0.02 μm (Fig. 3).

The cuticle cell's intercellular membrane complex had the same structure as reported by Seshadri & Bhushan [9].

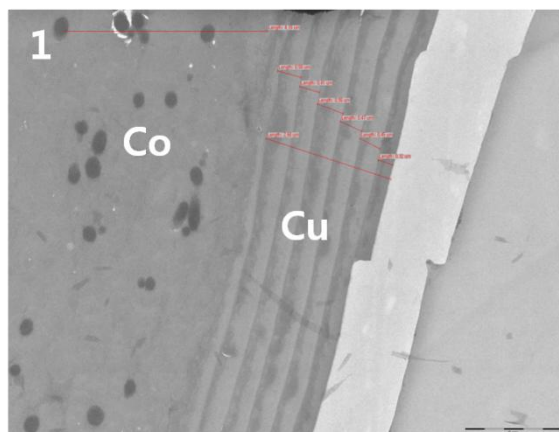


Figure 1. Transmission electron micrograph of the transverse sectioned newborn baby hair showing the cuticle layer(Cu) and cortex(Co).

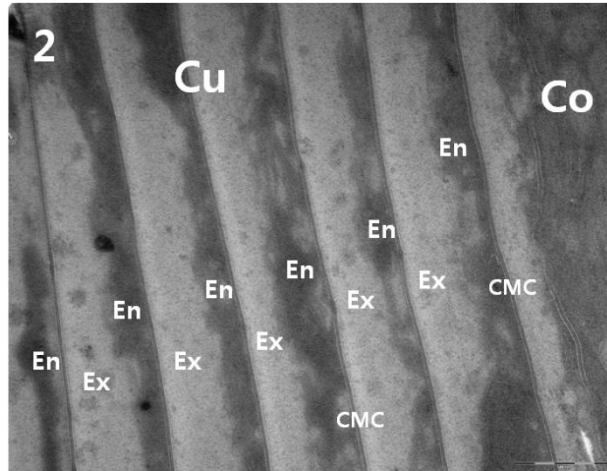


Figure 2. Transmission electron micrograph of the transverse sectioned newborn hair cuticle layer(Cu). Note that the cuticle cells are composed of intercellular membrane complex (CMC), endocuticles (En) and exocuticles (Ex). Co: cortex.

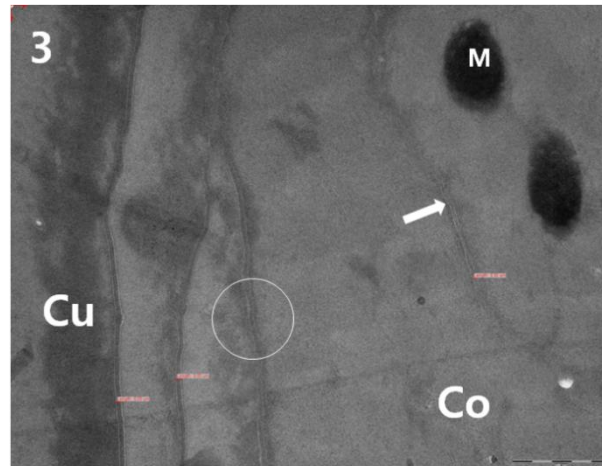


Figure 3. Transmission electron micrograph of the cortex (Co) and cuticle layer (Cu) of the newborn hair. Note the distinct cortical cell membranes (arrow) in the cortex. Circle: intercellular membrane complex of the cuticle cells. M: melanin granule.

The cortex is a region formed by the cortical cells being close to each other as they keratinized. Cortical cells are composed of macrofibrils, melanin granules, and cell remnants. Macrofibrils are long cylindrical structures in the microfibrils in the form of heterocysts and the amorphous matrix that fills them is surrounded by limited membranes [17]. Macrofibrils are surrounded by a single membrane approximately 0.5 μm in diameter and have a long cylindrical shape along the hair's long axis [14,18].

An amorphous matrix is a protein molecular component containing sulfur that strongly supports a nanometer, which is as thick as microfibrils [19,20]. Macrofibrils with these structural characteristics have the important function providing tensile strength, elasticity, and flexibility to hair.

In this study, the cortex of the newborn baby hair was filled with many small cortical cells. Inside the cortical cells, macrofibrils surrounded by limited membranes were clearly observed with

melanin granules among them. The cortical cells contained high electron density cell remnants with indeterminate structures.

However, in some cortical cells, large and small holes formed among the macrofibrils and near the cortical cell membranes (Fig. 4). According to Scanavez et al. [16], small holes naturally form between granules and macrofibrils in the cortex of normal adult hair.

In this study, large and small holes in the newborn baby hair cortex pushed to the scalp's surface because the hair generated from the hair follicles was keratinized, removing the moisture. It was thought to be formed by continuous physicochemical contact with the external environment.

The cortical cells in the cortex of the newborn baby hair were tightly joined to form a membrane complex (Fig. 4). The membrane complex of cortical cells had a diameter of 0.02 μm . In the transmission electron microscopic images, the cell remnants in the cortical cells had high electron densities and indeterminate structures. There were also melanin granules in the remnants (Fig. 4). The cell remnants were caused by keratinization as the active cortical cells became inactive and the cell organelles and nucleus in the cytoplasm were decomposed and concentrated. The region with the cell remnants in the cortex was weaker than other regions, forming large and small holes.

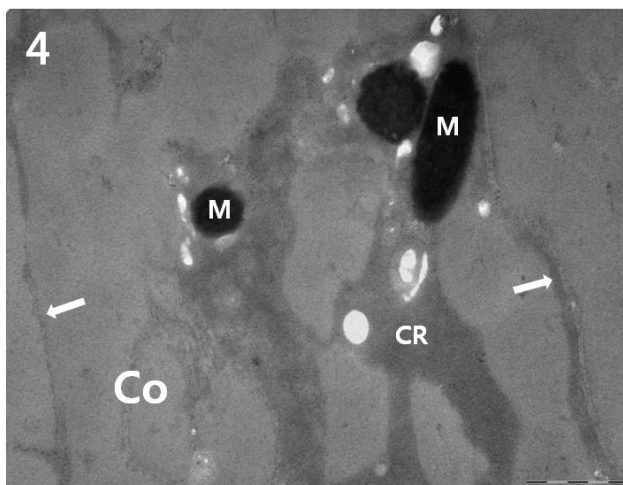


Figure 4. High-magnification transmission electron micrograph of the cortex(Co) showing cell remnants(CR) and cortical cell membranes(arrows). M: melanin granules.

Melanin granules in the cortex's cortical cell are present among the macrofibrils and inside the cell remnants [8, 16]. The melanin granules' transverse section had circular structures, and the vertical section had an elongated oval shape (Fig. 5).

At high magnification, the inside of the melanin granules formed small concentric structures (Fig.6). These structures appear when amino acids produce melanin granules with a peptide bond when melanocytes in hair follicles synthesize melanin granules using the tyrosinase enzyme as a pillar. After the melanin granules are produced, structures in the form of tyrosinase cores are present inside the granules [21,22], which have low electron density, making them easy to distinguish via high magnification transmission electron microscopy.

In this study, the melanin granules that formed in the elongated oval structure had an axial diameter of 500-670 nm and a uniaxial diameter of 250 nm and had the same structure as melanin granules observed in

normal adult hair (Figs. 5 and 6). The melanin granules in the hair cortex had a shape similar to long-grain rice.

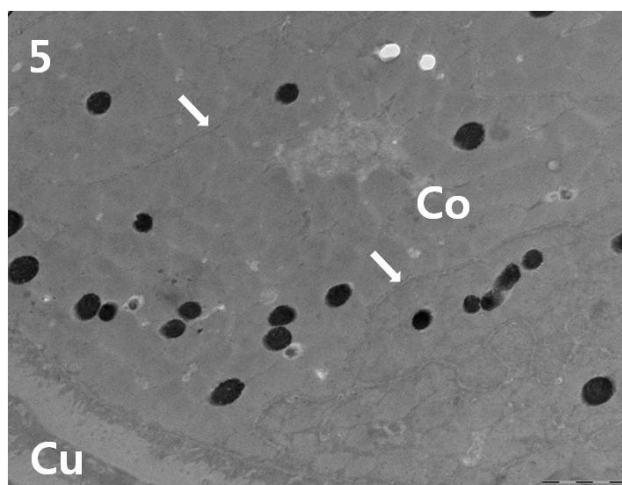


Figure 5. Transmission electron micrograph of the cortex (Co) of the newborn hair showing cortical cell membranes (arrows) and melanin granules. Cu: cuticle layer.

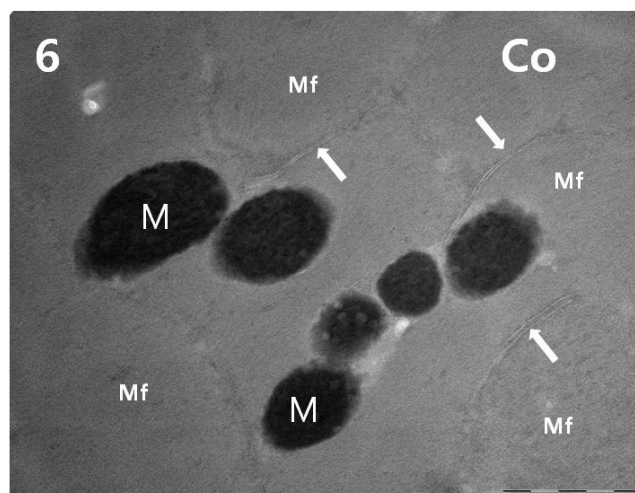


Figure 6. Transmission electron micrograph of the macrofibrils (Mf) and melanin granules (M) in the cortical cells (Co). Arrow: cortical cell membrane complex.

Conclusion

In this study, the internal microstructure of newborn baby hair was observed by transmission electron microscopy. The following results were obtained.

The newborn baby hair cuticle layers were measured by a transmission electron microscope. The cuticle cells were $0.32\mu\text{m}$ to $0.55\mu\text{m}$ in diameter and consisted of 4-6 cuticle cells. In the cuticle cells' cytoplasm, endocuticles and exocuticles were clearly distinguished by the differences in their electron densities. There were many melanin granules in the cortical cells close to the cuticle layer. The uniaxial diameter of the innermost cuticle cells in contact with the cortex in the cuticle layer was relatively large.

The cortex of the newborn baby hair was filled with many small cortical cells. The cortical cells were filled with macrofibrils, melanin granules, and cell remnants with indeterminate structures. Inside the high electron density melanin granules, there was a structure with a microscopic concentric tyrosinase core and a relatively low electron density. The remnants in the cortical cells' cytoplasm had a high electron density and indeterminate structures.

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