J Forensic Sci, November 2008, Vol. 53, No. 6
doi: 10.1111/j.1556-4029.2008.00868.x
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TECHNICAL NOTE

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The Ultrastructure of Tissue Attached to Telogen Hair Roots

ABSTRACT: Most tissues encountered in forensic biology laboratories have been previously characterized with electron microscopy due to their medical importance. Anagen hair root cells, epithelial cells, erythrocytes, neutrophils, osteocytes, and spermatozoa have received considerable research attention at the ultrastructual level. There is no literature indicating that cells attached to removed telogen hair roots have been characterized with transmission electron microscopy. Nonetheless, telogen hairs are a frequent submission item to forensic laboratories for DNA typing. The amount of tissue attached to a telogen hair root usually determines whether that hair is suitable for nuclear DNA typing methods or mitochondrial DNA typing methods. This study used transmission and scanning electron microscopy to characterize the tissues found in three commonly occurring telogen hair root forms. The tissues were found to consist of keratinized remnant follicle, nonnucleated epithelial cells, nucleated epithelial cells, and trichilemmal keratin. These findings were consistent with the known principles of hair follicle regression. The recognition of the root structures that contain these specific tissue types may assist in the DNA typing of telogen hairs inasmuch as the quality of tissue present may be more important than the amounts of tissue present.

KEYWORDS: forensic science, hair DNA typing, telogen, electron microscopy, hair histology, hair ultrastructure

The difficulty and success of telogen hair root DNA typing has been the subject of numerous forensic science reports (1–5). Telogen hair root morphology can be quite variable and is generally predictive of whether a hair is suitable for highly discriminating nuclear DNA typing or mitochondrial DNA sequence analysis (6). Anagen hair bulb and telogen hair club root ultrastructure have been previously reported but the fine structure of the tissue adhering to a telogen club has not (6). It is usually the amount of certain tissues attached to the telogen root that determines the success of nuclear DNA analysis for that type of hair. This report examines the content of the material attached to telogen club roots with electron microscopy to determine which tissues present on a telogen root may be most suitable for nuclear DNA typing.

Methods

Transmission Electron Microscopy (TEM)

Caucasian head, chest, and pubic hairs were obtained by plucking. After screening with a stereomicroscope, hairs with telogen roots were immersed in a fixative solution of 3% paraformaldehyde, 1.5% glutaraldehyde in phosphate buffer, and postfixed in 1% osmium tetroxide. The hair roots were dehydrated in a series of alcohols and propylene oxide was used as a transition fluid prior to embedment in 100% epoxy resin. The roots with a portion of hair shaft attached were oriented for horizontal sectioning in the resin blocks prior to overnight polymerization at 60°C. The hair roots were thin sectioned to 85–90 nanometers and stained with 4% uranyl acetate and lead citrate prior to examination with a Hitachi H-7650 transmission electron microscope (Hitachi High Technologies America, Inc., Pleasanton, CA).

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Received 21 Dec. 2007; and in revised form 8 Mar. 2008; accepted 29 Mar. 2008.

Scanning Electron Microscopy (SEM)

Previously described hair shafts with telogen roots were mounted on aluminum stubs, sputter coated with gold, and examined with a Hitachi S-3400N scanning electron microscope.

Results and Discussion

Three principal forms of telogen root clubs can be found in casework and they can be highly variable in the amount of tissue attached to the club. Telogen clubs with no tissue (Fig. 1), telogen roots with enlarged clubs that have a nipple at the end (Fig. 2), and telogen clubs with tissue either superior or inferior to the club (Fig. 3) are the most frequently seen variants. The fine structure of the enlarged telogen club is the most complex.

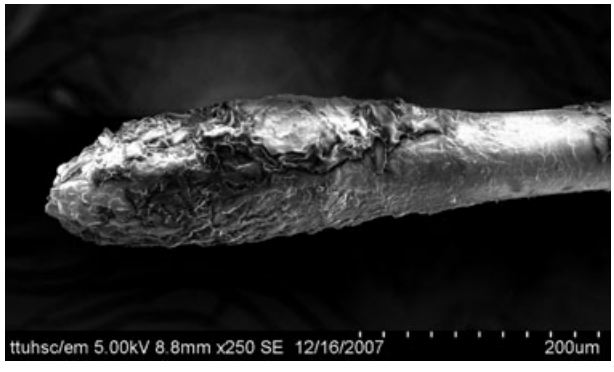

FIG. 1—*Head hair telogen root with no attached tissue. Root type suitable for mitochondrial DNA typing. Telogen roots absent tissue resemble cotton swabs when viewed with stereomicroscopy because the hard keratin shaft is straight and the club root appears white due to reflection of light from the nonpigmented keratin. These types of hairs are frequently referred to as "shed." The hair in this photograph was "plucked." (SEM 250×.)*

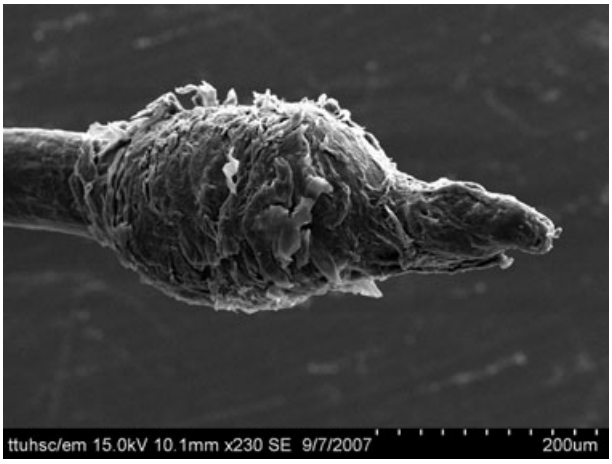

FIG. 2—*Pubic hair telogen root with apparent attached tissue and nipple. TEM cross sections of a nipple structure (right) can be found in Figs. 7 and 8. TEM cross sections of tissue attached to a club can be found in Figs. 5 and 6. SEM (original magnification 230×).*

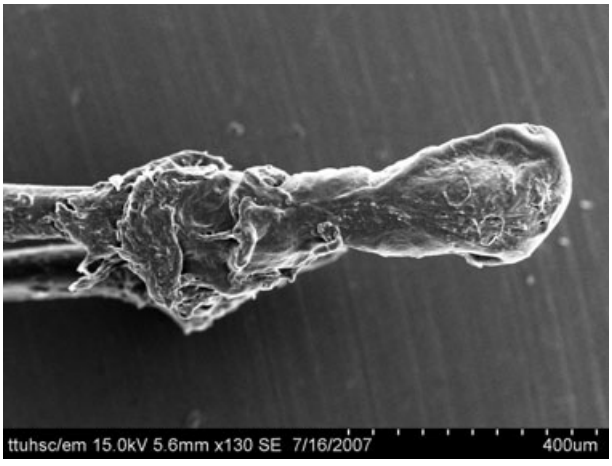

FIG. 3—*Pubic hair telogen root with sufficient attached tissue for nuclear DNA typing. The most tissue is superior (left of) to the club. TEM of this type of tissue mass can be found in Figs. 9 and 10. SEM (original magnification 130×).*

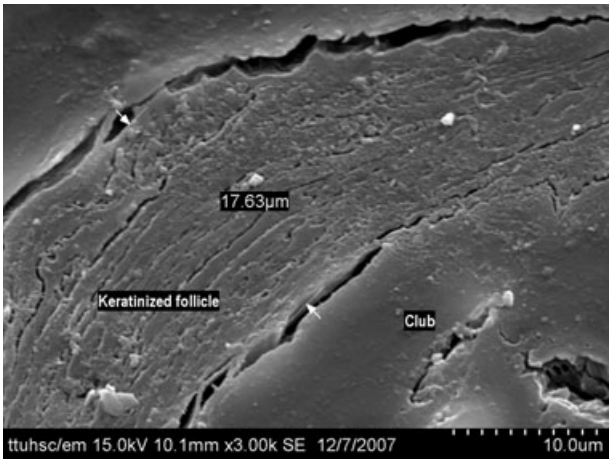

FIG. 4—*Horizontal section through telogen hair root club. The keratinized follicle is 17.63 microns thick. That would add about 36 microns to the diameter of this club root. The trichilemmal keratin club is lower right. SEM (original magnification 3000×).*

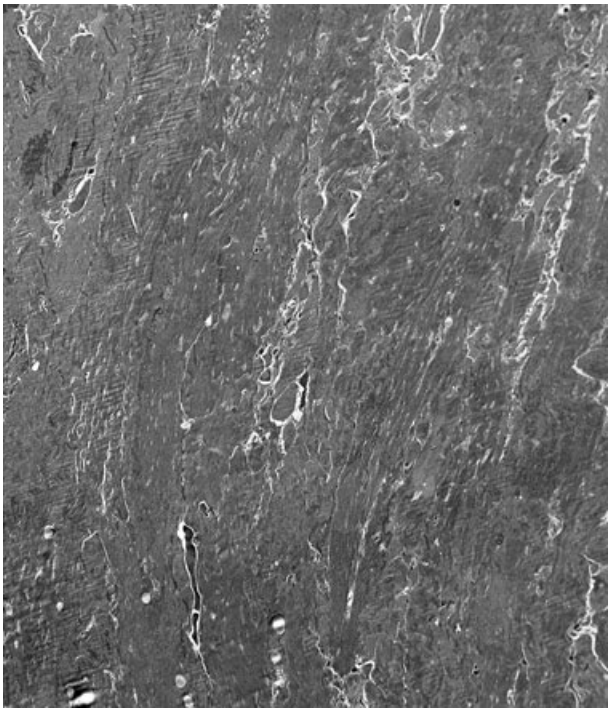

FIG. 5—*Keratinized follicle surrounding telogen club. The club is not shown. TEM (original magnification 1000×).*

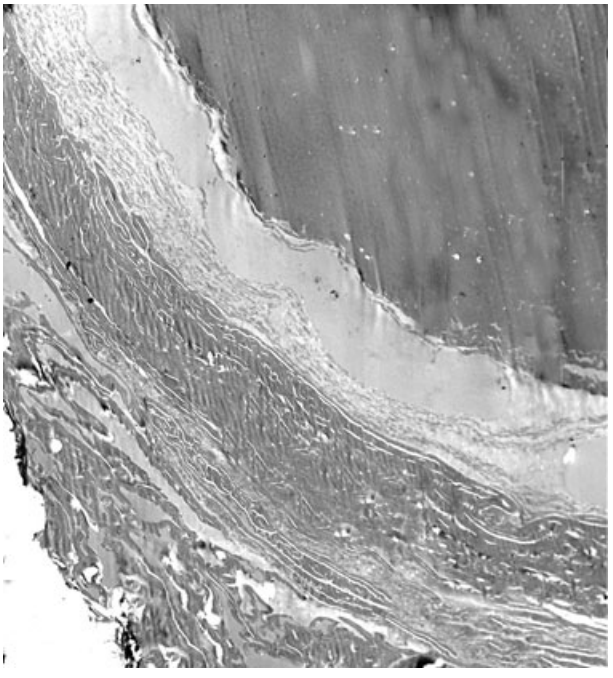

FIG. 6—*Horizontal section through telogen hair root club with attached tissue. The trichilemmal keratin club is upper right. TEM (original magnification 700×).*

Examination of horizontal sections through enlarged telogen root clubs shows that concentric rings of keratinized, nonnucleated material surrounding the club is responsible for the larger appearance of these roots (Figs. 4 and 5). The usual viable cell layers of the follicle (7–10) were not discernable but the rings were clearly

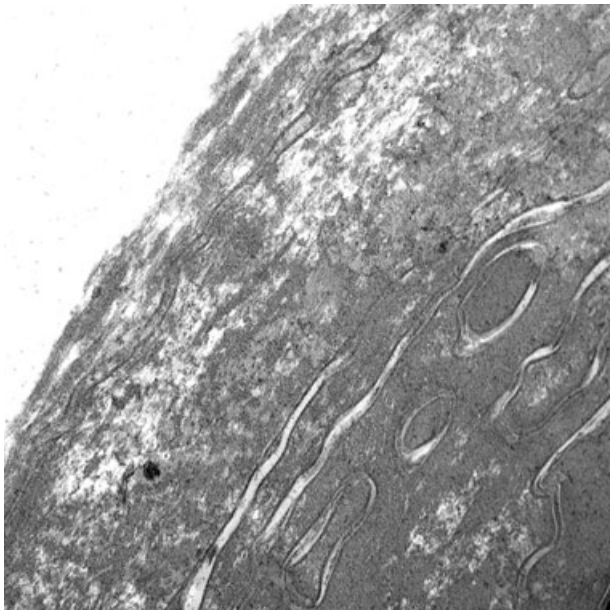

FIG. 7—*Horizontal section through club nipple. See Fig. 2. Outer edge of nipple is shown. TEM (original magnification 12,000×).*

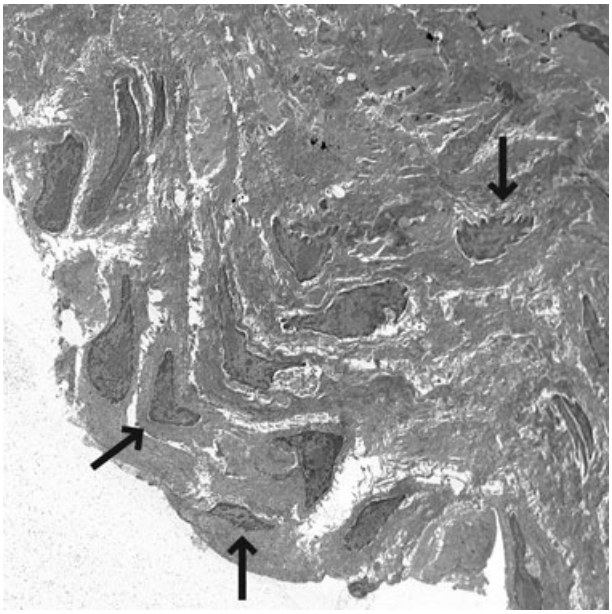

FIG. 9—*Horizontal section through a tissue mass that was superior to the club. See Fig. 3. Numerous nuclei indicated by arrows. TEM (original magnification 1000×).*

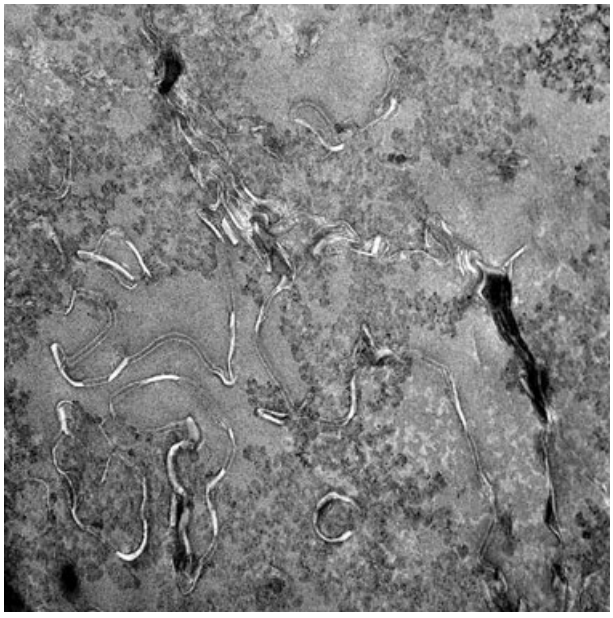

FIG. 8—*Horizontal section through club nipple. Center of nipple is shown. TEM (original magnification 10,000×).*

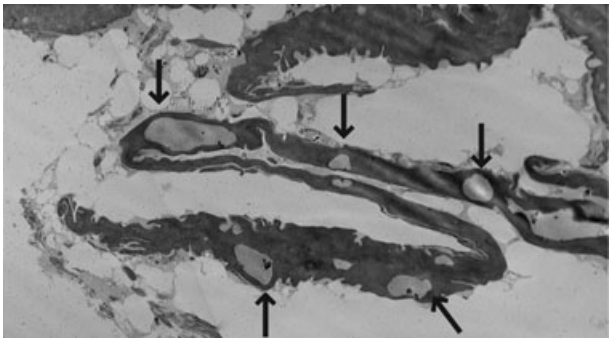

FIG. 10—*Horizontal section through a tissue mass that was superior to the club. Keratinized, nonnucleated epithelial cells resembling stratum corneum of the skin surface. Prominent trichohyalin granules indicated by arrows can be seen. Trichohyalin granules usually signal pending keratinization. TEM (original magnification 2500×).*

different from one another as demonstrated in Fig. 6. The well-defined layers, although keratinized, are consistent with hair follicle layers. The homogeneous, amorphous appearance of the trichilemmal keratin club is recognizable at the center of the concentric rings. A review of the biology of the follicle explains the attachment of keratinized, concentric layers of remnant follicle to the telogen club (7–10).

The hair follicle consists of several concentric layers of cooperating cell types that support and maintain the growing thread of protein we recognize as hair. In general, the numerous differentiated layers of the follicle can be categorized as inner root sheath (next to the hair shaft cuticle), outer root sheath, and dermal sheath (8). During catagen, or regression phase, the inner root sheath completely disintegrates through apoptosis and the telogen club is formed in the upper hair follicle near the level of the sebaceous gland duct. The first layer of the follicle inner root sheath is a cuticle that anchors the hair by interlocking in an opposing manner with the hair shaft cuticle. The nucleated cell layers of the inner root sheath after the cuticle are Huxley's layer and Henle's layer, respectively. Henle's layer keratinizes first and appears as corrugations in horizontal section during catagen (10). The cuticle of the inner root sheath keratinizes next and the inner root sheath ultimately crumbles and is replaced by trichilemmal keratin that is formed by the outer root sheath (8). Thus, the inner root sheath changes to an anchoring substance in the upper half of the lower follicle (8). The inner root sheath layers are followed by additional concentric cell layers that include a companion layer, outer root sheath, and a connective tissue follicle (dermal sheath) (7). It appears the tissue surrounding the hair root club (Fig. 6) is a

combination of trichilemmal keratin from the inner root sheath, outer root sheath, and dermal follicle elements that have cornified and shrunken to the root club within the epithelial capsule to form the trichilemma.

Telogen hair root clubs are frequently found with a nipple of tissue at the end (Fig. 2). TEM of horizontal sections of these nipples showed them to consist of trichilemmal keratin (Figs. 7 and 8). Again, during catagen, the hair root stem and bulb are digested through apoptosis leaving the lower follicle to shrink and cornify with the nipple representing the lowest level of visible remnant follicle. The nipple is therefore primarily composed of the connective tissue follicle from the outer root sheath. No nuclei were seen in the nipple structures or in the previously described trichilemmal structure around the club during these examinations.

The final telogen root variant structure examined in this study was the type with a tissue mass either superior or inferior to the root club. The tissue mass shown in Fig. 3 is primarily superior to the root club, although a lesser amount can be seen surrounding and inferior to the club. Above the hair root club and sebaceous gland of the telogen follicle, there are cells resembling stratum corneum overlying spinous cells (9). The tissue mass in this study was found to consist of nucleated (Fig. 9) and nonnucleated (Fig. 10) epidermal cells consistent with the types of cells found above the level of the telogen club in skin. The cells in the tissue mass most likely have origin from the upper layers of the outer root sheath and the dermal sheath that remain continuous with the skin surface epidermis (8). When a telogen hair is removed from the skin, this soft tissue can slide and be found in various locations on the extruded hair shaft/root. A hair is considered by dermatologists to be in telogen phase if the trichilemmal keratin club is formed, regardless of how much soft tissue may be attached to it (8).

Hairs are the only structures in the human body that completely regenerate themselves. The complexity of telogen root ultrastructure reflects the multitude of cellular changes that occur to make such regeneration possible. Numerous different cell types and tissue remnant types were identified in this study. One would expect telogen hair roots with tissue superior or inferior (excluding nipple structures) to the club to be most suitable for nuclear DNA typing because these cells have origin in the upper outer root sheath and dermal sheath. These areas of the follicle are unaffected by the apoptotic/keratinization changes that occur during catagen phase.

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