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Ultramicroscopic observations on morphological changes in hair during 25 years of weathering

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Abstract

Weathering or long-term burial may cause profound morphological and histological changes in hair, which may affect the results of forensic and archaeological investigations. We therefore used ultramicroscopic techniques to assay the changes in weathering hair shafts caused by burial for up to 25 years. We found that the middle portion of hair shafts from living individuals shows the expected histological hair structure, while the cuticle layer was absent from the terminal portion of the same hairs, which may be due to the increased weathering experienced by the terminal portion. In hair samples taken 5 years after death, no significant changes in morphology were observed. By 15 years after death, however, we observed losses in various layers of the hair, including the cuticle layer. At 25 years after death, hair shafts showed a number of pores extending into the medulla, with only some hair shafts retaining their cortical layers. To our knowledge, this is the first ultramicroscopic study on weathering of hair for up to 25 years after death. Our results may therefore provide a basis for similar studies in the fields of forensic science and physical anthropology.

Keywords: Hair; Electron microscope; Weathering; Cuticle; Cortex; Medulla

1. Introduction

Hair samples are good resource biomaterials in forensic science and physical anthropology because various chemicals and biological substances that accumulate in hair can be detected and measured. Furthermore, the basic chemical composition of hair is not influenced by changes in blood chemistry or by exposure to chemicals after hair formation [1–3]. Therefore, hair samples are frequently used for autopsy toxicology, including the detection of drugs of abuse, the forensic genetic identification of relations, and personal identification [4,5].

In archaeological burials, hair is a unique resource for capturing a snapshot of life. More so than other biological

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samples including bone, hair is formed at a constant rate and, once formed, does not show further biogenic turnover. In contrast to other soft tissue samples, which are degraded after burial for long periods of time, hair samples have been found to be relatively resistant to degradation. Although hair samples from archaeological burials look inert to the naked eye, these samples are also subject to biodegradation [6]. Because hairs found during archaeological excavations have varying degrees of degradation, depending on the conditions and duration of burial, it is important to determine the time-dependant morphological changes in hair.

To understand the morphological changes that occur in hair during long-term burial, it is necessary to consider changes in histological morphology of the hair caused by various factors. For example, environmental factors including sunlight, air pollution and wind, have been found to induce histological changes in the hair cuticle and cortex, ultimately leading to the destruction of the hair shaft [7–10]. In addition, histological changes in hair structures are caused by various pathological states [11–13]. For example, in individuals with alopecia areata, keratinocytes in the hair are damaged by an autoimmune mechanism, and these defects in cortical cells within the hair shaft correlate with the pathophysiology of the disease [9,14].

Many hair samples taken from sites of archaeological or forensic investigation have undergone weathering for long periods of time. Although changes in the histological structure of the hair have been documented during various conditions, there have been few studies on morphological changes of hair that occur during prolonged weathering. Thus, an ultramicroscopical study on the morphological changes that occur in weathering hair samples would be useful. We therefore undertook to document the weathering pattern of hairs taken from living individuals and from corpses entombed for long periods of time.

2. Materials and methods

Ten hair samples 20–25 cm long were taken from the middle and terminal portions of the hair shaft of five living females. As the weathering on a hair is greater in the terminal portion than in the middle portion, owing to the differences in duration of exposure to the external environment, each hair sample served as its own control.

Hair samples weathered for 5–25 years were also taken from deceased individuals in a number of tombs in Kangneung, Korea. All of these deceased individuals were males, aged 50–60 years old. Five to 10 hair samples were collected from 3 to 5 tombs of individuals buried for 5, 10, 15, 20, and 25 years. The hair samples were washed in a graded ethanol series and their general appearance was observed under a stereomicroscope (Olympus, SZH-ILLD).

For transmission electron microscope (TEM), the method used has been described previously [15,16]. Briefly, hair samples were immersed in 2.5% paraformaldehyde–glutaraldehyde in neutral 0.1 M phosphate buffer for 1 h. Tissues were postfixed for 1 h in 1% (w/v) osmic acid dissolved in phosphate buffered saline (PBS), dehydrated in graded ethanol, and embedded in Epon 812 (EMS, Fort Washington, PA). General morphology was observed by light microscopy of toluidine blue stained semi-thin sections. Ultrathin sections were cut and mounted on nickel grids coated with Formvar film and viewed under a JEOL 100 CX-II TEM (Tokyo, Japan) TEM after uranyl-lead counter staining.

For scanning electron microscope (SEM), the method used has been described [15,16]. Briefly, hair samples were prefixed by immersion in 4% paraformaldehyde, 0.1% glutaraldehyde in neutral 0.1 M phosphate buffer, and post-fixed for 2 h in 1% (w/v) osmic acid dissolved in phosphate buffered saline (PBS). Samples were treated in a graded ethanol series and isoamyl aceteate, dried in a critical point dryer (Hitachi SCP-II), gold coated using an ion coater (JFC-1100), and observed under a JSM-840 A SEM.

3. Results

In the case of middle portion of hair shafts from living individuals, the terminal portions showed much more severe weathering patterns due to the longer period of exposure of the latter, compared with the middle portion of the hair shafts. In the middle portion of the hair shaft, the structures of the cortex, medulla and cuticle were well preserved (Fig. 1A), and, on magnified images, melanin granules were observed to be evenly spread within the cortex (Fig. 1B). Under SEM, the shaft appeared smooth, and the keratinized squamous epithelium was well arranged like roof tiles, allowing it to serve as a protective barrier (Fig. 1C). Typical ultramicroscopic morphology was shown more clearly by TEM, in that the cuticle, cortex and medulla of the transverse section of the hair shaft were well preserved (Fig. 1D). The cuticle layer was composed of electron-dense exocuticles and electron-lucent endocuticles (Fig. 1E), whereas there were many macrofibrils and fewer melanin granules in the cortex (Fig. 1F).

The terminal portion of the hair shafts of living individuals showed considerable weather-damage due to the chemical and physical environments of the subject. The clearest differences between the middle and terminal portions of the hair shaft of living individuals were related to the preservation of the cuticle. In the terminal portion, the cortex was exposed to the environment because the cuticle was not intact (Fig. 2A and B). SEM showed a transverse fissure in the cuticle layer and within the exposed cortex, as well as melanin granules on the surface (Fig. 2C).

When compared with the middle portion of hair shafts taken from living individuals (Fig. 3A and B), the histological morphology of the middle portion of the hair 5 years after death did not differ markedly in cross section (Fig. 3C and D). By 15 years after death, however, the surface of the

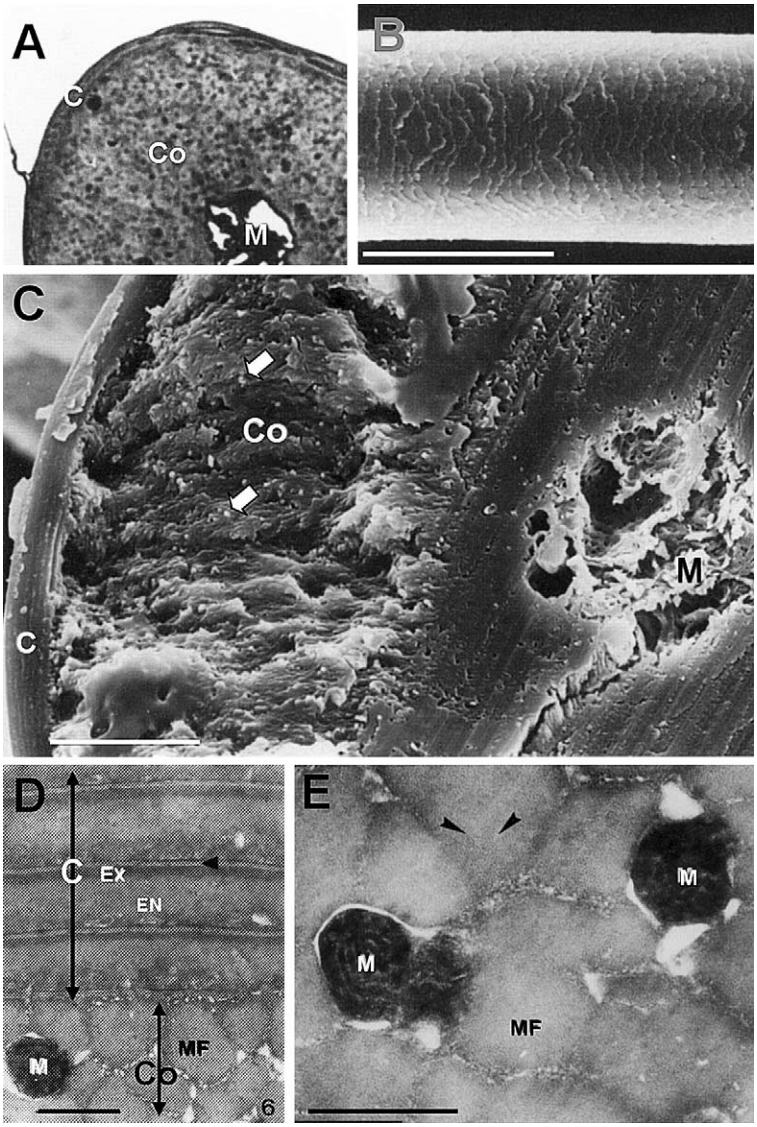

Fig. 1. The middle portion of the hair shaft from a living individual. (A) A cross section showing the cuticle (c), cortex (Co) and medulla (M). Melanin pigment is observed in the cortex layer. (B) Scanning electron microscopy (SEM) showing well preserved cuticular scales. (C) SEM of the cross section, with the cuticle (c), cortex (Co) and medulla (M) clearly visible. Arrows indicate melanin pigment granules. (D) Transmission electron micrograph (TEM) of the cross section of a cuticle, showing its arrangement in a regular concentric form with endocuticle (EN) and exocuticle (EX). Note that the cuticle layers are smooth and flat. The arrowhead indicates the intercellular membrane complex. M, melanin pigment; MF, macrofibril. (E) Highly magnified TEM image of the cortex. Arrowheads and MF indicate microfibrils in the cortex. Melanin pigment granules (M) are distributed between macrofibrils. Scale bars: (B) 100 μm, (C) 10 μm, (D) and (E) 0.5 μm.

hair was rougher, and the cuticle layers were removed (Fig. 3E and F). At 20 years after death, most of the cuticle layer was damaged, leaving the underlying cortex exposed to the environment. In addition, tissue loss of the medulla layer was greater after 20 years than at previous stages (Fig. 3G and H). At 25 years after death, a number of holes had formed in the hair shafts (Fig. 3I and J).

These light microscopic findings were confirmed by electron microscopy. Five years after death, the hair shaft and root appeared intact by SEM (Fig. 4A and B). By 10 years after death, however, we observed that the scales of the cuticle layer had started to detach from their underlying structures (Fig. 4C) and, at least in this case, the hair root was severely damaged, in that the outer and inner root sheath were detached and the underlying keratinized stratified squamous epithelium was exposed (Fig. 4D). At 15 years after death, although the general structure of the hair shaft was maintained and the scales on the hair shaft were intact, a

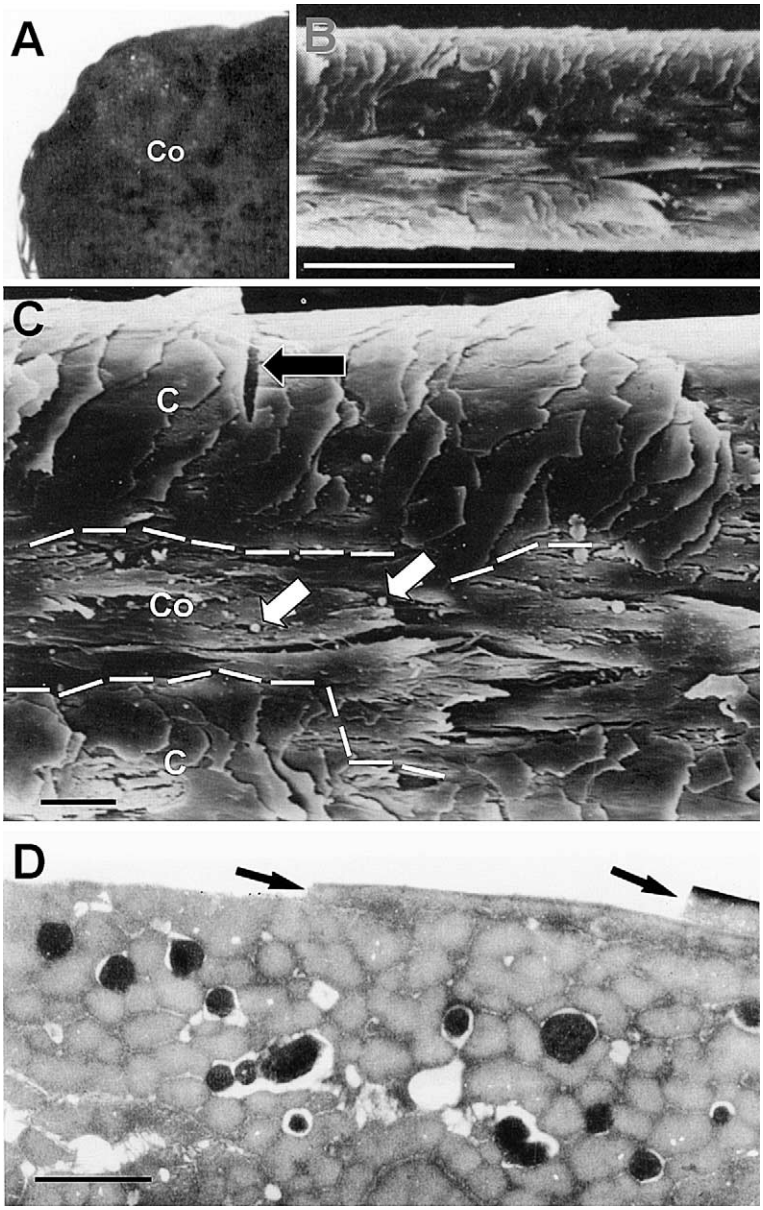

Fig. 2. The terminal portion of the hair shaft from a living individual. (A) A cross section showing an irregular shape and a thinner cuticular layer than in Fig. 1A. (B) SEM data showing loss of the cuticle layer and exposure of the underlying cortex. (C) Magnified image of the SEM showing loss of the cuticle and exposure of the underlying cortex. The black arrow indicates a transverse fissure in the cuticle. White arrows indicate melanin pigment granules in the cortex. (D) TEM image of the cuticle and cortex layers. Arrows indicate the loss of cuticle scales. Scale bars: (B) 100 μm, (C) 10 μm, and (D) 1 μm.

number of small sized pores were observed (Fig. 4E and F). The number and size of the pores in the hair shaft increased at 20 years (Fig. 5A), when the cuticle layer had detached totally and when the macrofibrils of the keratinized stratified epithelial cells around the pores in the hair shaft had been exposed (Fig. 5B). When we examined these pores in detail, we found that they extended into the cortex and medulla of the hair (Fig. 5C). TEM of a cross section of a hair shaft at 20 years showed that the cuticle layer was almost lost and that there were spaces between macrofibrils in the cortex (Fig. 5D and E). Finally, at 25 years after death, only the outer cortex remained, whereas the other structures of the inner cortex and medulla had disappeared (Fig. 5F and G). In TEM images at 25 years, and in contrast to earlier samples, the spaces between macrofibrils increased and the cuticle layer disappeared (Fig. 5H).

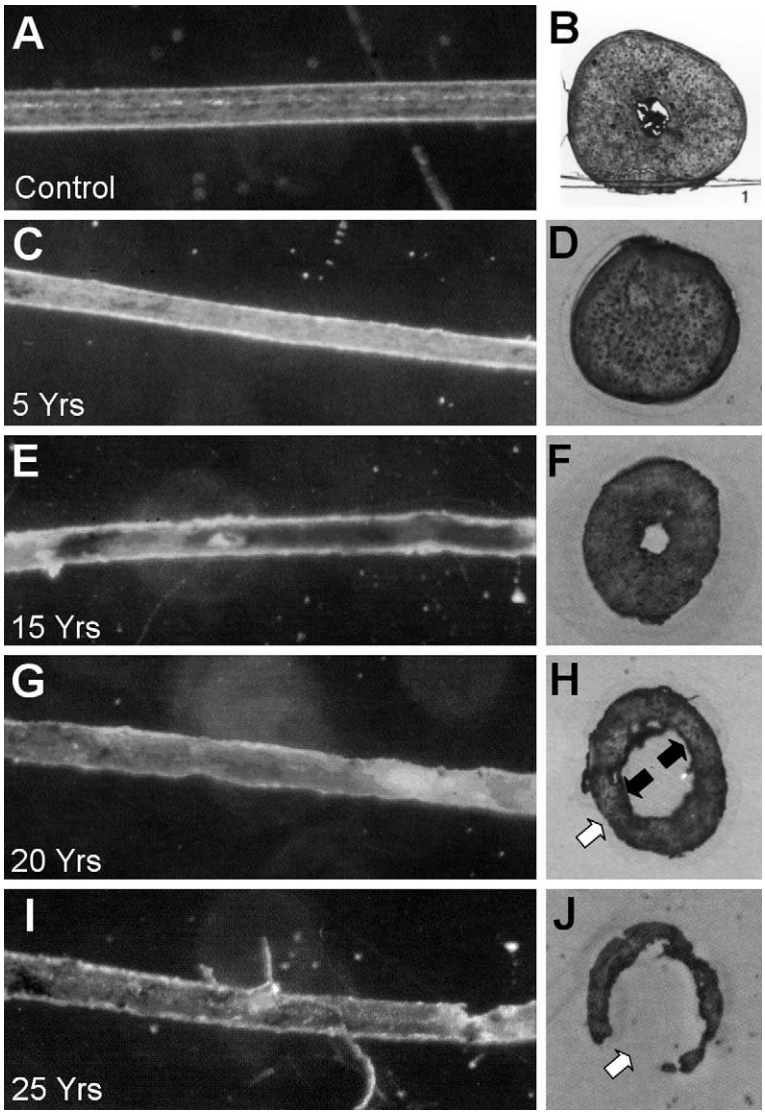

Fig. 3. Histological changes of hair shafts during 25 years of weathering. (Left) Representative external morphology under the stereomicroscope. (Right) A representative toluidine blue stained cross section under the light microscope. (A) and (B) Controls, showing that the general structure of the middle portion of hair from a living individual is well preserved. (C) and (D) Five years after death. Histological appearances similar to (A) and (B). (E) and (F) Fifteen years after death, showing rougher surface and partial detachment of the cuticle layers. (G) and (H) Twenty years after death, showing damage to most of the cuticle layer and exposure of the underlying cortex to the environment (white arrow in H). Weathering damage proceeded from the medullar to the cortical layer (black arrows in H). (I) and (J) Twenty-five years after death, showing a hole in the hair shaft (white arrow in J), which seems to have been formed by fusion between damage in the cuticle and outer cortical layers and damage in the medulla and cortical layers.

4. Discussion

Because our study dealt with changes in hair morphology during long-term burial of hair samples, we must consider the normal structure of the hair. Each hair is composed of a root and a shaft, and cross sections show the cuticle, cortex and medulla layers. The hair cuticle layer is formed by stratified squamous epithelium, which is arranged as roof tiles with their ends projecting like scales. The cortex contains melanin pigment and macrofibrils, while the medulla layer forms the central region of the hair shaft [17,18]. This normal structure has been shown to be degraded by repeated exposure to physicochemical stimuli, including sunlight, air pollution, wind, and salt water [19,20]. Hair damaged by weathering exhibits discoloration, weakness and fragmentation, although hair samples can be

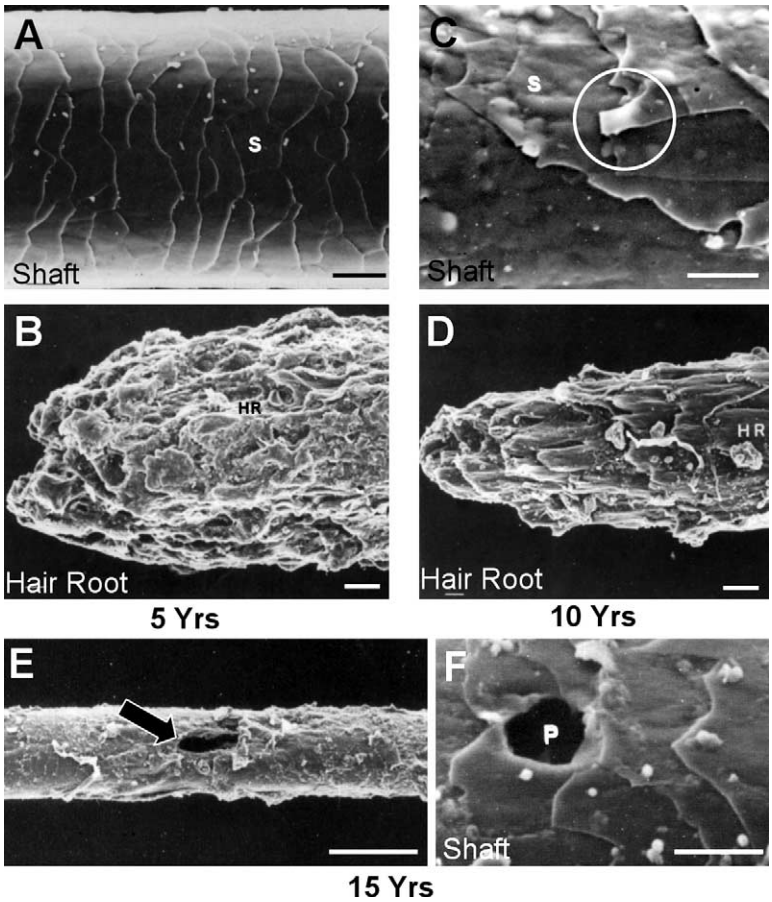

Fig. 4. TEM and SEM images of samples 5, 10 and 15 years after death. (A) and (B) SEM of a sample 5 years after death, showing that the hair shaft and root (HR) appear intact. S, scales in the cuticle. (C) and (D) SEM of a sample 10 years after death, showing that the scales (s) on the cuticle layer have started to detach from the underlying structures (circle). The hair root also appears severely damaged, and, in this sample, the outer and inner root sheaths had detached, exposing the underlying keratinized stratified squamous epithelium. (E) and (F) SEM of a sample 15 years after death, showing retention of the general structure of the hair shaft, as well as the generation of several small pores (arrow in E, P in F). Scale bars: (A)–(D) and (F) 10 μm, and (E) 100 μm.

recovered after burial for long periods of time. In addition, electron microscopy has shown that weathering changes occur in normal human hair, starting as a detachment of hair scales from the cuticle layer. Following detachment of the cuticle layer from the hair shaft and the exposure of the underlying cortex, hair weathering is accelerated. Therefore, exposure of the cortex due to the disappearance of the cuticle layer is regarded as one of the most important factors in hair weathering [19,20].

We have shown here that hair samples taken from corpses buried for long periods of time show patterns of degradation similar to those seen in weathering hairs. That is, following exposure of the cortex layer, the ultrastructure underwent extensive changes over time. Hair samples taken 5 years after death showed few morphological changes in comparison with the middle portion of hair shafts of living individuals. In contrast, hairs taken 10–25 years after death showed tissue losses in the cuticle and medulla layers, finally forming holes in the hair shaft. Although many more samples may be needed to determine whether all hair samples could be expected to survive long periods of burial, our results suggest that all hair samples undergo similar patterns of degradation at each stage.

Hair samples are frequently used as a resource biomaterial in forensic science and physical anthropology. Since the degree of degradation of hair increases with increasing burial time, our results suggest the need for ultramicroscopic description of hair samples taken during archaeological or forensic studies, rather than relying solely on appearance to the naked eye.

The reason for the greater decay of the inner layer of the medulla and the inner cortex relative to the outer cortex could not be determined from our results. Studies on the chemical composition of the hair, however, may provide an explanation. For example, the presence of hydrophilic amino acids in the inner cortex and medulla has been shown to facilitate water absorption and to accelerate hair degradation

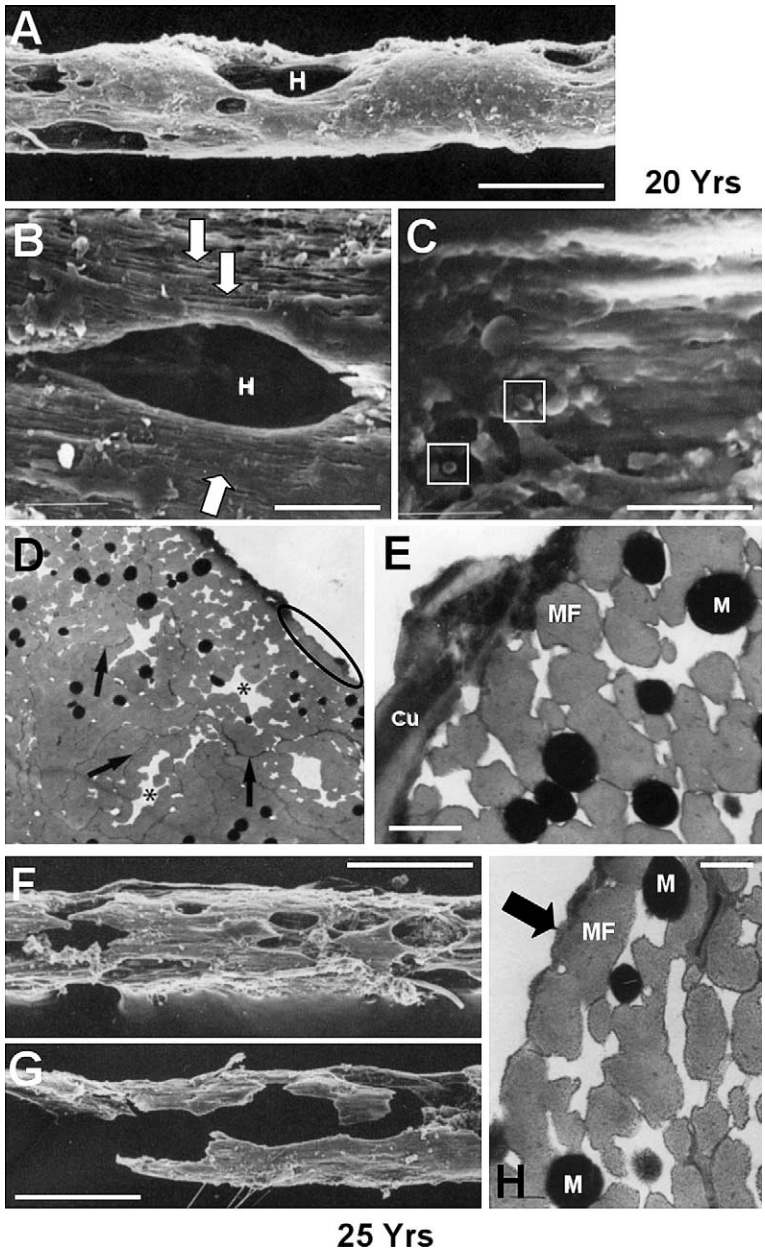

Fig. 5. TEM and SEM images of samples 20 (A–E) and 25 (F–H) years after death. (A) SEM image, showing an increase in the number and sizes of pores in the hair shaft 20 years after death. (B) SEM image, showing detachment of the entire cuticle layer and macrofibrils (arrows) of exposed keratinized stratified epithelial cells around the pores (H) in the hair shaft. (C) SEM image, showing extension of the pores into the cortex and medulla of the hair. (D) TEM image of the hair shaft cross section. The cuticle layer was almost lost (ellipsoidal circle) and there were spaces between macrofibrils in the cortex (asterisk). Arrows indicate the cortical cell membrane. (E) Highly magnified TEM image, showing spaces around the macrofibrils (MFs) in the cortex and the appearance of melanin pigment granules (M) between macrofibrils. Cu, cuticular cell fragment. (F) and (G) SEM 25 years after death, showing that only the outer cortex remained, whereas the inner cortex and medulla had disappeared. (H) TEM comparisons with younger samples, showing that the space between the macrofibrils increased and the cuticle layer had disappeared. Scale bars: (A), (F) and (G) 100 μm, (B) and (C) 10 μm, (D) 1 μm, (E) 0.5 μm, and (H) 0.25 μm.

[8]. In addition, fungi are considered the major decomposers of hair in the burial environment. During fungal tunneling of the hair, fungal hyphae make their way into the hair through a hole, causing small areas of destruction in the medulla and finally causing the hair fiber to be brittle and fragmented [6]. Our results suggest that when collecting hair samples that have survived long periods of time, care should be taken not to break the hair samples.

5. Conclusions

To our knowledge, this is the first report on morphological changes occurring in hair over a 25-year period following death. The findings of this study could therefore be useful in forensic science and physical anthropology. Although individual degradation patterns may vary, our findings may provide a basis for studies on the ultrastructure of hair samples subjected to long-term weathering in the grave.

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