Microscopical Characterization of Known Postmortem Root Bands Using Light and Scanning Electron Microscopy

Jack Hietpas^a, JoAnn Buscaglia^b, Adam H. Richard^c, Stephen Shaw^d, Hilda S. Castillo^e, and Joseph Donfack^f

^a Visiting Scientist Program, Counterterrorism and Forensic Science Research Unit, Federal Bureau of Investigation Laboratory Division, 2501 Investigation Parkway, Quantico 22135, Virginia, USA; jack.hietpas@ic.fbi.gov

^b Counterterrorism and Forensic Science Research Unit, Federal Bureau of Investigation Laboratory Division, 2501 Investigation Parkway, Quantico 22135, Virginia, USA; joann.buscaglia@ic.fbi.gov

^c Visiting Scientist Program, Counterterrorism and Forensic Science Research Unit, Federal Bureau of Investigation Laboratory Division, 2501 Investigation Parkway, Quantico 22135, Virginia, USA

^d Trace Evidence Unit, Federal Bureau of Investigation Laboratory Division, 2501 Investigation Parkway, Quantico 22135, Virginia, USA; stephen.shaw@ic.fbi.gov

^e Visiting Scientist Program, Counterterrorism and Forensic Science Research Unit, Federal Bureau of Investigation Laboratory Division, 2501 Investigation Parkway, Quantico 22135, Virginia, USA

^f Counterterrorism and Forensic Science Research Unit, Federal Bureau of Investigation Laboratory Division, 2501 Investigation Parkway, Quantico 22135, Virginia, USA; joseph.donfack@ic.fbi.gov

Corresponding author:

JoAnn Buscaglia, PhD
Counterterrorism and Forensic Science Research Unit
Federal Bureau of Investigation Laboratory
2501 Investigation Parkway
Quantico, Virginia 22135 USA
(703) 632-4553
(703) 632-4557 (fax)
joann.buscaglia@ic.fbi.gov

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3 **Abstract**

4 A postmortem root band (PMRB) is a distinct microscopic feature that is postulated to occur in
5 hair remaining in the follicle during the postmortem interval [1]. The scientific validity of this premise
6 has been highlighted in two recent high-profile criminal cases involving PMRBs [2, 3]. To better
7 understand the fundamental aspects of postmortem root banding, the microscopical properties of
8 known PMRBs¹ were characterized by light microscopy and scanning electron microscope (SEM) imaging
9 of microtomed sections of hairs showing root banding. The results from this study show that the
10 appearance of the PMRB may be due to the degradation of the chemically labile, non-keratin
11 intermacrofibrillar matrix (IMM) in the pre-keratin region of anagen hairs. In addition, this degradation
12 is confined to the cortex of the hair, with no apparent damage to the layers of the cuticle. These results
13 could provide valuable information for determining the mechanism of band formation, as well as
14 identify a set of microscopical features that could be used to distinguish hairs with known PMRBs from
15 similarly looking environmentally degraded hairs.

16 **Introduction**

17 Detailed examination of hair evidence using light microscopy can provide valuable associative
18 information that may establish linkages between victims, suspects, objects, and crime scenes [5]. In
19 addition to questions of comparison (between questioned and known hairs), microscopical analysis of
20 hair evidence can also provide valuable information concerning somatic origin, and characteristics of

¹ In this paper, a *known PMRB* is defined as a dark elliptical band in a hair (when viewed in transmitted light) in the pre-keratin region of the hair root and is also confirmed to have been recovered from the tissue of a deceased person during the postmortem interval. This is equivalent to a Stage 2 band as defined by [4] Koch SL, Michaud AL, Mikell CE. Taphonomy of Hair-A study of postmortem root banding. J Forensic Sci. 2013:s52-s9, [4] *ibid*.

ancestry, disease, damage, growth stage, and color alteration[6]. Observations made using light microscopy have also identified distinct root characteristics that have been interpreted to be indicative of the hair being shed from a deceased person[7]. These characteristics have been termed “dead man’s root,” “dead man’s ring,” “putrid root” or “post mortem root band (PMRB);” the later term is used throughout this paper. A PMRB is defined by the Scientific Working Group for Materials Analysis (SWGMA) as “the appearance of an opaque microscopic band near the root area of hairs from a decomposing body”[8]. Microscopic hair features (e.g., PMRB (Fig. 1)) that reliably indicate the hair donor was deceased may provide valuable information for reconstruction purposes that require making meaningful associations between people and/or locations with a known sample from a deceased person [9]. However, the scientific validity of whether or not a hair showing the characteristics of a PMRB can be positively identified as deriving from a deceased person is still under question [2, 3, 10], largely due to the lack of a definitive mechanism for PMRB formation.

This paper focuses on the ultrastructural characterization of PMRBs using microscopical techniques, which may provide fundamental information for defining the potential mechanism(s) of band formation. The second paper of this series will address aspects of the mechanism of PMRB formation. Before the details of PMRB characteristics are discussed, a detailed review of human hair biochemistry, structure, and growth is presented. A strong foundation in these topics is required for meaningful interpretations of the microscopical and ultrastructural observations of hairs that display PMRBs.

The Biochemistry of Human Hair

Human hair is a multicellular protein fiber composed of several morphological units, each having a specific chemical composition [11]. The hair fiber is composed primarily of protein (keratin and

keratin-associated proteins) and lipids. Proteomic studies have identified ~340 proteins in samples of human head hair [12, 13]. Extensive disulfide bonding between thiol functional groups (-R-SH) present in the amino acid cysteine (thus forming cystine following disulphide bonding) is the reason for hair's significant resistance to physical and chemical degradation [11].

Hair Structure

The complex structure of hair has been elucidated in several fundamental studies that relied on high-resolution transmission electron microscopy [14-19]. The hair and associated follicle consists of several distinct structural layers including the external root sheath and internal root sheaths, cuticle, cortex and medulla (Fig. 2). Each of these units will be discussed in detail below.

The Root Sheaths

The function of the outer root sheath is not definitively known, but it may contribute to shaping the hair fiber [20]. Surrounding the hair is the inner root sheath, which is composed of three concentric layers: the inner root sheath cuticle, the Huxley layer, and the Henley layer [21]. All three layers are fully keratinized "soft" keratin (e.g., stratum corneum) [21]. This type of keratin differs from "hard" keratin (e.g., hair, nails, and beaks) in having a lower cysteine content (2.6% vs. 7.9%, respectively) [22]. The inner root sheath is truncated at the point where the sebaceous duct intersects the hair shaft [21]; it is thought that proteases secreted in the sebaceous duct are responsible for limiting the height of the root sheath [21]. The outer root sheath extends from the lower end of the hair bulb to the entrance of the sebaceous duct where it changes into epidermis; at this location, the outer root sheath becomes keratinized [21].

The Cuticle

The cuticle has a layered structure (Fig. 3) composed of overlapping scales [23, 24]. In addition, the individual scales also have a layered structure composed of four major units, each differing in disulphide and isopeptide bonding [11]. The epicuticle is a hydrophobic layer approximately 5 nm thick that is present on the outer surface of the scale. The next layer, called the “ α -layer,” is keratinous and contains a very high amount of sulfur [24]; the covalent disulphide bonds give this layer resistance to physical and chemical attack [11]. The third layer, the exocuticle, also contains a high amount of sulfur (~11%) [25]; thus, this layer (through disulphide bonding) provides physical and mechanical resistance and has been shown to be resistant to proteolytic digestions [19]. The inner most layer, the endocuticle, is non-keratinous and consists mainly of protein and cellular debris. It contains very little cysteine/cystine but large amounts of free acidic and basic groups [24]. Swift and Bews [19] reported that the endocuticle is solubilized by proteolytic enzymes; this layer is hydrophilic and consequently swells in water [23]. The cell membrane complex (CMC), which contains very little protein and is composed of polysaccharides and lipids, functions to cement the cuticle layers together [24].

The Cortex

The cortex accounts for the bulk of the hair fiber weight; it contains fibrous and matrix proteins, which are responsible for the mechanical properties of the hair fiber [14]. The cortex is composed of CMC and three cortical cell types: ortho-, para- and meso-cortex cells, each differing in their crystallinity [11]. The cortical cells are spindle-shaped and are approximately 100 μm long and 25 μm wide [25]. These cells contain bundled aggregates of macrofibers that are embedded in a non-keratin material, termed the intermacrofibrillar matrix (IMM) (Fig. 3). A cortical cell typically contains 5-8 macrofibers, each with a diameter of 300-500 nm [11]. The IMM accounts for 1-3% of the fiber weight [26] and its composition is a complex mixture of protein and organelles leftover from the keratinization process.

More specifically, the IMM is primarily composed of nuclear and cytoplasmic remnants, endoplasmic reticula, Golgi apparatus, and mitochondria, among other minor cellular components [11]. The IMM is one of the three non-keratinous components of hair (the CMC and endocuticle being the other two [26]). Like the endocuticle and CMC, there is little cysteine/ cystine present in the IMM and the region is hydrophilic, consequently swelling in aqueous solutions. In addition, the IMM, along with the CMC and endocuticle is a pathway for aqueous solutions and components (e.g., hair dyes) to enter the cortex [26].

Each macrofiber is composed of 500-800 bundles of microfibrils (Fig. 3) embedded in a matrix composed of keratin-associated proteins [11, 14]. Each microfibril is composed of 7-8 single or paired protofilaments; the subunit of a protofilament is a four-chain α -helix structure [11].

The Medulla

The medulla, which is contained within the cortex, consists of hollow cells with a skeleton of amorphous proteins and fine filaments [11]. The medulla is typically filled with gas, thus the region commonly appears dark in transmitted light microscopy. The function and formation of the medulla is not well understood; however, it does not contribute to the mechanical or cosmetic properties of human hair [27].

Hair Growth

Hair growth can be divided into three major phases. Anagen is the active growth phase during which follicle development occurs and the hair shaft is formed. Approximately 80-95% of all human head hairs are in the growing phase at any one time [28]. The anagen phase can vary from 2 to 6 years,

and an approximate value for the rate of growth is 400 $\mu\text{m}/\text{day}$ [25]. At the end of the anagen phase, the hair enters the catagen phase, which lasts approximately 1-2 weeks. During this phase, the follicle slowly begins programmed cell death (apoptosis). The telogen phase follows the catagen phase and normally lasts about 2 to 3 months [29]. During this time the hair does not grow but stays attached to the follicle while the dermal papilla stays in a resting phase. Approximately 5-15 % of all hairs are in the telogen phase at any one time. The hair is still held in the skin through friction, but can be shed (exogen phase) through mechanical abrasion or pushed out by an underlying actively growing (anagen phase) hair follicle. Most people naturally shed approximately 50-150 scalp hairs per day [29, 30].

PMRBs: Conditions for and Progression of Formation

A review of the literature pertaining to the conditions in which PMRBs form notes several common observations. First, PMRBs have been reported primarily in anagen-phase hairs [1, 4, 31]. However, Linch and Prahlow [31] and Koch et al. [4] observed PMRBs in catagen phase hairs. No PMRBs have been observed in hairs that are in the telogen phase [1, 31]. Consequently, empirical data suggest that hair must be in either the active growing phase (anagen) or early stages of quiescence (catagen) prior to an individual's death to be susceptible to PMRB formation. Given this apparent growth cycle constraint, human hair from the scalp (large proportion of anagen growth phase) is the most common type a hair to display a PMRB. Hair from other somatic origins (e.g. pubic, limb, body) are more likely to be in the telogen phase and thus not susceptible to PMRB formation.

The second reported observation is that the hair must be in the skin while the body is decomposing for a hair to display root banding [32]. Studies have been conducted to attempt to replicate PMRBs by exposing hairs to various environmental factors [32-34]. Although some of these studies have concluded that some PMRB characteristics could be produced by environmental factors,

none have proven to replicate characteristics that an experienced hair examiner would identify as PMRBs. The primary differences in characteristics between hair that is environmentally degraded and hair displaying a true PMRB are the morphology and location of root degradation.

The final factor influencing PMRB formation is the length of the postmortem interval (PMI). The time observed for the onset of a PMRB varies widely [10] from as little as 8 hours [1] to “a minimum of 7 days” (expert witness testimony [3]). Approximately one week is a commonly cited PMI for PMRB formation, which is supported by Koch et al. [4]. In addition to these three factors, Koch et al. [4] and Collins [32] reported a correlation between temperature and occurrence of PMRBs; an increase in temperature at the location of cadaver decomposition decreases the time for the occurrence of PMRBs.

Two studies have described stages that broadly define the progression of PMRB formation. Linch et al. [35] reported that following the formation of a typical PMRB, the band further develops (inferred continued degradation to the hair) to produce either a “brushy end,” where the proximal end is highly frayed, or a “hard point,” where the soft keratin of the hair is completely removed leaving behind the more resistant hard (keratinized) region of the hair shaft. Koch et al. [4] presented a similar PMRB development scheme; however, they define four major stages. Stage 0 hair from a deceased person shows no observable differences in the characteristics of the hair roots from antemortem hair samples. Hair in stage 1 shows some limited darkening of the pre-keratin region of the hair root. Hair in stage 2 shows the typical dark band that is confined to the pre-keratin region of hair (i.e., the “typical” PMRB). Finally, stage 3 (last phase of hair decomposition) hair has a “brushy” or “hard point” proximal end [1, 4, 31]. In addition, both Koch et al. [4] and Linch and Prahlow [31] have reported that the hair shaft darkens in the root region during PMRB formation.

It is also important to note that the abundance of hairs from a cadaver that display stage 2 PMRBs is highly variable. Based on the data from 21 cadavers, the average percentage of hairs displaying stage 2 bands is $5 \pm 9\%$ (1σ) with a range of 0-31% [4]. However, 99% of the hairs from one additional test cadaver (that was buried in soil for 3 years) displayed stage 2 PMRBs [4]. These data may suggest that the conditions for PMRB formation are quite strict. Experiments to investigate potential mechanisms/ conditions for PMRB formation will be the focus of the second paper of this research.

Scientific Validity that a PMRB is an Indicator of Derivation from a Deceased Individual

The scientific validity of PMRBs has received considerable attention. In the case of *People v. Kogut* a hair displaying a PMRB, and microscopically consistent with hair from a deceased victim, was found in the trunk of the defendant's vehicle [3]. The finding of the victim's hair (which reportedly displayed a PMRB) in the vehicle was argued to be from either police negligence or misconduct. These interpretations were based on the appearance of the band and the short postmortem interval (on the order of a few hours). The United States District Court, Eastern District of New York held a *Daubert* hearing [36] to determine the validity of the PMRB evidence; the court's ruling allowed testimony concerning PMRBs to be included at the subsequent wrongful conviction case.

Postmortem hair root banding was a significant piece of evidence in the high-profile "Casey Anthony Case" [2]. A single PMRB hair that was microscopically consistent with the victim was found in the trunk of the defendant's vehicle. A *Frye* hearing [37] was held with the defense challenging the scientific validity of PMRB evidence. The defense argued that experts do not know the exact or potential causes of root banding other than death, and that science leaves too much interpretation to the forensic hair examiner. The *Frye* hearing to exclude the testimony concerning PMRB was denied. During trial testimony, Federal Bureau of Investigation's hair examiner correctly stated [we] "cannot assert that characteristics of decomposition seen in hairs are due to them being shed from a deceased individual."

179 However, the examiner also stated “to date, no research projects have been able to replicate the
180 characteristics of postmortem rooting banding, but we don’t know that nothing else can cause those
181 characteristics.”

182 Several research projects have been undertaken to assess the potential to induce PMRB
183 characteristics in antemortem hairs via exposure to various environmental conditions (e.g., soil, water
184 immersion, vehicle trunk), thus testing the premise that hairs with root bands are solely derived from
185 deceased individuals [33, 34, 38, 39]. Delgado [33] and Domzalski [34] cautioned that *some* antemortem
186 hairs subjected to different environmental conditions could be falsely identified as hair containing a
187 PMRB, but also acknowledged that additional studies comparing environmentally exposed antemortem
188 hairs to postmortem hairs should be conducted. A blind-study by Shaw 2012 [39] showed that hair
189 examiners correctly identified known PMRBs (true positive) for 99.5% of the evaluated samples; no
190 errors occurred when quality assurance practices requiring verification by a second examiner were
191 included. In addition, examiners correctly differentiated environmentally degraded antemortem hairs
192 (true negative) from hairs containing known PMRBs.

193 To help shed light on the issues presented above, this research was focused on better
194 understanding PMRBs at the light microscopical and ultrastructural spatial scales. A detailed
195 understanding of the physical characteristics that define a PMRB will provide vital information for
196 shedding light on potential mechanisms of band formation and may also provide metrics to differentiate
197 true PMRBs from environmentally degraded antemortem hair.

198 **Methods**

199 Individual hairs ($n \approx 250$ hairs from eight donors) displaying known PMRBs were obtained from
200 the Koch et al. study [4]; the determination of known PMRB was a consensus of several qualified hair
201 examiners and by this research team. The Koch et al. study involved placing cadavers in different

positions and in a variety of environmental conditions to investigate the occurrence and formation of PMRBs. Hair samples were collected over varying time periods (some collected daily for 2 weeks, while others for only 1 week due to scalp detachment). Detailed examinations of the hair samples from this study were performed using a Nikon Eclipse E600 POL microscope with a QImaging Micropublisher 5.0 RTV CMOS camera. In addition, the physical dimensions of the PMRBs were quantified using ImagePro Plus. Linear dimensions were calibrated using a NIST-traceable stage micrometer.

Approximately 15 hair samples (from 3 donors) displaying stage 1 or stage 2 banding (as defined in the Koch et al. study [4]) were selected for sectioning and detailed examination using scanning electron microscopy (SEM). Hair samples were removed from the slide preparations using xylene to dissolve the Permunt. The hair samples were then dehydrated in 5 minute steps using a graded series of ethanol solutions (50%, 70%, 90%, and 95%). After dehydration, hair samples were embedded in either Buehler Epo-thin® or Electron Microscopy Sciences Embed-812®. Hair samples were subsequently sectioned using a Leica EM UC6 ultramicrotome with a histology diamond knife. Both longitudinal and cross-sections of 0.5-3 μm thickness were gold-coated and examined using a Zeiss-Leo 1450VP SEM.

Results

Light Microscopy

A PMRB is a dark band when viewed in transmitted light; however, it can also appear white in dark field or reflected illumination (Fig. 1). The band consists of a complex network of interwoven elongated elliptical void spaces that are oriented parallel to the hair shaft (Fig. 4). The quantitative microscopical characterization of known PMRBs was first reported by Petraco et al. [1]. They reported that a PMRB occurs approximately 0.5 mm distal to the root tip and 2 mm from the skin surface; our findings are consistent with these reported values. In addition, using image analysis, we measured the

length of stage 2 and 3 bands (with intact proximal ends) in 220 hair samples from eight cadavers. The average length was determined to be $252\text{ }\mu\text{m} \pm 48\text{ }\mu\text{m}$ (1σ).

The overall morphology of the band is broadly elliptical. At the proximal end, the transition from nondegraded hair to the band is abrupt (Figs. 1, 5-7); however, at the distal end, the transition from the banded region to the nondegraded portion of the hair is more gradual or diffuse (Figs. 1, 5-7). The banded region is constrained to the cortex of the hair and there is no observable damage to the cuticle (Figs. 5-6). The band generally develops such that it affects all available space contained within the cuticle, thus the width of the band is typically that of the hair shaft. In some instances, the banded region is slightly bulged outward, giving the hair a swollen appearance in the banded region (Fig. 5). This observation may be due to one or more of the following: 1) the region of the hair is coincidentally slightly wider than the rest of the hair shaft; 2) the degradation that results in PMRBs causes internal expansion; or 3) the structural integrity of the hair is compromised in this region, allowing the area to expand slightly during sample handling and mounting for microscopy.

In addition, the most proximal end of the band (hypothesized start of the band) coincides with the region where the cuticle is first discernible (Fig. 5). In this study, no known PMRBs were observed to extend significantly below this region, and this provides a good reference point for determining a PMRB in a hair of unknown origin. It was also observed that in several instances, hairs displaying PMRB in stages 1 or 2 also contained intact “soft” keratin root sheaths (Fig. 7). This finding may have important implications for understanding the mechanism of PMRB formation and will be further developed in the next paper of this series.

Scanning Electron Microscopy

Microtomed longitudinal sections were examined at 100-50,000x magnification. The accelerating voltage was kept at a moderate range of 5-15 keV to enhance spatial resolution. The

location of PMRB damage is readily apparent (Fig. 8). Consistent with light microscopy observations, the “banded” region consists of a complex collection of void spaces. These voids are roughly elliptical in shape, with their major axis parallel to the hair fiber length. Although the cortex of a normal, healthy hair may contain small void spaces (e.g., cortical fusi), the morphology and the extreme high density of the spaces in the PMRB are significantly different (compare Fig. 2 to Figs. 8-10). This is further supported by TEM-scale ultrastructural studies from the cosmetics and textiles industries, which do not report large networks of void spaces in normal healthy hair [14, 17, 25, 40-42].

The ~200-600 nm fibers that encompass the void spaces are interpreted to be macrofibers. The IMM that binds macrofibers to one another appears to be preferentially degraded in the PMRB region. However, we cannot exclude additional degradation to the cellular wall of the cortical cells that contain the macrofibers; to make this assessment, higher resolution transmission electron microscopy (TEM) would be needed. Apparent remnants of the IMM can be seen as the jagged “saw-tooth” patterns between the separated macrofibers (Fig. 9). The morphology of these structures may suggest a mechanical “pull-apart” method of degradation (this is further addressed in the Discussion). There appears to be insignificant cross-fiber (perpendicular to the hair length) degradation. The damage is oriented parallel to the hair length, effectively “unzipping” the macrofibers from one another. In addition, the void spaces are present in several stages of band development. In the center of the “band,” the void spaces are well developed with a significant amount of IMM degradation. However, the distal and, to a lesser extent, the proximal ends of the “banded” region show partially opened void spaces (i.e., less degradation of the IMM) (Fig. 9).

The degradation mechanism that is responsible for PMRBs does not appear to affect the cuticle (Figs. 5, 6, 10). The “band” shows a distinct and abrupt termination at the interface with the cuticle. At

high-magnification (30,000-50,000x), no degradation to the non-keratin CMC or endocuticle layers of the cuticle was observed (Fig. 10).

Observations made on cross-sectional views (Fig. 11) show that the void spaces are randomly scattered throughout the cortex of the hair and the cuticle layers are intact, consistent with the findings from the longitudinal sections. In addition, high-magnification examination of the cut ends of the individual macrofibers does not show indication of degradation to the microfibers. However, verification of this finding would require using higher magnification transmission or field-emission electron microscopy.

Discussion

The results from this study show that the characteristics of a PMRB may be due to the preferential degradation of the IMM within the pre-keratin/ keratogenous region of anagen and possibly catagen hairs. The reason for the apparent localized degradation to the IMM is likely due to its hydrophilicity; the region is wet (water is liberated from the biosynthesis of proteins), and the region is not fully keratinized [43]. During the anagen phase, the cortical cells above the pre-keratin/ keratogenous zone become stabilized due to coalescence, consolidation, and hardening; these processes are facilitated by cortical cell dehydration, hydrogen bond formation, and disulphide bonding [44]. These cellular modifications are hypothesized to limit the distal extent of the PMRB because the mature cortex is more resistant to degradation (empirically shown in the study by Chang et al. [45]). In addition, we hypothesize that the PMRB does not extend below the pre-keratin/ keratogenous zone because the IMM (material leftover from the keratinization process) is not present, and thus not available for degradation.

We find no evidence to suggest that any “causative agent(s)” that ultimately produce PMRBs significantly degrade the resistant keratin protein fibers. In support of this is the lack of degradation

perpendicular to the hair shaft (Figs. 4, 8-9), the absence of observable damage to the endocuticle or cuticle CMC layers (Fig. 10), and no burrowed fungal pathways through the cuticle [46]. There is also no evidence for external microbes entering the hair shaft either through the cuticle or from the soft root tip. This is consistent with the observation that many hairs with complete stage 2 PMRBs have fully intact root sheaths, which are made of labile “soft” keratin (Fig. 7). The root sheaths are a readily accessible, nutrient-rich material that bacteria would assuredly digest prior to attacking the IMM.

The style of degradation to IMM may provide clues to potential causative agents for PMRB formation. The appearance of the void spaces does not show transverse directionality (e.g. progression inwards or outwards, nor from one side of the hair shaft to the other). This suggests that the causative agents that lead to PMRBs are not degrading through the cuticle as they penetrate the hair shaft. However, the band is more fully developed at the proximal end (near the start of the cuticle) than at the distal end; this could suggest distal propagation parallel to the hair shaft.

The isolated, partially opened void spaces that are observed in both the distal and proximal ends of the PMRB are interpreted to be the result of incomplete degradation of the IMM and/or failure of the void spaces to connect to the central mass of degradation (the “band”). In addition, the vulnerability of the IMM may vary depending on the localized degrees of hydration and keratinization in the distal region of the “band”. Thus, some portions of the pre-keratin/keratinogenous region can be degraded, while others appear unaffected; this may explain the diffuse nature of the transition from “band” to apparently unaffected cortex (Figs. 4-9). On the basis of the observed degradation to the IMM, both chemical and mechanical attacks are viable mechanisms for PMRB formation. The “pull-apart” structures in Fig. 9 may suggest mechanical degradation. The IMM appears to be stretched transversely to the point to where it is eventually ruptured, thus producing the characteristic “saw tooth” patterns. One possible origin for this apparent mechanical damage would be the trapping and build-up of

decomposition gases within the pre-keratin region. However, chemical or enzymatic attack to the IMM or follicle components is also possible; such causative agents may be components secreted by bacteria, released during autolysis, or perhaps unregulated biological pathway processes (e.g., proteases, other enzymes, pH changes, or other water soluble components). All these phenomena, and others, might occur. Experiments designed to test the efficacy of these potential causative agents in the formation of PMRBs are the focus of the next paper in this series.

Conclusions

The results from this study show that the manifestation of postmortem root banding is the result of the apparent preferential degradation of the intermacrofibrillar matrix (IMM) that cements together adjacent macrofiber bundles. The degradation of this material facilitates the development of elliptical-shaped void spaces that are confined to the pre-keratin region of the cortex of the hair. No significant damage was observed to the layers of the cuticle. In addition, the proximal end of the PMRB coincides well with the location where the cuticle layer of the hair is first recognized as a distinct structure.

This study provides valuable descriptive and quantitative information that could be used in assessing whether a questioned hair contains a PMRB. In addition, the results reported may provide a foundation from which to differentiate environmentally degraded hairs that display characteristics similar to those of hairs with true PMRBs. In the next papers of this series, known PMRBs will be examined using high-resolution TEM analysis to further refine the identity of the IMM component(s) (e.g., nuclear remnant, cellular remnants) as well as to assess potential degradation of the cortical cell wall, which may also contribute to PMRB formation. Additionally, several potential “causative agents” that may be responsible for PMRB formation will be investigated in an attempt to discern mechanistic information concerning PMRB formation.

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341 Figure 1. Photomicrographs of a hair displaying a known postmortem root band in transmitted (left) and
342 oblique (right) illumination. The difference in appearance suggests that the “band” is a collection of air
343 (gas) pockets [1].

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348 Figure 2. Schematic illustration of a hair follicle and SEM images of longitudinal sections of a normal
349 antemortem head hair. IRS: inner root sheath, ORS: outer root sheath, CL: cuticle layer, FS: follicle
350 sheath, SG: sebaceous gland, AP: arrector pili. Schematic illustration used with permission from Karger
351 Publishing [47].

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Figure 3. Schematic illustration showing the complex ultrastructure of hair. Note that the macrofibers are held to each other by the intermacrofibrillar matrix (IMM). Image used with permission from J. Wiley and Sons [11].

Figure 4. Photomicrograph of the distal end of a PMRB. Note that the elongated voids are not necessarily inter-connected and that they are oriented parallel to the hair fiber length.

Figure 5. Photomicrographs of hair displaying known PMRBs. Note the intact cuticle that surrounds the “band”. In addition, note that the most proximal end of the “band” starts very close to where the cuticle is first discernible.

Figure 6. Light micrograph of a hair displaying a known PMRB (left) and corresponding SEM image (right) of the external cuticle surface at the location of the band (white rectangle). Note there is no discernible degradation to the external surface of the cuticle. However, the cuticle can display a “curtain-like” appearance with advanced stages of hair decomposition.

Figure 7. Photomicrographs of two hairs displaying known full stage 2 PMRBs. Note the intact condition of the soft keratin root sheaths present. It is common, though not required, for a hair that displays a PMRB to also have a root sheath.

Figure 8. SEM image map of ultramicrotomed longitudinal section of a hair displaying a known PMRB. Note that the epoxy resin has separated from the hair section. Left inset: light micrograph of the sectioned hair. Right inset: note the significant degradation parallel to the length of the hair fiber. Compare extensive damage observed in the PMRB with the same region of a healthy antemortem hair in Fig. 2.

Figure 9. SEM images of longitudinal sections of the “banded” region from a hair displaying a known PMRB (same hair shown in Fig. 1). The top two images show the characteristic “saw-teeth” pattern of apparent remnant IMM. The bottom left image was taken from the midpoint of the banded area, and the bottom right one from the distal end of the banded region.

Figure 10. SEM image of the cuticle-cortex (black arrow) interface for the hair shown in Fig. 7. Degradation is constrained to the cortex of the hair, while the layered structure of the cuticle does not appear to be affected.

Figure 11. Light micrograph of a hair displaying a known PMRB (left) and SEM image (right) of the corresponding cross-section. Note that the damage to the hair is randomly distributed across the hair section with no apparent directionality. In addition, the cuticle is unaffected by the process that produces PMRBs. Note that the hair section has pulled away from the epoxy and has slightly wrinkled.

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