6

Taphonomic Alterations to Hair and Nail

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6.1 Introduction

This chapter explores the nature of changes that hair and nail can undergo in different depositional environments, drawing upon archaeological, forensic and experimental observations. It is important to distinguish between circumstances where naturally shed, forcibly removed fibres or cut fingernails may be found isolated from a body, and those instances where a body is encountered, for which broader taphonomic variables affecting the differential decomposition of body tissues can help to explain changes (Janaway *et al*. 2009; Wilson 2008; Wilson *et al*. 2007b).

Both hair and nail grow incrementally, yet these cellular structures are in essence dead once they emerge from the skin as hardened 'keratinised' structures. The only living portion of hair – the follicle – is seen to undergo distinct changes of morphology. The pattern of development follows predictable phases of growth (anagen), transition (catagen) and resting (telogen) that give rise to the hair growth cycle (Paus 1998). This cyclic pattern of hair growth, together with the fact that we do not moult, instead having a mosaic pattern of growth, explains the fact that roughly a hundred hairs are naturally shed from the body each day (Courtois *et al*. 1996).

Normal development of the nail plate, which grows at different incremental rates between fingernails and toenails (Kapickis 2003; Yaemsiri *et al*. 2010), will continue unchecked unless traumatic injury affects the nail bed. In cases of crush or avulsion injury, the nail may be shed with regrowth, which can take between 6 and 9 months, depending on the integrity of the nail bed (Zook 1985). Growth of both hair and nail is usually kept in check through normal grooming practice, which means that shed hair and nail clippings are often recoverable from domestic dwellings and in association with personal effects, which may be particularly relevant as ante-mortem reference samples as part of a missing persons investigation, or Disaster Victim Identification (DVI).

Hair and nail are relatively robust tissues and so long as the depositional environment is relatively dry and stable, isolated fibres and nail clippings can survive indefinitely given the right conditions. Of course, a multitude of environmental conditions can be expected in different depositional environments, which justifies using archaeological experience to consider variables affecting hair and nail degradation over extended timescales. The fact that within the archaeological record, hair and nail will normally only survive under extreme environmental conditions, emphasises the dominant role that microbial biodeterioration plays in their destruction. Keratin is recognised as being recalcitrant to many common proteolytic enzymes, and as such, only keratinolytic microorganisms, which produce keratinase enzymes, have the capacity to exploit keratin as a sole nutrient source, unless these structures have already undergone some alteration (Gupta and Ramnani 2006). Furthermore, the age of the specimen is largely irrelevant in relation to taphonomic alteration, since it is the localised

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environment that will determine the extent of biological activity, based on the key variables of temperature, light, aeration, moisture, salinity and pH. This normally means that within the wider environment, these tissues survive long term where they are shielded from light and as a consequence of desiccation, waterlogging or extreme cold, which are all conditions under which microbial activity is retarded or ceases altogether.

Hair and nail are recognised as valuable repositories of information (Wilson 2005; Wilson and Gilbert 2007). Traditional methods of morphological characterisation of hair (Tridico *et al*. 2014) use comparison of external features, such as cuticle scale pattern and fibre diameter, and internal features, such as medulla, pigment density and cortical fusi, using light microscopy of fibre whole mounts, scale casts and fibre cross-sections. Whilst the follicle used to be the preferred source of DNA, the hair shaft and nail plate are now recognised as a source of biomolecular information using both DNA and proteomics (Bengtsson *et al*. 2012; Edson *et al*. 2013; Solazzo *et al*. 2013). Furthermore, hair and nail are important because of the incremental way in which these tissues develop. Of key importance is the fact that once formed, these tissues do not undergo biogenic alteration. In the case of terminal scalp hair, it grows at a fairly constant rate of roughly 1 cm per month (Wilson and Gilbert 2007), although this varies between population groups (Loussouarn 2001). The incremental nature of hair growth offers a timeline of recent lifeways information, such as sequential data on health, physiology nutrition and starvation, using stable light isotopes and chronic drug use mass spectrometry (Thompson *et al*. 2014; Wilson *et al*. 2013). Ultimately, the amount of potential information is dependent on the fibre or nail length and the sample requirements of different analytical methods. It is worthwhile also noting that by harnessing an understanding of the formation times of different body tissues – from hair/nail and muscle (recent), to bone (lifetime average) and teeth (childhood) – it may be possible to create a composite picture of lifeways information that can augment a biological profile for unidentified remains.

6.2 Structure of Hair and Nail

Although markedly different in appearance, hair and nail share a common chemistry and origin, which are detailed elsewhere (de Berker 2013; McCarthy 2004; Robbins 2002; Ryder 1973). Both tissues derive from the ectoderm during late embryonic development and have a heterogeneous structure based on the arrangement of keratinised cells.

The nail plate, which comprises multiple layers of flattened keratinised cells, varies markedly in thickness (Mogensen *et al*. 2007) and rate of growth, between fingernails and toenails. Both have a hard resistant structure, with some elasticity and translucency. The nail plate comprises three main layers – ranging from the hard, dense, smooth outer (dorsal) surface (~200 μm thick), only a few cells thick, to the thinnest lower (ventral) layer that connects the nail plate with the underlying nail bed. The main bulk of the nail plate comprises the intermediate layer, with a fibrous structure that is oriented perpendicular to the direction of nail growth.

Terminal human scalp hair shares features common with fibres from other mammalian species (Thomas *et al*. 2012) – comprising a protective outer cuticle, responsible also for the optical properties of the fibre; the cortex, the main bulk of the hair shaft, offering strength, resilience and flexibility to the fibre and the central medulla, which may be continuous, discontinuous or absent. Key distinctions between species include variations in fibre diameter; the thickness or number of cells comprising the cuticle and the diameter and form of the medulla. Outwardly, protection of the hair fibre is afforded by the lipid-protein complex of the exocuticle, which conveys hydrophobicity (Bringans *et al*. 2007).

The three-dimensional architecture of macrofibrils within individual cortical cells gives rise to the mechanical properties of human hair (Harland *et al*. 2014). The bilateral arrangement of cortical cells

into orthocortex and paracortex, which is more distinct in wool, results from the concentration of cysteine and the higher proportion of ultra-high sulphur proteins in the tightly packed paracortex, compared with the less densely packed orthocortex (Plowman *et al*. 2007). In physical terms, these distinctions give rise to the amount of curl in a fibre, with the paracortex on the concave inside of the crimp wave and the orthocortex on the convex outside of the crimp wave. A third, intermediate type of cell has also been described as the mesocortex (Plowman *et al*. 2007). A further distinction is the amount of matrix material in each, with greater electron density of the matrix relative to the intermediate filaments, observed under transmission electron microscopy and attributed to more disulphide bonds within the matrix.

6.3 Changes to Hair and Nail

Nutritional deficiency, especially protein intake, can affect the growth of hair and nail (Cashman and Sloan 2010; Goldberg and Lenzy 2010). Diseases of the hair/scalp and nailbed can affect the strength of hair and nail, including dermatophytic infections, or relatively rare developmental conditions with associated hair fragility, such as monilithrix, which results in changes to hair proteins comparable to oxidative damage due to chemical insult from cosmetics (Sinclair *et al*. 2012). Both disease and aggressive grooming practice can affect the condition of hair in life and make these tissues more susceptible to changes that can occur within the depositional environment.

6.3.1 Physical Alteration

Hair can undergo physical change in living individuals – largely as a direct result of aggressive grooming practice. Frequent washing and brushing of hair can result in physical abrasion of the outer cuticle, initially causing damage to the margins of individual cuticle cells. Aggressive brushing and combing will lead ultimately to breakage and loss of the cuticle. Where the cuticle is lost in its entirety to expose the underlying cortex, there is little to safeguard against separation of cortical cells – essentially the changes responsible for split ends. Hair and nail have a significant water content, and the use of heat tongs at the wrong setting is known to cause swelling and thus physical damage to hair, due to the effect of excess heat on this moisture (Christian *et al*. 2011). Because of the incremental nature of hair, growth changes are more pronounced towards the distal end due to greater cumulative damage to these, the oldest parts of the hair shaft. Such progressive changes are termed 'weathering' (Bottoms *et al*. 1972; Dawber 2002) and can physically weaken the fibre, exposing it to a broader range of insults – including chemical and biological attack. Weathering has been demonstrated to have an effect on stable light isotope values in cattle tail hair (Auerswald *et al*. 2011). The progress of morphological change can be seen using various histological means of assessment, combining fibre cross-section techniques, such as high resolution light microscopy and transmission electron microscopy, and fibre whole mounts, using both light microscopy and fluorescence microscopy with different staining techniques and scanning electron microscopy (Wilson *et al*. 2010b).

6.3.2 Chemical Alteration

The over-use of hair care/cosmetic products, including bleach, chemical straighteners, perming, colourants or highlights, can have a deleterious effect on hair (Gavazzoni Dias 2015; Miranda-Vilela *et al*. 2013; Tate *et al*. 1993). Whilst many hair care product ingredients are intended to provide positive benefits, such as improved tensile strength and hydrophobicity (Oshimura *et al*. 2007), even seemingly innocuous styling sprays and mousses that are alcohol-based, can have a drying effect on hair in the long term.

Hair and nail can tolerate a wide range of pH conditions. Since the skin is mildly acidic, it is not surprising to note that alkalis have a more marked effect on keratin structures, although both strong acids and strong alkalis can denature hair and nail. Under controlled parameters, high pH conditions are used within cosmetic treatment, as with the use of sodium hydroxide to bring about chemical straightening of hair. Given that such chemicals can also be found in many cleaning products, including drain cleaners, the potential dramatic effect on hair and nail is noteworthy. The structural changes that can be brought about have been seen to affect hair differently, according to race (Kuzuhara 2005; Lee *et al*. 2014).

Given that hair and nail are normally found associated with bodies in the archaeological record where other biomaterials also survive (bone, teeth and potentially other soft tissues), there is often comparative data on how aggressive conditions have been within the depositional environment, by examining the extent of differential decomposition across these tissues.

There are exceptional circumstances where all that remain are keratotics, such as hair, nail and proteinaceous clothing items (woollen textiles and hides). These situations usually occur where low temperature and/or waterlogged acidic pH conditions persist, and include findings of cut hair in organic-rich deposits, such as at Deer Park Farms in Ireland (Wilson 2010a) and from permafrost in Greenland (Rasmussen *et al*. 2010). Specialised microenvironments may develop, such as within oak coffin burials where the acidic conditions, brought about by formation of tannic acids derived from the oak coffin, destroy mineralised structures such as bone and teeth. Examples include the remains of a woman found at Quernmore, Lancashire in the UK, where only hair, fingernails and a woollen cloak survived (Turner 1995) and with the Egtved woman from Denmark (Frei *et al*. 2015), where hair, textile and a hide cloak were all that remained.

These rare situations also emphasise the robust nature of hair in relation to putrefactive change and mild acidic conditions that are otherwise destructive to other tissues. However, these observations do not mean that hair and nail is immune from degradative change. These structures are subject to hydrolytic and oxidative change and this is evidenced by work on wool fibres from wet depositional environments (von Holstein *et al*. 2014). It is evident that variations in localised depositional conditions are responsible for different degrees of chemical breakdown, such as deamidation of the alpha keratin structure (Solazzo *et al*. 2014).

Gerontobiology shows us that in addition to fundamental changes to the hair growth cycle (Messenger 2011; Van Neste and Tobin 2004), the underlying substructure of the fibre is affected by ageing – with hair follicles reprogramming matrix keratinocytes to 'increase production of medullary, rather than cortical keratinocytes', as evidenced by increased growth and medullation to fibres alongside decreased pigmentation (Trueb 2009). These physical rearrangements to the hair shaft can offer a greater surface area to chemical and biological attack, as well as reducing the quantity of pigment, which is also more resistant to microbial attack.

All hair contains a mixture of eumelanin and phaeomelanin, which are responsible for the black-brown and red-yellow colouration of hair respectively, alongside factors such as pigment density. Whilst there is a sound basis to explain potential changes to hair colouration, based on chemical alteration to hair pigmentation under both oxidising and reducing conditions – this generally requires either extreme desiccating conditions or extreme waterlogging, which even under archaeological conditions accounts for a small proportion of the surviving examples. In each case, the less stable eumelanin, either decolourises (oxidising conditions) or turns red (reducing conditions), resulting in an overall enhancement of the red-yellow phaeomelanin (Wilson *et al*. 2001). Of course, other parameters need to be taken into account, such as changes to the optical properties of the fibre, based on cuticle condition and the infiltration of material from the depositional environment, for example humic material from waterlogged conditions. Many examples of dark pigmented hair have been recovered from the archaeological record (Wilson and Tobin 2010).

6.3.3 Biological Alteration

Although only keratinolytic species can exploit hair and nail in its native state as a sole nutrient source, these organisms have fairly widespread geographic distribution, having been linked to contact with animals and other humans (Chermette *et al*. 2008; Nweze 2011; Okafor and Ada 2000; Spiewak and Szostak 2000; Weitzman and Summerbell 1995) and a wide range of different soil environments (Abdel-Fattah *et al*. 1982; Deshmukh and Verekar 2014; Filipello Marchisio *et al*. 1991; Kaul and Sumbali 1997). Infections caused by Dermatophytes (typically members of the *Microsporum*, *Trichophyton* and *Epidermophyton* genera) and non-dermatophytic infections affecting hair and nail, have been extensively reported within the clinical literature (Nenoff *et al*. 2014a; Segal and Frenkel 2015; Shemer *et al*. 2015) for which targeted therapies are recommended (Nenoff *et al*. 2014b, 2015). Some bacteria and actinobacteria can also exploit keratin as a sole nutrient source, of importance in managing keratinous waste – such as rendering waste poultry feather (Brandelli *et al*. 2010; Kornillowicz-Kowalska and Bohacz 2010, 2011).

One distinctive feature of keratinolytic fungi is that they produce boring hyphae. These penetrating organs, often with an 'anvil-shaped' form, produce keratinase enzymes that attack the disulphide bonds of the keratin substrate (Deshmukh and Agrawal 1982, 1985; English 1963; Kunert and Krajci 1981). The term keratinolytic fungi emphasises their lytic activity that results in characteristic focal destruction (Figure 6.1). These ovoid lesions have erosive margins and the openings of so-called 'fungal tunnels' (DeGaetano *et al*. 1992) that initially run perpendicular to the main fibre axis, before allowing the hyphae to exploit inherent weaknesses as they branch out laterally within the cortex and medulla (Figure 6.2). Microbial alteration to hair will change the appearance of the fibre in several ways – by either destroying tissue or physically disrupting the layered structure of the fibre, particularly the outer cuticle, which is largely responsible for the optical properties of the fibre. The structures have varied resistance to enzymatic attack, with melanin pigment granules being most resistant (Figure 6.3) (Wilson *et al*. 2007a). Once either biological or chemical change has been initiated, this physically opens these tissues up to exploitation by a wider range of bacteria and fungi, which can then gain access past the protective outer cuticle, and can make use of the chemically less-resistant structures and breakdown products (Wilson 2010b; Wilson *et al*. 2007a).

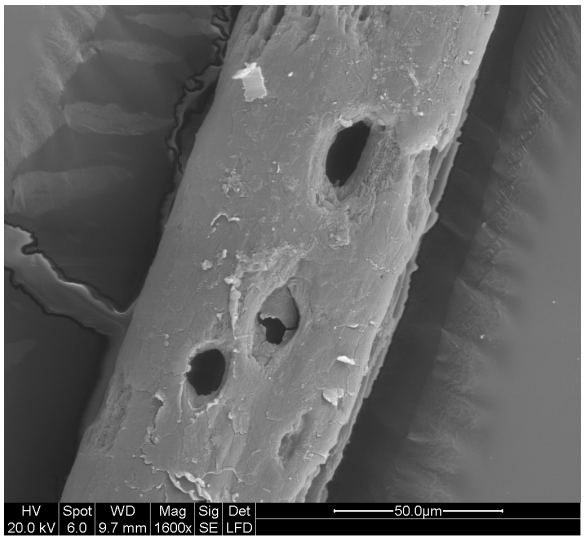

Figure 6.1 Scanning electron micrograph of scalp hair affected by keratinolytic fungi, evident from characteristic ovoid lesions created by fungal tunnelling. Bar = 50 µm

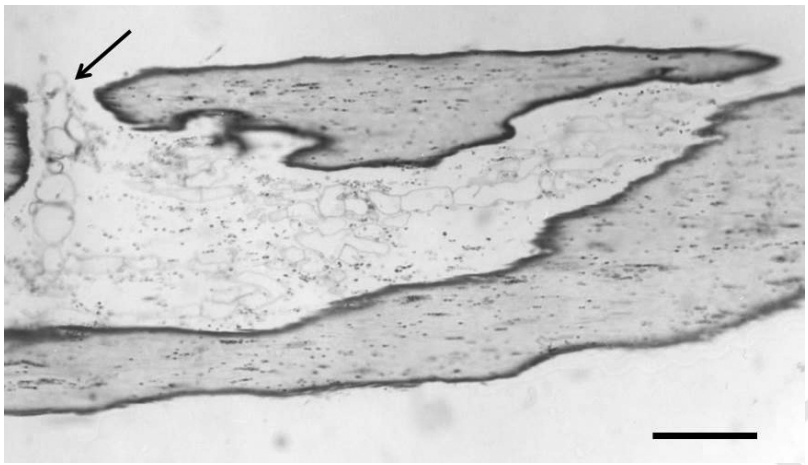

Figure 6.2 Transverse semi-thin section of scalp hair stained with toluidine blue in borax showing fungal tunnelling in progress (arrow), with fungal hyphae extending within the hair shaft. Bar = 15 μm

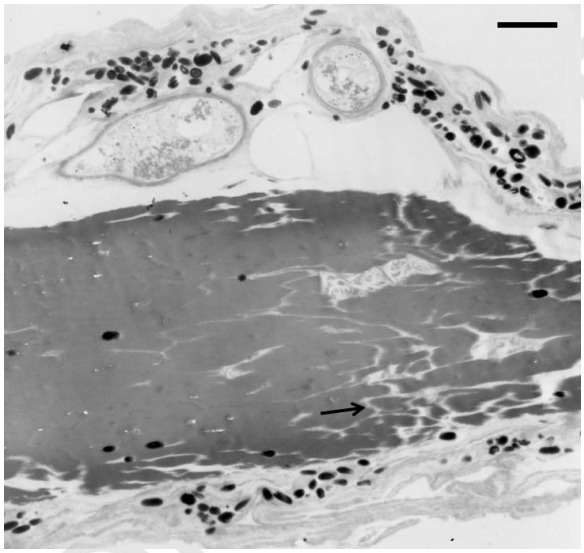

Figure 6.3 Transverse ultra-thin transmission electron micrograph, showing enzymatic attack resulting in separation of macrofibrils (arrow). The circular black structures aggregated within the fungal hyphae are melanin pigment granules which are chemically distinct from keratin and more resistant to enzymatic attack. Bar = 5 μm

6.3.4 Changes Associated with Body Decomposition

During active putrefactive change, the integument undergoes a process commonly termed skin slippage (Dix and Graham 2000; Janaway *et al*. 2009). During this wet phase of decomposition, the hair and nail can be lost as the skin is weakened and sloughs off the body.

Hair recovered from decomposed or partially decomposed human remains has been shown to undergo alteration, with characteristic changes evident to the proximal end of fibres that were in anagen and catagen (Koch *et al*. 2013). Post-mortem root banding is a feature that is associated with putrefactive changes and is visible in the 'soft' unkeratinised region of the hair shaft (Koch *et al*. 2013). The onset of post-mortem root banding, which is hastened by conditions conducive to putrefaction such as warm temperatures, has also been observed to have been delayed where putrefaction

is similarly retarded in aqueous environments and conditions which influence desiccation, such as air-conditioning and cold weather (Koch *et al*. 2013).

The phenomenon of post-mortem banding is influenced by staining from putrefactive changes to the skin, and the infiltration of degradative products to the porous structure of the developing proximal fibre. It is a function of the structural differentiation during hair shaft formation, where initially intermediate filaments are aggregated into fibrils. These regions of the developing fibre have a more open structure, prior to the synthesis and insertion of matrix proteins that have been shown to aggregate as 'blocks', which subsequently disperse between the intermediate filaments as differentiation advances within the zone of keratinisation (Rogers 2004).

In archaeological cases where hair is encountered with human remains, it is often devoid of scalp tissue (particularly in temperate 18/19th century contexts). Commonly, short embrittled fibre lengths may be encountered without discernible root, their embrittled nature characteristic of breakage caused by fungal tunnelling action by keratinolytic fungi, which produce ovoid lytic lesions disrupting the hair shaft. In the best surviving examples involving exceptionally preserved bodies in archaeology, principles of conservation often override the potential for using invasive methods, such as plucking hair or excising tissue that will potentially damage the remains, and are in favour instead of cutting hair close to the scalp. With both situations, it is unlikely that the most proximal portion of the fibre will be available and so post-mortem root banding will not be evident on examination.

In terms of entomology, late stage arrivals to a corpse include insects from the family Dermistidae (e.g. *Anthrenus spp*. – carpet beetles; *Dermestes maculatus* – the hide beetle) and the family Tineidae (the clothes' moths). The common names for these insects emphasise the special adaptations that their larvae have in order to be able to exploit keratin – including use of chewing mouthparts and keratinolytic enzymes in order to digest keratin (Cohen 2015). It is possible to clearly distinguish between breakage of fibres due to forcible trauma and entomological activity using SEM (Mazzarelli *et al*. 2015).

6.4 Processing and Storage of Hair

Despite the range of possible insults to hair and nail, dry samples can generally be stored under stable conditions at room temperature. Acid-free paper bags are preferable to plastic, since biomolecular evidence is an important consideration, with long-term contact with plasticisers a potential issue for analysis by chromatography.

Hair and nails can retain valuable microscopic evidence, including particulates, such as gunshot and explosives residues or testate amoebae, pollen and soil that serve as valuable ecological indicators. Recommendations exist for processing hair for this type of evidence at autopsy (Wiltshire 2006).

When hair and nail is recovered from a body in casework, it is possible for hair to be saturated in blood, putrefactive by-products or other moisture/nutrient-laden sources, depending on the circumstances of the case. If it is recovered damp, it is important that this hair is either processed immediately or frozen on recovery. Since this text is intended both for forensic practitioners and those in other related disciplines such as archaeology, it is important to highlight that poor practice in terms of packaging and curation has seen fungal activity re-established on samples stored damp from excavation, where soft tissue or putrefactive fluids were present with the hair (Wilson and Tobin 2010).

6.5 Conclusion

The evidential or research value of hair and nail continues to expand with new biomolecular approaches, many of which exploit the incremental nature of hair and nail growth. Hair and nail

are robust biomaterials that will persist over considerable time depths unless exposed to various different taphonomic variables. Only a discrete range of 'keratinolytic' organisms can exploit hair and nail as a sole nutrient source. Characteristic biological and chemically induced changes to hair are usually readily identifiable as the cause, and can be distinguished from weathering in life. These alterations can provide access to a wide range of insults when the protective structures such as the cuticle are breached, hastening further alteration.

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