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Selective biodegradation in hair shafts derived from archaeological, forensic and experimental contexts

A.S. Wilson,*† H.I. Dodson,* R.C. Janaway,† A.M. Pollard‡ and D.J. Tobin*

*Medical Biosciences and †Archaeological Sciences, School of Life Sciences, University of Bradford, Bradford, West Yorkshire, U.K.
‡Research Laboratory for Archaeology, University of Oxford, Oxford, U.K.

Correspondence
Andrew Wilson.
E-mail: a.s.wilson2@bradford.ac.uk

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Conflicts of interest
None declared.

Summary

Background Hair is degraded by the action of both dermatophytic and nondermatophytic microorganisms. The importance of understanding hair sample condition in archaeological and forensic investigation highlights the need for a detailed knowledge of the sequence of degradation in samples that have been either buried or left exposed at the ground surface.

Objectives To investigate the sequence of biodegradative change to human terminal scalp hair from archaeological and forensic contexts.

Methods Cut modern scalp hair from three individuals with caucasoid-type hair was inoculated with soil microorganisms through soil burial in the field and under laboratory conditions to produce experimentally degraded samples. The degraded hair fibres were subjected to detailed histological examination using a combination of high-resolution light microscopy, transmission electron microscopy and scanning electron microscopy to investigate the nature and sequence of degradative change to hair structural components.

Results/discussion Degradation was found to occur first within the least structurally robust components that afford the least resistance to microbial/chemical attack. The sequence of degradation (most to least-reflecting degree of vulnerability) in the hair cuticle was as follows: (1) intercellular δ-layer (cell membrane complex); (2) endocuticle; (3) cell membrane β-layers; (4) exocuticle; (5) epicuticle; and (6) A-layer. In the hair cortex this was as follows: (I) intercellular δ-layer (cell membrane complex); (II) cell membrane β-layers; (III) intermacrofibrillar matrix/nuclear remnants; (IV) microfibrils; (V) intermicrofibrillar matrix; and (VI) pigment granules (the hair fibre component that was the least vulnerable to degradation).

Conclusions The selective progress of degradation in the hair shaft has been charted and this provides a basis for further histological work in better understanding the condition of hair fibres derived from archaeological or forensic contexts as well as being relevant to investigation of diseased hair, in particular hair infected by dermatophytes and hair weakened by genetic hair shaft abnormalities.

Study of the human hair shaft is assuming increasing importance in several key areas of archaeological[1] and forensic science,[2] addressing questions of dietary reconstruction,[3,4] investigation of seasonality,[5,6] assessment of geographic mobility,[7] environmental toxicology,[8] drug analysis,[9,10] DNA comparisons[11,12] and as a trap of particulate matter such as pollen or gunshot residues.[13] Many of these important developments stem from several key, and unique, features of hair growth. In contrast to bone and teeth (the most commonly analysed human tissues in bioarchaeology), the hair shaft does not undergo further biogenic change post-keratinization. The hair is one of the more robust nonskeletal tissues in decomposed bodies of forensic interest. In addition, the unique biology of hair growth ensures excellent chronological resolution along individual fibres, which is key for the types of studies indicated above. As the study of single fibres or portions of individual fibres is now technically feasible, investigations utilizing hair can be considered both noninvasive and minimally destructive.

The survival of hair under only very specific burial conditions, over archaeological timeframes, points to microbial activity as the major cause of its destruction. As such there is

a need to define changes that may occur during breakdown of the hair's structure and to derive a means for assessing and measuring these histological changes—the subject of ongoing investigation—to ensure the quality of samples presented for further analytical study.[14,15]

Traditionally, tissues such as bone or teeth have been exploited for archaeological investigation. Because they have a mineral as well as a protein component, they survive in a broad range of depositional environments even when the protein component may be severely altered. Degradative processes affecting these tissues have been studied via histology, microbiology and by chemical means.[16–24] The complex structure of the hair shaft and the fact that the biogenesis of bone and teeth is very different to that of hair demonstrates the need for a separate detailed understanding of the nature and processes that lead to degradation of the hair fibre.

There have been some limited attempts to understand the process of hair fibre degradation over forensic timescales, but much of this has been restricted to gross observations, usually at the fibre surface.[25–31] Fungal tunnelling, first recognized as a feature of bone degradation,[24] has also been described for hair recovered from forensic casework,[32] where the ends of degraded hair fibre are more severely degraded than the mid-shaft region.[33]

Much of our understanding of hair degradation is drawn from the literature on fungal infections of the hair, skin and nails *in vivo*, which are common infections in humans and other mammals.[34–41] In addition to dermatophytes, a variety of different microorganisms can exploit the hair fibre as a nutrient source.[42–44] Most of these are present in soil, and can colonize and exploit different structures within the hair fibre. Affected targets range from the destruction of keratin, and the resultant use of by-products of keratin digestion by other microorganisms, to the breakdown of external lipids. This phenomenon has been exploited in a number of commercial areas including processing of poultry waste[45,46] and the manufacture of fertilizer.[47,48]

The aim of the current study was to investigate in detail the progress of biodegradation in human scalp hair samples that were degraded experimentally in burial and laboratory environments to mimic the range of preservation encountered in different types of depositional environment. A range of morphological approaches were used for this study including high-resolution light microscopy (HRLM), transmission electron microscopy (TEM) and scanning electron microscopy (SEM).

Materials and methods

Experimental degradation of hair fibres

Nondyed caucasoid scalp hair was obtained from three healthy volunteers (two females and one male aged 22–28 years) during routine visits to the hairdressers. The cut fibres at their distal end (i.e. furthest from scalp) were approximately 3 cm in length. In order to address any variation in sample condition due to different hair lengths and hence any marked differences in weathering, each individual served as their own control with a subsample of hair fibres from each individual retained as 'undegraded' control. The hair samples were subdivided into small bundles of fibres, placed into nylon mesh bags (minimum 100-μm mesh) and buried in pits at three contrasting field sites (in peat at a moorland site; in loam at a deciduous woodland site; and in loam at a pasture site) in West Yorkshire in northern England as part of a large-scale study to examine change to hair under simulated archaeological/forensic depositional conditions.[49]

Field burials and surface exposure of hair fibres

Labelled hair samples were buried above and below (30 and 60 cm, respectively) the head/torso of adult pigs (*Sus scrofa*)—as human body analogues[49]—and at equivalent depths within control pits (Fig. 1). Hair fibre samples were also left exposed at the ground surface at the moorland and woodland sites and recovered at intervals (1, 3, 6, 12 and 30 months).

Laboratory-based hair fibre degradation

In order to promote accelerated degradation in modern samples, to examine in more detail the impact of keratinolytic microorganisms on hair ultrastructure, hair was buried between nylon mesh in glass beakers filled with field-wet soil from the pasture site and incubated under laboratory conditions at room temperature for 5 weeks. The hair was then removed from the nylon mesh. In this way, the hair was inoculated with microorganisms from the soil with minimal contamination by adherent soil particles. The inoculated hair was incubated in a moist environment (autoclaved glass wool was moistened with sterile water) at room temperature to promote further microbial growth.

Fig 1. Pasture fieldsite (loamy soil) being prepared for two pigs and the experimental hair samples.

Microscopic analysis of hair samples

Scanning electron microscopy

Hair fibres were retrieved from both field and laboratory 'burials', rinsed in 70% alcohol and mounted on aluminium pin-stubs (Agar Scientific, Stansted, U.K.) for SEM using self-adhesive carbon mounts (Agar Scientific). The mounted samples were gold-coated using a sputter coater (BOC Edwards, Crawley, U.K.) and then examined and photodocumented using both a Cambridge Stereoscan 150mk2 scanning electron microscope (Cambridge, U.K.) and FEI Quanta 400 ESEM (Cambridge, U.K.) in high vacuum mode using secondary electron imaging.

High-resolution light microscopy and transmission electron microscopy

Freshly excavated fibres were fixed immediately post-excavation and processed as previously described.[50] Briefly, fibres were fixed using half-strength electron microscopy fixative according to the method of Karnovsky,[51] post-fixed with 2% osmium tetroxide (Agar Scientific), and then dehydrated in graded alcohols. The fixed hair fibres were cut into proximal, mid-shaft and distal portions and infiltrated with Araldite epoxy resin, arranged into silicone moulds and cured for 72 h at 60 °C.

Serial 0·5-μm 'semi-thin' sections (HRLM) were stained using toluidine blue in borax, examined and photodocumented. Serial 100-nm 'ultra-thin' sections (TEM) were stained using Reynold's method of lead citrate and uranyl acetate[52] and examined using a Jeol 1200EX transmission electron microscope and photodocumented.

Results and discussion

Variable degradation of human scalp hair by microbial activity was observed in samples incubated under both field and laboratory experimental conditions. The presence of tunnels within the degraded hair shaft, similar to those found by Rowe and DeGaetano,[25,32] indicates that much of this alteration was mediated by fungi. Fibres recovered from the loam soil at the pasture field site featured localized erosive lesions to the cuticle, with a small hole tunnelled into the hair shaft cortex at their centre (Fig. 2a). Other fibres had an intact cuticle despite also exhibiting significant fibre collapse internally (Fig. 2b). Transverse and longitudinal sections through such fibres showed that microbial destruction had occurred to the underlying cortex. Here there was evidence that the microbial activity had spread laterally from the initial site of tunnelling (Fig. 3a–c). Degradation was selective according to the structures comprising the hair shaft.

Degradation of hair fibre structural components proceeds sequentially

The progress and extent of degradation was found to occur in a predictable sequence, dependent upon the composition and hence relative resistance of hair structural components to degradation by microorganisms. Degradation corresponded to the extent of keratinization and was characterized by the initial degradation of cystine-poor structures in both the cuticle and cortex (Table 1).

In the cuticle, the cystine-rich epicuticle and exocuticle A-layer were more resistant to degradation than the endocuticle and cell membrane complex. Destruction of the cell membrane complex resulted in delamination of cuticle cells. Structural breakdown occurred in the following sequence (Fig. 4): (1) cell membrane δ-layer (the intercellular cement of the cell membrane complex); (2) endocuticle; (3) cell membrane β-layers (protein–lipid complexes of the cell membrane complex that are situated either side of the δ-layer); (4) exocuticle; (5) epicuticle; and (6) A-layer.

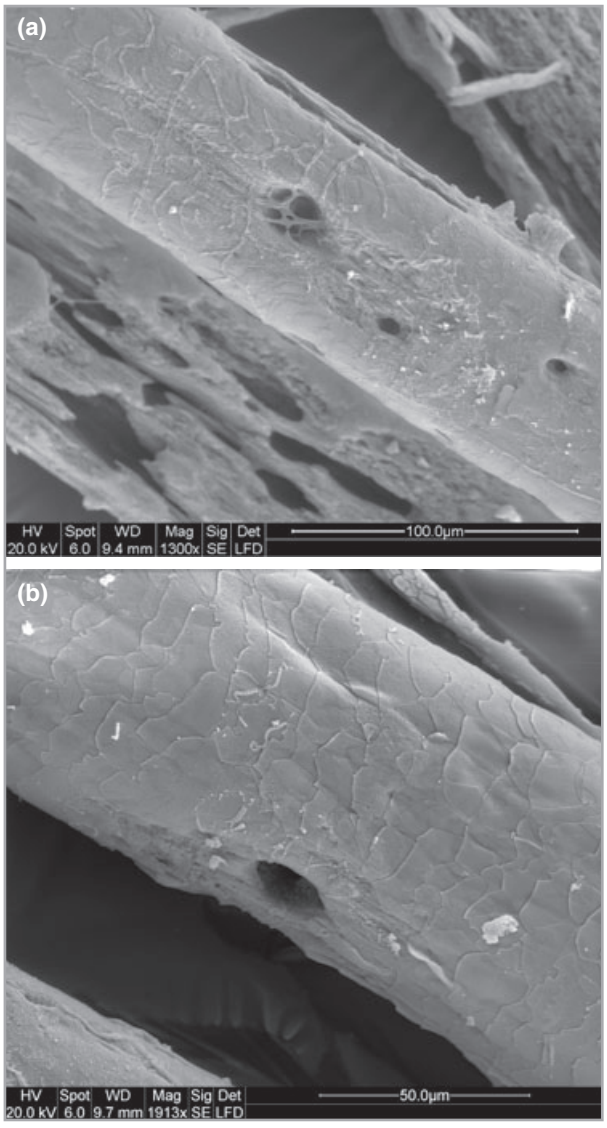

Fig 2. (a) Localized erosive lesions characteristic of 'tunnels' produced by fungal damage; (b) fibre exhibits both tunneling and grooving. The later reflects fibre collapse suggesting degradation to the underlying cortex, despite an apparently intact cuticle.

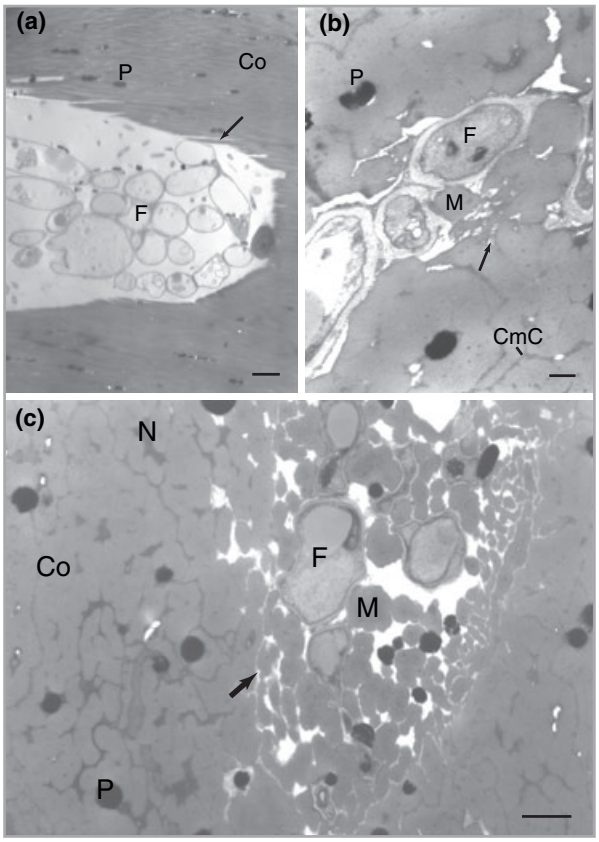

Fig 3. Transmission electron micrographs (a) longitudinal section; (b) transverse section; (c) transverse section. All show destruction of hair cortex by fungi with separation of macrofibrils (arrows). The ragged appearance of the macrofibrils (arrows) is characteristic of selective degradation, involving destruction of the microfibrils while the intermicrofibrillar matrix remains. CmC, cell membrane complex; Co, cortex; F, fungal structures; M, macrofibril; N, nuclear remnant; P, pigment granule. Scale bar: (a) 2 μm, (b) 200 nm, (c) 1 μm.

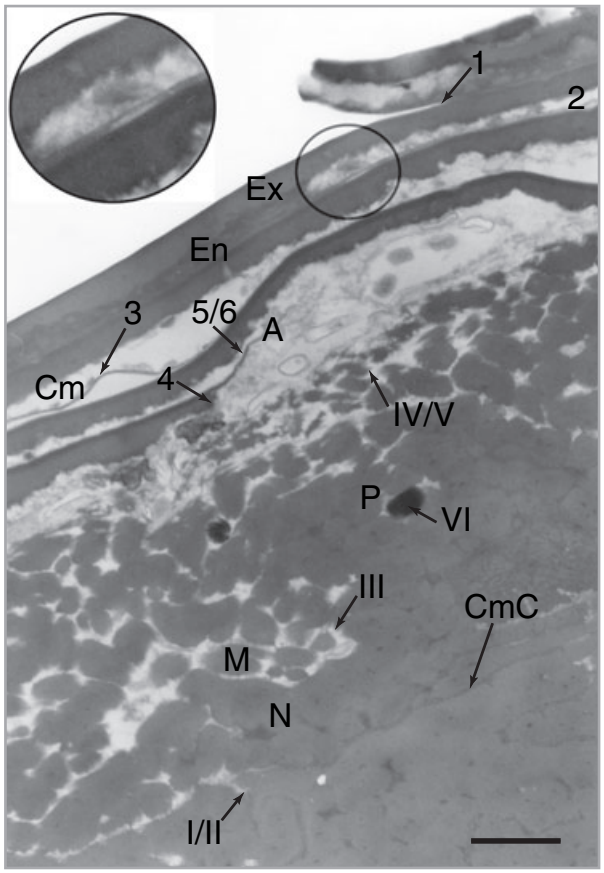

Fig 4. Sequence of ultrastructural change in hair shaft transverse section due to microbial degradation. Arabic and Roman numerals relate to the progress of attack in the cuticle and cortex respectively using the descriptors in Table 1. The lifting of individual cuticle scales results from breakdown of the intercellular δ-layer (see detail inset). Note the increased osmophilia (i.e. electron density) in the most severely degraded regions. Cuticle: A, A layer; Cm, cell membrane; En, endocuticle; Ex, exocuticle. Cortex: CmC, cell membrane complex; M, macrofibril; N, nuclear remnant; P, pigment granule. Scale bar: 1 μm.

Table 1 Relative resistance of hair shaft components to microbial degradation

Cuticle	Cortex	Resistance
1 Intercellular δ-layer cell membrane complex	I Intercellular δ-layer cell membrane complex	Least
2 Endocuticle	II Cell membrane β-layers	▾
3 Cell membrane β-layers	III Intermacrofibrillar matrix/nuclear remnants	▼
4 Exocuticle	IV Microfibrils	▼
5 Epicuticle	V Intermicrofibrillar matrix	
6 A-layer	VI Melanin granules	Most

Similarly, in the cortex, the cell membrane complex, intermacrofibrillar matrix and nuclear remnants were degraded first resulting in the separation of individual macrofibrils, similar to changes observed by T. Kaaman and B. Forslind (1985, unpublished results).[53] The hexagonal-packed microfibrils were degraded in advance of the surrounding cystine-rich intermicrofibrillar matrix in which they were embedded, leaving a ragged appearance to the macrofibrils. Structural breakdown occurred as follows (Fig. 4): (I) cell membrane δ-layer; (II) cell membrane β-layers; (III) intermacrofibrillar matrix/nuclear remnants; (IV) microfibrils; (V) intermicrofibrillar matrix; and (VI) pigment granules.

Importantly, the hair fibre's internal structure was often damaged despite the outwardly well-preserved external appearance of the hair cuticle. This reflects the protective function of the hair cuticle[54] and further highlights the fact that the sequence of hair degradation is dependent on the variable chemistry (and therefore varied resistance) of the hair fibre's structural components.

Melanin structures degrade differently from keratin structures in the hair shaft

Melanin granules were broadly resistant to microbial degradation, whereas keratinaceous structures were readily degraded.

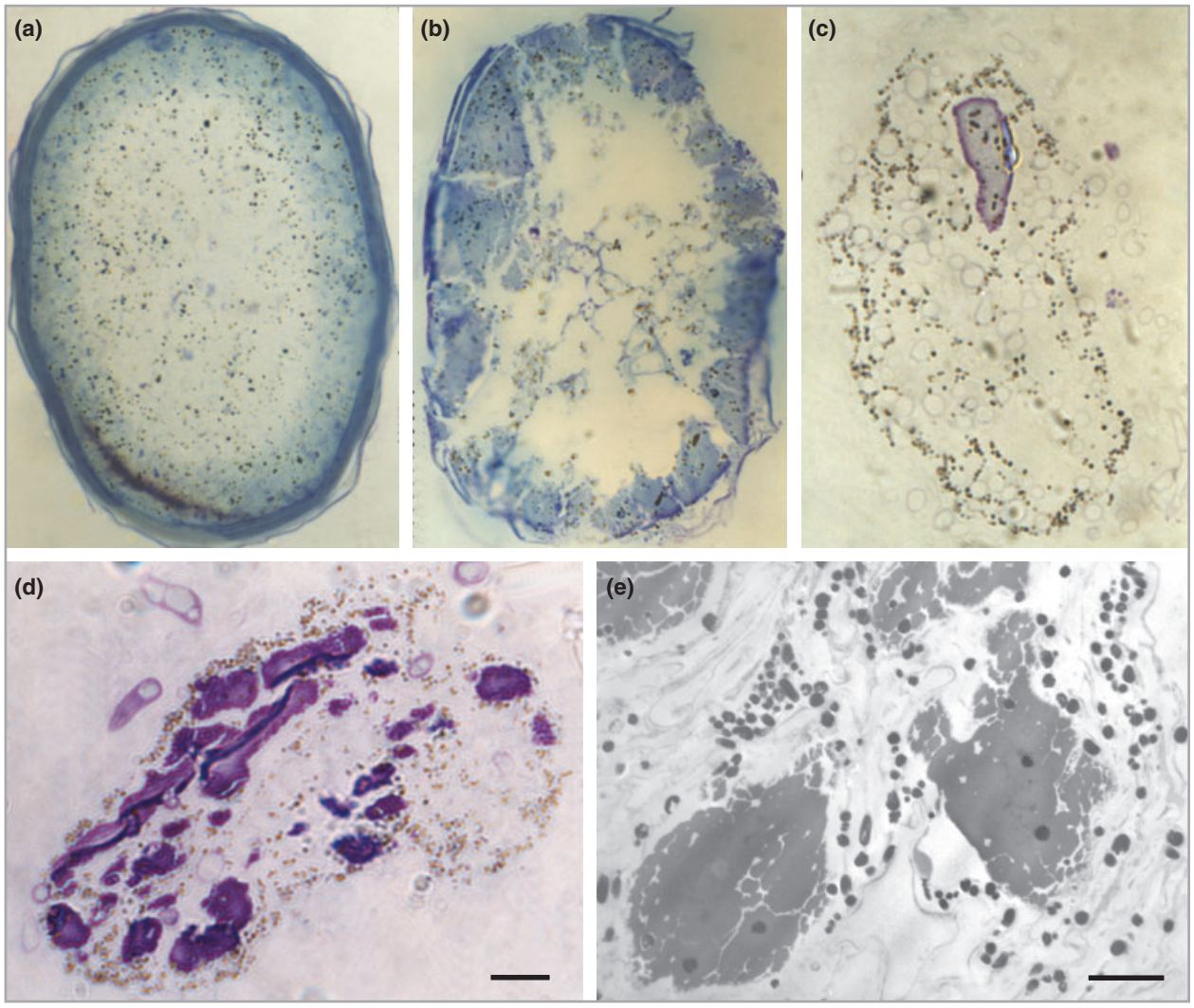

Fig 5. Transverse sections (high-resolution light microscopy, HRLM) showing the progressive change from (a) intact fibre to (b) degraded fibre, which ultimately results in total destruction of keratin structures to form (c) a 'ghost fibre'. Detailed examination of these severely degraded fibres using (d) HRLM and (e) transmission electron microscopy shows survival of remnant cortex and aggregation of separate melanin pigment granules embedded within a mass of hyphal structures. Scale bar: (a–d) 10 μm; (e) 3 μm.

Indeed, in the most severe cases of microbial destruction to the hair shaft, the complete loss of keratinaceous material was associated with concomitant survival of melanin pigment granules. The progressive nature of degradative changes to the hair was readily appreciated in transverse sections of hair fibres (Fig. 5a–c). These range from intact and compacted fibre organization (Fig. 5a) to severely degraded fibres with little cortical bulk and cuticular encapsulation remaining (Fig. 5c). These 'ghost fibres' were observed with remnant cortex fragments and separated melanin granules apparently held together by microbial networks (Fig. 5d,e). The latter was noted especially in hair fibre samples that were incubated under laboratory conditions.

Hair fibres that were retrieved at intervals from the ground surface at the moorland site were influenced by sun exposure whereas those recovered from the woodland site were more influenced by microbial attack (Fig. 6a,b). Photodegradation of hair fibres was localized and depended on the orientation of the fibre in relation to sunlight. Pigment granules in the photobleached region of fibres seen in cross-section were largely lost and presented as circular apparent 'holes' within the cortex. Alteration to the remaining keratin structures was evidenced by dense staining in histological section.

Much of the literature has been concerned with destruction of keratinaceous structures at the expense of melanin pigment survival.[25,26] Yet we demonstrate here that the most extreme example of this differential breakdown of hair structural components involves the preferential survival of melanin pigment granules embedded between microbial structures to form 'ghost fibres'. These changes to the hair fibre can occur over relatively short timeframes, as evidenced by the extent of degradation observed in hair fibres incubated under laboratory conditions. The resistance of melanin to degradation evidenced by this study is also

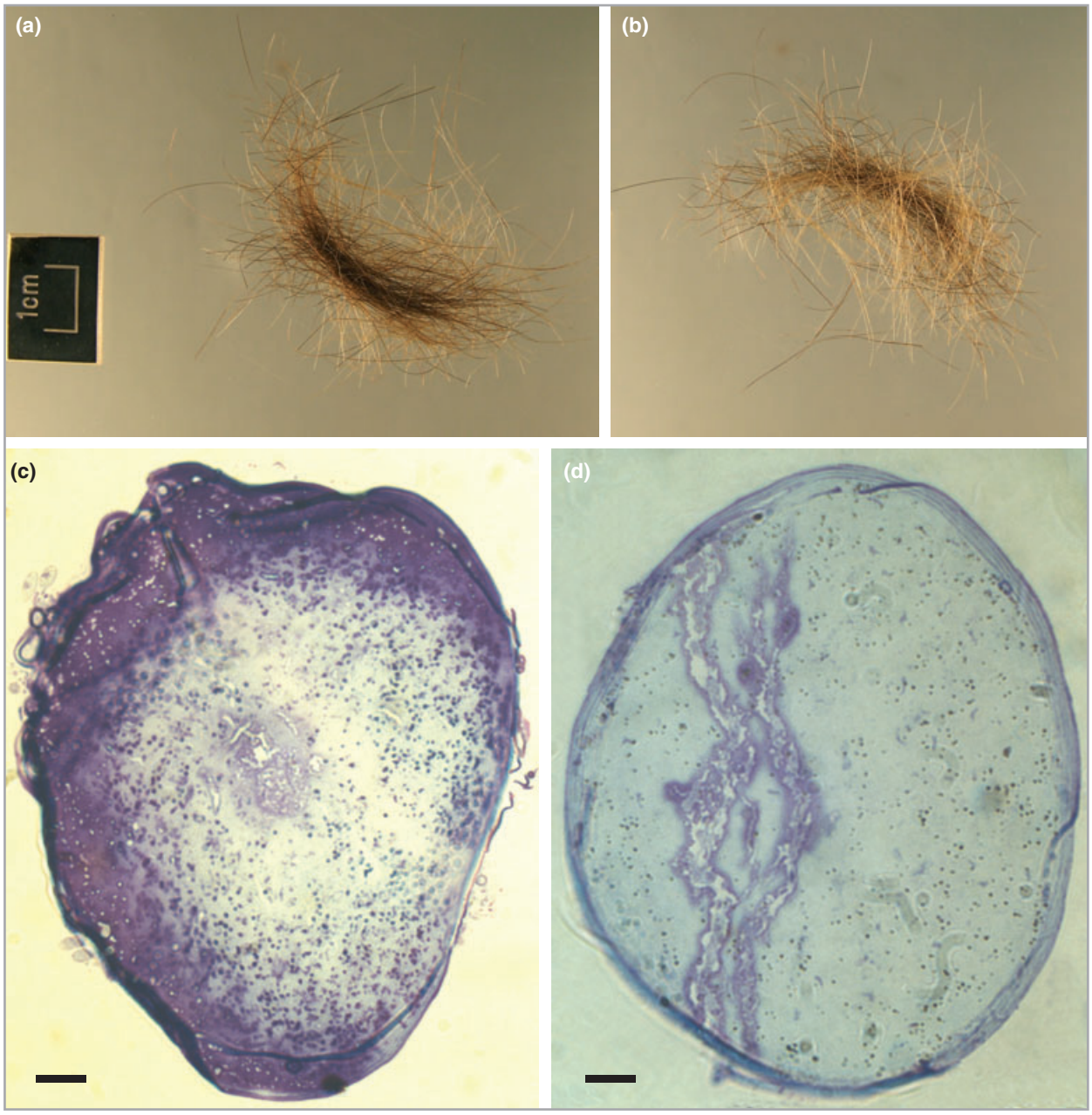

Fig 6. Hair sample obtained from one individual, recovered after 12 months' surface exposure at the moorland site. (a) The undersurface retains the natural hair colour of this individual, whereas (b) the uppermost sun-exposed surface of the same sample is faded. Differential uptake of toluidine blue stain in the cortex of this sample (c) at the uppermost surface (arrows) and loss of melanin pigment granules is the result of photo-oxidation. Conversely, damage caused by microbial degradation (d) was the major change in samples from the woodland site exposed at the ground surface over the same 12-month time scale. Scale bar: 15 μm.

supported by work at the Anthropological Research Facility at the University of Tennessee, Knoxville, TN, U.S.A., where melanin derived from skin pigmentation was recovered from soil samples taken from beneath decomposing human cadavers.[55] In addition, melanin granules can be preferentially isolated from hair using enzymatic digestion.[56] In fact, melanin is considered to function as part of the innate immune defence system by inhibiting growth of microorganisms.[57]

Environment and the presence of keratinolytic microorganisms define the progress of hair degradation

The keratinolytic ability of microorganisms exploiting hair as a nutrient source defines the speed and progress by which hair fibre breakdown occurs. Some keratinolytic microoganisms do not appear to be restricted by the natural boundaries of individual hair component structures as evidenced by 'fungal tunnelling', which is largely mediated by enzyme activity. The

process of selective degradation of hair fibres is dependent on the micro-environmental conditions under which these micro-organisms will flourish. In fact, in contrast to the findings of Chang *et al.*,[58] environment, not time, is a more important driver of ultrastructural change to the hair shaft. Fibres left on the ground surface at two field sites were degraded differently from those buried at these sites. Fibres recovered from the open sun-exposed moorland site exhibited intensive but localized staining to the cortex margin and localized dissolution of melanin pigment granules in HRLM thin sections. Localized degradation to the fibres indicated that photo-oxidation had affected only the uppermost ultraviolet radiation-exposed fibre surfaces.

Conclusion

This study was concerned with the nature and sequence of ultrastructural alteration to the hair fibre under conditions of surface deposition or burial in laboratory and field settings. The principles discussed in this paper may also be relevant to dermatologists who are presented with diseased hair (particularly hair subject to dermatophytic infection) and hair weakened by genetic abnormalities of the hair shaft. The main findings of this study are that degradation of hair fibres was found to follow a predictable sequence, characterized by differential changes to the cortex resulting in preferential survival of melanin over keratin structures. These new insights into the sequence of events that occur during hair degradation are key to our understanding of hair survival over forensic and archaeological timescales. Importantly, total destruction of hair can occur over a relatively short timeframe and it is the progress or inhibition of these initial changes within the depositional environment, which depend on site conditions, that are key to defining the survival or degradation of hair over the longer timeframe.

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