

Resistance of degraded hair shafts to contaminant DNA

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Received 24 September 2004; received in revised form 15 February 2005; accepted 17 February 2005

Available online 26 April 2005

Abstract

We have investigated the susceptibility of degraded human hair shaft samples to contamination by exogenous sources of DNA, including blood, saliva, skin cells, and purified DNA. The results indicate that on the whole hair shafts are either largely resistant to penetration by contaminant DNA, or extremely easy to successfully decontaminate. This pertains to samples that are both morphologically and biochemically degraded. We suggest that this resistance to the incorporation of contaminant DNA relates to the hydrophobic and impermeable nature of the keratin structures forming the hair shaft. Therefore, hair samples represent an important and underestimated source of DNA in both forensic and ancient DNA studies.

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Keywords: Contamination; Degradation; Hair; mtDNA

1. Introduction

Forensic scientists have successfully amplified DNA from almost all sources of human hair, including eyebrow, pubic, and torso hair [1]. Such success is not limited to modern samples—mitochondrial DNA (mtDNA) has been successfully extracted from both degraded and old hair samples, including burnt specimens [2], 100-year-old Native American samples [1], bighorn sheep (*Ovis canadensis*) dating to more than 9400 years old [3], and permafrost-preserved bison (*Bison bison*) samples dating to over 64,800 years old [4]. Therefore, hair is a promising source of DNA for both forensic and ancient DNA studies.

Nevertheless, old specimens containing degraded DNA are often at risk of contamination by exogenous DNA. Even low amounts of modern contaminant DNA may mask or modify results if the quantity of endogenous DNA is small enough. Consequently, the naturally low levels of DNA present in hair [5,6] suggest that this material may be particularly vulnerable to problems of contamination. It is therefore significant that several studies have demonstrated that it is possible to successfully decontaminate modern hair shafts that have been contaminated with human saliva and blood [7,8].

We report here the findings of a study that investigated whether degraded hair specimens are susceptible to contamination by exogenous sources of DNA. We attempted to contaminate degraded and control hair shafts through both direct handling, bathing in fresh human blood, bathing in human saliva, and bathing in solution containing naked DNA. Following these procedures samples were treated with a simple decontamination protocol prior to DNA extraction and sequence analysis using molecular cloning techniques.

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The results indicate that, as with ‘fresh’ hair samples [5,8], on the whole, degraded hair shafts are either naturally resistant to contamination with exogenous DNA easy to decontaminate, or both.

2. Materials and methods

2.1. Contamination of samples

Contamination experiments were carried out on 10 different samples of human hair shafts. The hair had originally been sampled from one donor individual (Table 1). The donor’s mitochondrial DNA sequence has been previously characterised, and differs from the Cambridge Reference Sequence [9] between nucleotide positions (n.p.) 16,209 and 16,356 by a thymine to cytosine transition at n.p. 16,304. Pre-sampling, the different hair samples had been used as part of a field experiment at Shelf, West Yorkshire, England, in an experiment that was used to model how hair and textiles survive and/or alter within the temperate ‘buried body’ environment [10,11]. Accordingly, the different samples exhibited a range of different biochemical and morphological preservation, making them ideal specimens for the investigation.

The condition of the hair was assessed using histology (for details refer to supplementary data), and their preservation spans the range of the hair histological index [12]. As histology appears to be one of the best current indicators of sample preservation [12] it can be hypothesised that these samples accurately reflect the range of specimens that might be utilised for DNA analysis.

Hair shafts from all 10 original samples were used in contamination experiments (Table 1). Initially, one shaft was taken from each sample, and subdivided into three sections. The first subsection was vigorously handled in order to simulate the contamination which may occur when hair shafts are handled through the deposition of skin cells onto the sample. The handler’s mtDNA sequence differs from the

Cambridge Reference Sequence by a cytosine to thymine transition at n.p. 16,298. A second portion of each hair sample was bathed for 5 min in sterile water containing 10^{-12} g/l naked commercial Φ X174 Hae III DNA (Sigma, UK) to investigate the contamination risk presented by purified DNA. For comparison, the mass of DNA within a single human mitochondria has been estimated at approximately 3×10^{-15} g [6]. The third portion of each hair was left uncontaminated as a control. After treatment, the samples were left to air-dry for 24 h at room temperature, then decontaminated by immersion in 50% strength (final strength 3% sodium hypochlorite) commercial bleach solution (Clorox) for 30 s followed by a rinse in sterile ethanol.

Following the initial experimentation, six further hair shafts were taken (from 6 of the 10 original sources of hairs, chosen so as to provide samples that spanned the complete histological index). The hairs were subdivided and the halves were subjected to two additional contamination regimes—exposure to fresh blood and exposure to fresh saliva (Table 1). The saliva and blood samples were provided by the same handler described above. The shafts exposed to blood were bathed in the freshly drawn blood for 5 min, removed and allowed to dry as above. Once dried, the hair shafts were washed in 95% ethanol to remove obvious residue, then decontaminated in bleach solution as above. The shafts exposed to saliva were bathed in saliva for 5 min, removed, allowed to dry and then decontaminated as above.

2.2. DNA extraction and analysis

DNA preparation and extraction methods followed strict ancient DNA specific requirements [13]. Following decontamination, samples were digested in a 0.5 ml extraction buffer (modified from [14]) containing 0.01 M Tris buffer, 0.01 M NaCl solution, 1% SDS, 0.5 mg/ml proteinase K, 10 mg/ml DTT and 0.001 M PTB (*N*-phenacylthiazolium bromide). After incubation at 55 °C for 24 h, the DNA was extracted following a standard phenol:chloroform DNA

Table 1
Experimentally degraded hair samples used in artificial contamination experiments

| Sample | Hair histology | Clones sequenced (H, B, S) | Amplifiable DNA | Evidence of human contamination | | | Φ X174 contamination |
|--------|----------------|-------------------------------|-----------------|---------------------------------|-----------|------------|------------------------------|
| | | | | Handled (H) | Blood (B) | Saliva (S) | |
| 8 | 0 | 47, 12, 12 | Yes | No | No | Yes | Yes |
| 26 | 3 | 47, 12, 12 | Yes | No | No | No | No |
| 27 | 2 | 41, n/a, n/a | Yes | No | n/a | n/a | No |
| 47 | 1 | 71, 12, 16 | Yes | No | No | Yes | No |
| 63 | 1 | 47, n/a, n/a | Yes | No | n/a | n/a | No |
| 71 | 2 | 41, 12, 12 | Yes | No | No | No | No |
| 94 | 4 | 43, 12, 12 | Yes | No | No | No | No |
| 95 | 4 | 9, n/a, n/a | Yes | No | n/a | n/a | No |
| H081 | 5 | 8, 12, 12 | Yes | No | No | No | Yes |
| H082 | 5 | 42, n/a, n/a | Yes | No | n/a | n/a | No |
| Cex- | – | – | No | No | No | No | No |
| Cex- | – | – | No | No | No | No | No |

Cex-: DNA negative extraction controls, n/a: not tested.

extraction protocol (after [14]), concentrated by centrifugal dialysis (Millipore, UK) to yield a final volume of approximately 100 μ l. One blank control extraction was performed for every four samples extracted.

Human mtDNA was amplified from each sample using primers (L16209-H16356 [15]) that target an 189 bp fragment within the mitochondrial hypervariable region 1 (HVRI) following [16]. All amplified human products were cloned and sequenced. The sequences were compared to that of the donor and the handler in order to screen for the presence of contaminant DNA within extracted samples.

The survival of Φ X174 DNA in samples that had been deliberately contaminated was also determined using PCR with the enzyme Platinum Taq Hifidelity (Invitrogen, UK). Primers Φ X1F (5'ctg ccg ttt tgg att taa cc) and Φ X1R (5'ttt gaa tgt tga cgg gat ga) amplify a product of 207 bp and primers Φ X3F (5'cat gac ctt tcc cat ctt gg) and Φ X3R (5'caa tgg aga aag acg gag ag) amplify a product of 129 bp. PCR conditions for both primer sets were: Initial enzyme activation of 94 °C for 90 s, followed by 40 cycles of denaturation at 94 °C for 15 s, annealing at 56 °C for 45 s, extension at 68 °C for 90 s, with a final extension at 68 °C for 10 min.

3. Results

Although the samples of hair shaft analysed spanned the full range of the histological preservation index [10], authentic cloned human mtDNA sequences (i.e. those matching the donor) were obtained from all hair samples (Table 1). Furthermore, despite sequencing a large number of clones per sample, in most samples we observed no evidence for the survival of contaminant human DNA (i.e. any sequence different to that of the donor, and in particular that of the handler). The exception was with the two most degraded samples (hairs 47 and 8, histology 1 and 0, respectively) that had been bathed in saliva prior to decontamination. In both these cases, a majority of the clones sequenced contained the donor sequence (9/16 and 7/12 clones, respectively). For full sequence information refer to supplementary data. There is the possibility that in the other samples some contaminant sequences were present in the extract in undetectable amounts. However, in such a scenario the levels (i.e. maximum contaminant level at a frequency of approximately below 1/8–1/72 of the total DNA in the extract) would be too low to affect the outcomes of a PCR analysis. Furthermore, it might be argued that our findings of DNA survival in the samples could be explained by contamination of the hairs by the donor. However, this is unlikely because the sole contact between the donor and the degraded samples was at excavation (where the samples, in nylon net bags, were collected using gloves and forceps), and because the donor has never been to the molecular analysis laboratories in Oxford.

In contrast to the results of the handling test, faint Φ X174 DNA products could be amplified from 2 out of 10 hair extracts using 1 primer set (Φ X1F/ Φ X1R) (Table 1, Fig. 1).

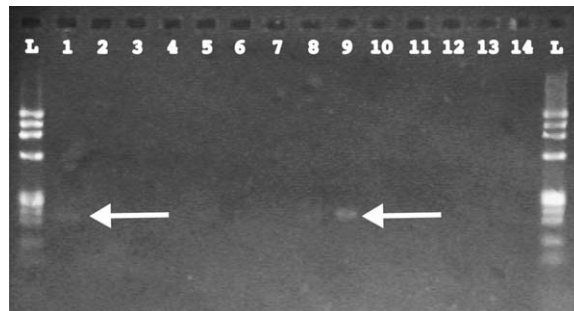


Fig. 1. This is a photograph of a 2% TA electrophoresis gel stained with 2 μ l ethidium bromide, exposed under ultra-violet light, on which 10 μ l of samples PCR amplified with primer set Φ XF1/ Φ XR1 were loaded and electrophoresed. The two lanes marked 'L' contain Φ X174 HaeIII digest molecular weight marker. Lanes marked 1–12 contain amplifications from samples 8, 26, 27, 47, 63, 71, 94, 95, H081, H082 and two extraction blanks respectively. Lanes 13 and 14 are PCR negative controls. Evidence of extract contamination can be seen as weak PCR products in lane 1 (sample 8) and lane 9 (sample H081) as indicated with the white arrows.

Curiously, while the histological preservation of one of these samples was minimal (0), the second extract was from a hair identified as very well preserved (5).

4. Discussion

It is probable that the survival of mtDNA in degraded hair samples, and its protection from external sources of contaminant DNA derives from the unique manner in which hair grows during life. As precortical cells keratinize to form the cortex, they undergo loss of cell cytoplasm, organelle destruction and dehydration. This cell death, associated with the programmed terminal differentiation of cortical keratinocytes, has been likened to apoptosis, a hallmark of which is the protracted retention of organelle integrity, most specifically mitochondrial integrity [17]. Thus while nuclear fragmentation and lysis will expose nuDNA to degradation, the protracted maintenance of mitochondrial membrane integrity may be more likely to protect the mtDNA.

The hydrophobic nature of the proteins filling hair cuticle cells (due to inherent chemical cross-links along alpha-helical polypeptide chains e.g. S–S, glutamic acid–lysine isopeptide bonds) and the keratin packing of the cells [18,19] helps provide a watertight seal around the hair cortex [18], and suggests a plausible explanation as to how samples may be on the whole resisting the penetration of contaminant DNA. With histological degradation some of the protection against water and contaminants conferred by the cuticle, medulla and cortex will be lost. Nevertheless, with only a couple of exceptions, samples exhibiting even low levels of histological preservation do not appear any more susceptible to DNA contamination. Presumably this is due to the survival of highly keratinised (thus impermeable) cuticle

and cortex fragments. Additionally, it is possible that the porosity that arises with hair degradation is large enough to provide an easy route for bleach to access, and thus degrade, any contaminant DNA. It is interesting that the contaminant DNA could not be removed from the most degraded, saliva-bathed samples. One possible explanation is that these two specimens were not bathed for a sufficient period in bleach to fully remove the contaminant, and that some property of the saliva (that is absent in the other sources of contaminant) thus protected the contaminant DNA. As with our results, hairs (presumably well preserved) have previously been demonstrated to be resistant to contamination from saliva [8], although in this previous study a different decontamination protocol was adopted. Whether this alternative protocol is suitable for decontaminating highly degraded hair shafts remains to be seen.

It is difficult to explain our observation of the persistence of naked contaminant DNA in two samples. It cannot be ruled out that, despite the lack of evidence for contamination in PCR and extraction blanks, the Φ X174 DNA observed in these samples entered the extractions at some stage after decontamination. Such sporadic contamination, termed the ‘carrier effect’ has been documented in other studies on degraded sources of tissue (e.g. [20,21]).

5. Conclusion

We have demonstrated that endogenous mtDNA can survive in degraded hair shaft samples. We have also demonstrated that contaminant DNA, in particular that derived through direct handling of specimens, immersion in blood, and immersion in saliva, can generally be removed from such samples, although we caution that seriously degraded hairs that are bathed in fresh saliva may be problematic. However it seems unlikely that such a scenario would be common (or even realistic). As other forms of tissue often found at archaeological and forensic sites (such as bone and teeth) have now been shown to be very susceptible to liquid bourn sources of contaminant DNA (cf. [22]), hairs represent a more reliable and more contaminant-resistant tissue for use in both forensic and ancient DNA analyses.

Acknowledgement

M.T.P.G., A.S.W., and A.C. would like to thank the Wellcome Trust for funding their research (grants 061610, 024661, and 053966).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.forsciint.2005.02.021](https://doi.org/10.1016/j.forsciint.2005.02.021).

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