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The relative DNA-shedding propensity of the palm and finger surfaces

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ABSTRACT

The relative DNA shedding propensity of palmar and finger surfaces has not previously been examined. In the study presented here, palm and fingermarks of six volunteers were analysed for DNA recovery, after deposition at a pressure of approximately 4900 Pa onto glass plates or slides, respectively. The marks were swabbed; DNA extracted using a modified Chelex® method, and then quantified using qPCR, followed by genotype analysis. To assess the availability of DNA-containing material on the skin surface, DNA was analysed by directly swabbing the palm and fingerprint areas of the skin. A further set of palm and fingermarks was subjected to microscopic examination.

The results demonstrated that the quantity of DNA shed from the palmar surface is significantly less than from two fingers. Single donor DNA profiles were obtained from deposited fingermarks by applying a low copy number protocol (32 cycles). DNA retrieved from palm and fingers may be degraded, as suggested by reduced peak intensity and allelic dropout amongst the larger STR loci. These findings suggest that, owing to the low levels of DNA deposition, when palmar marks are found at crime scenes, every effort should be made to recover friction ridge detail to use as an identification metric, with collection for DNA analysis performed afterwards.

1. Introduction

Since the first successful attempt by van Oorschot and Jones in 1997 [1], the application of DNA profiling to fingermark residues has widened the use of fingerprints in personal identification. Marks that are smudged, distorted or otherwise lacking adequate detail may not be useful for conventional pattern comparison but can still be used as a possible DNA source in forensic investigations [2].

The source of DNA in fingermarks was assumed to be the outer layer of the epidermis [3]. However, the majority of epidermal cells from the finger are nucleus-free keratinocytes [4]. Through a long process of differentiation and migration towards the skin surface, averaging 39 days, the cells show shrinking of the nucleus and condensation of chromatin [5]. During this process large organelles, including the nucleus, disintegrate and the cells fill with keratin [5]. Thus, cells from the outer epidermal layer may not be the sole source of DNA in fingermarks.

Several attempts have been made to identify the sources of DNA present in human skin. Balogh et al. [4] and Alessandrini et al. [3] suggested that the epithelial cells obtained from fingerprints were represented as nucleus-free corneocytes, nucleated corneocytes and stripped nuclei. They found that the occurrence of nucleated cells and/or stripped nuclei was theoretically sufficient to generate a full DNA profile. In contrast, the results of Kita et al. [6] showed that a small amount of cell free single stranded DNA is present on the top-most layer of skin. They theorised that the DNA from touched objects originates from the corneal layer sloughed from the surface, as well as DNA excreted through the skin by sweat and sebaceous glands. However, many researchers concur that a variety of factors such as shedder status [7], personal habits [8], substrate [9], perspiration [10], hand washing [11], and person's age or season [12] have a measurable effect on the number of shed cells and the amount of DNA, inasmuch as such factors can alter the turnover time of keratinocytes [13].

These findings suggest that DNA might easily be left behind in the crime scene by transfer of DNA-containing materials in fingerprint residues. The amount of DNA detectable on a touched object following handling was found to be variable and ranged between 0 and 169 ng [1,5,9,14,15]. Moreover, full DNA profiles might be produced from fingerprints in about 80% of the general population (i.e., 18.6% heavy shedders plus 60.5% intermediate shedders) [2].

This investigation aims to identify the potential of different parts of the volar surface, namely the palm and the distal phalanx of the fingers, to shed DNA. We demonstrate that the amount of DNA that is transferred from different parts of the hand volar surface varies with anatomical location and apply this knowledge to partly address the issue raised by Ferraro [16] concerning whether to develop a scene mark for pattern analysis or sample it for DNA.

2. Materials and methods

2.1. Preparation of deposition surfaces

All materials used in the DNA deposition and recovery experiments were treated thoroughly to remove contaminating DNA. Plain glass

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slides 76 × 26 mm, 0.8 to 1.0 mm thick (Fisher Scientific FB58620, UK) or glass plates (200 × 150 × 4 mm, E H Harris, Wolverhampton) were washed with detergent (PHS Direct, UK), thoroughly rinsed with tap water, then distilled water and finally ethanol, before being left to air dry, prior to treatment with UV irradiation for 30 min in a UV crosslinker (CL-1000, UVP, USA). Plastic, portable equipment such as scissors and pipettes and other consumables were treated with UV irradiation for 30 min. All sampling work was carried out in a custom made UV hood.

2.2. Sampling

Six male volunteers were asked to wash their hands with liquid soap (PHS Direct, UK) followed by rinsing with running tap water to remove any contaminating DNA-containing material. Volunteers waited for approximately 60 min without touching any surface with their hands prior to mark deposition. Volunteers were asked to touch either a glass slide for 15 s with their middle and ring distal phalanx (fingers) or a glass plate (palms). Deposition pressure was controlled to be approximately 4900 Pa by calculating the average surface area of either fingers or palm for that donor and then exerting a force onto a top-pan balance resulting in the mass reading equivalent to the required pressure.

2.3. DNA recovery

Six volunteers deposited a total of 24 fingermark pairs (distal phalanx of middle- and ring finger) and 24 palm marks. Each deposition was carried out on independent days. DNA samples were recovered from deposited marks using the double swab technique [17]. Both swab heads were cut off using scissors and placed into a 2 ml microcentrifuge tube. DNA isolation was carried out using the Chelex® based DNA extraction technique described by Linacre et al. [18] with an addition of 0.1 mg proteinase K (Sigma). Extracts were concentrated to a final volume of 20 μl using an Amicon Ultra-0.5, Ultracel-50 Membrane (Millipore Corporation, USA).

Participant reference samples were collected using a buccal swab. DNA extraction was conducted using the EZNA® Blood DNA Kit (Omega Bio-Tek) following the manufacturer's protocol for buccal swabs. Blank swabs, slides and glass plates were included as negative controls with each batch of extractions.

2.4. Direct swabbing of volar surfaces

For a direct assessment of the potential differences in the amount of DNA present on the volar surface of fingers and palms, each hand was washed as described previously. Four samples from finger pairs (first phalanx of middle and ring finger) and palm were collected from each of two volunteers on different days by directly swabbing the donor skin using the double swab technique [17]. Both swab heads were cut off from the stem using scissors and were placed into a 2 ml microcentrifuge tube. DNA extraction from these samples was carried out using the Chelex® based protocol indicated earlier.

2.5. DNA quantification

The Investigator® Quantiplex Quantification Kit assay (QIAGEN, Crawley, UK) was used for quantification of recovered DNA and was carried out according to the manufacturer instructions, using an ABI® 7500 Real-Time PCR System (Applied Biosystems, USA). The analysis of results was performed using SDS 1.9.1 software (Applied Biosystems, USA). Quantification analysis was performed in triplicate.

2.6. DNA profiling

DNA samples of palm mark and two fingermark samples were pre-analysed using singleplex amplifications of the STR loci VWA and TH01 according to Schmerer et al. [19] using 34 cycles, to test the amplification potential of STR loci from resulting DNA extracts in a more sensitive singleplex assay. This preliminary analysis supported quantification estimates and demonstrated that recovered DNA was suitable for the amplification of STR loci.

DNA recovered from fingermarks requires a different approach compared to standard STR profiling, as very small amounts of DNA-containing material are deposited. PCR was performed for 32 cycles on all samples using the AmpFLSTR® SGM Plus® PCR Amplification Kit (Applied Biosystems, USA), and the GeneAmp® PCR System 9700 (Applied Biosystems, USA). PCR products were subjected to electrophoresis using an ABI 310 Genetic Analyser (Applied Biosystems, USA) and profiles were analysed using GeneMapper® ID v3.2 Software (Applied Biosystems, USA). A minimum peak threshold of 100 relative fluorescent units (RFUs) was applied.

2.7. Microscopic examination

To examine whether latent finger or palm marks contain nucleated cells, direct visualisation of nuclei was performed via in-situ staining and microscopic analysis. Finger and palm marks were deposited on poly-L-lysine coated microscope glass slides (Polyscience Inc., USA). Slides were fixed using the wet fixation technique with pre-cooled methanol (−20 °C) followed by air drying, according to the protocol of Keebler and Facik [20] with the slight modification of dripping fixative onto the slides rather than immersion. Care was taken not to move slides vigorously, to prevent dislodging of DNA-containing material from the slide surface. Three drops of absolute methanol (−20 °C) were applied to the glass slides for 10 min before leaving them to air dry at room temperature. Then, slides were stained with iron haematoxylin-anol solution for 10 min to visualise the nucleus. Excess stain was removed using filter paper, before slides were left to air dry at room temperature for 10 min. The entire surface of the stained slides was examined by light microscopy (×400 magnification).

3. Results and discussion

3.1. DNA extraction

Several DNA extraction methods have been used to retrieve DNA from fingerprints. The common ones are organic solvent (usually phenol–chloroform mixtures) [5], Chelex® [18], silica based [21] and magnetic bead-based methods [22]. Each one has advantages and disadvantages with respect to time, complexity and resources required. A modified Chelex® extraction [18] was chosen for this study as it is considered more rapid and involves fewer tube transfers compared to other methods, reducing the risk of sample contamination and DNA loss [23]. In our hands, the modified Chelex® method yielded total DNA recovery from middle and ring fingerprints of 0.0–1.57 ng, which concurs with the DNA level recovered by Daly et al. [9].

A DNA profile obtained from a sample containing DNA from a finger mark pair is shown in Fig. 1. In general no template DNA controls were clean. The results demonstrated that single donor DNA profiles can be obtained using the methodology described in this paper. In general, DNA samples over 250 pg resulted in good quality profiles. The profile shows that, in common with all samples yielding full or partial profiles, nearly all of the DNA originates from the donor, with very little contamination (with contamination being defined as the presence of mixed profiles). Occasional random drop-in was observed, which was limited to individual loci.

The results may also suggest that the DNA recovered from finger marks was degraded, as the majority of allelic dropout occurred within the large STR loci (200–350 bp) and the intensity of the signal was generally reduced with an increase in length of the amplified STR showing the characteristics ski slope pattern as described by Whitaker et al. [23]. This degradation might, at least in part, be attributed to the action of deoxyribonuclease during keratinocyte migration towards the outer