Forensic Science International: Genetics 22 (2016) 44–53

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Research paper

A molecular exploration of human DNA/RNA co-extracted from the palmar surface of the hands and fingers

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ARTICLE INFO

Article history:
Received 2 November 2015
Received in revised form 28 December 2015
Accepted 19 January 2016
Available online 21 January 2016

Keywords:
Touch DNA
Hand
DNA/RNA co-extraction
mRNA profiling
DNA mixture

ABSTRACT

"Touch DNA" refers to the DNA that is left behind when a person touches or comes into contact with an item. However, the source of touch DNA is still debated and the large variability in DNA yield from casework samples suggests that, besides skin, various body fluids can be transferred through contact. Another important issue concerning touch DNA is the possible occurrence of secondary transfer, but the data published in the literature in relation to the background levels of foreign DNA present on the hand surfaces of the general population are very limited.

As the present study aimed at better understanding the nature and characteristics of touch DNA, samples were collected from the palmar surface of the hands and fingers ("PHF" samples) of 30 male and 30 female donors by tape-lifting/swabbing and subjected to DNA/RNA co-extraction. Multiplex mRNA profiling showed that cellular material different from skin could be observed in 15% of the PHF samples. The total amount of DNA recovered from these samples (median 5.1 ng) was significantly higher than that obtained from samples containing skin cells only (median 1.6 ng).

The integrity of the DNA isolated from the donors' hands and fingers as well as the prevalence of DNA mixtures were evaluated by STR typing and compared with reference STR profiles from buccal swabs. DNA integrity appeared significantly higher in the male rather than in the female subsample, as the average percentage of the donors' alleles effectively detected in PHF profiles was 75.1% and 60.1%, respectively. The prevalence of mixtures with a foreign DNA contribution ≥20% was 19.2% (30.0% in the female PHF samples and 8.3% in the male PHF samples).

The obtained results support the hypothesis that transfer of cellular material different from skin may underlie the occasional recovery of quality STR profiles from handled items. These results also suggest that gender may represent an important factor influencing the propensity of individuals to carry and transfer DNA through hand contact, possibly because of the differences in personal and hygiene habits between males and females.

1. Introduction

The increasing sensitivity of multiplex PCR assays for the simultaneous amplification of Short Tandem Repeat (STR) loci enables forensic investigators to recover DNA profiles even from tiny stains and highly degraded samples, including cellular residues left behind when a person touches or comes into contact with an item (i.e., the so-called "touch DNA"). The first report on the possibility to generate genetic profiles from swabs taken from handled objects dates back to 1997 [1]. Since that pioneering study, however, considerable uncertainty has remained about several aspects of touch DNA, i.e., its biological sources, composition, transfer dynamics and ability to withstand different environmental conditions [2,3].

It is often assumed that touch DNA derives from a limited number of shed nucleated cells and of stripped nuclei dispersed among mostly anucleate corneocytes, which can be found on the surface of the outermost layer of the epidermis [4,5]. It has recently been suggested that DNA recovered from touched surfaces may not entirely originate from epidermal cells, but that it could also consist of cell-free DNA from extracellular secretions like sweat

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http://dx.doi.org/10.1016/j.fsigen.2016.01.012
1872-4973/

and sebum [6,7]. Moreover, since skin is by nature constantly exposed to the external environment, it is expected to carry on its surface body fluids that do not derive from the epidermis and its annexes, and to consequently transfer them to objects through handling. For instance, saliva seems to be ubiquitous in the human environment, as it is diffused via droplets originating from the mouth [8]; in fact, it was demonstrated that a static speaking individual can rapidly deposit in the surrounding area a sufficient amount of DNA for a positive identification [9]. Hence, wearing protective masks at the crime scene is mandatory to prevent contamination [10]. Because of the relatively high cellular content of human saliva, DNA yields from hand surfaces subjected to primary transfer of the same fluid are significantly higher than those obtained from freshly washed, bare palms [11]. It can therefore be hypothesized that, rather than the individual propensity to transfer DNA (i.e., the so-called "shedder status" [12]), the presence on human hands of body fluids different from skin may be the reason for the high quantities of human DNA that are sporadically detected on handled objects (both experimental and casework samples) [1,13–16].

Another important issue concerning touch DNA is the possible occurrence of secondary transfer, i.e., the indirect transfer of the donor's DNA to a surface via an intermediary who, in turn, has either come into direct contact with the donor or held an object previously touched by the donor [1]. Therefore, to better understand the possible extent of secondary transfer events, an assessment of the background levels of foreign DNA present on the hand surfaces of the general population appears to be crucial. Nevertheless, previous studies have almost exclusively focused on the prevalence of DNA mixtures under fingernails [17–19], largely ignoring the palm and finger surfaces of the hand, which are the anatomical parts more frequently involved in object handling and in the transfer of touch DNA.

Recently, several robust protocols for DNA/RNA co-extraction from stains have been described and validated for forensic purposes [20], thus enabling the single pipeline analysis of both STRs and mRNA profiles to identify body fluids. In particular, the availability of skin-specific mRNA markers [21,22] now offers the possibility of better exploring the tissue origin of touch DNA. Hence, the aim of the present study was to investigate the characteristics of the human biological material present on the palmar surface of the hands and fingers of individuals from the general population through combined DNA/mRNA profiling. The mRNA profiling results were then put in relation with other factors that are relevant for the interpretation of touch DNA dynamics such as DNA quantity and integrity, prevalence of DNA mixtures and percentage contribution of foreign DNA to mixtures.

2. Materials and methods

2.1. Sample collection

After the Ethical Committee of the University of Turin granted approval for the study, consenting participants were recruited among university students and laboratory personnel. Although donors were informed of the nature and purpose of the experiments, they were not notified in advance of the exact date and time of sampling so as to prevent changes in their activities and social interactions in the period immediately preceding sample collection.

Samples ($n = 120$) were collected from the palmar surface of the hands and fingers of 60 different donors (30 males and 30 females) and are referred to from now on as "PHF" samples. All donors were sampled once, providing separate PHF samples from each hand. Three different sampling methods were employed: dry sterile cotton swab (10 males and 10 females); sterile cotton swab moistened with RNase-free water (10 males and 10 females); tape lift by means of "stubs" that are currently used for gunshot residue collection (10 males and 10 females). A buccal swab was also collected from each individual to establish reference STR profiles. Participation in the study was anonymous and the only information recorded at the time of sampling included age, gender and hand dominance. Donors were also asked to complete a questionnaire (Supplementary material, Table S1) to record their latest activities, social interactions, personal and hygiene habits, etc.

2.2. DNA/RNA co-extraction

After collection, swabs/stubs were air-dried and stored at room temperature for 6–24 h before being submitted to nucleic acid isolation. Total DNA and RNA were extracted using the AllPrep DNA/RNA Micro Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. For the disruption and homogenization of the starting material, swabs/stubs were placed in a spin basket (Investigator Lyse & Spin Basket Kit, Qiagen) with 345 μl RLT buffer, 5 μl Carrier RNA (4 ng/μl) and 13.8 μl DTT (1 M), and incubated 3 h at 56 °C. Lysate collection was then performed by centrifugation at maximum speed. DNA and RNA were finally eluted in 50 μl of buffer EB and 13 μl of RNase-free water respectively.

Genomic DNA was isolated from buccal swabs using the ChargeSwitch gDNA Normalized Buccal Cell Kit (Invitrogen, Carlsbad, CA, USA) and the KingFisher mL Magnetic separator (Thermo Fisher Scientific, Vantaa, Finland).

2.3. mRNA profiling

RNA extracts were treated with DNase (TURBO DNA free, Ambion, Carlsbad, CA, USA) and cDNA was synthesized using the RETROscript Kit with Random Decamers (Ambion), according to the manufacturer's instructions.

Sixteen tissue markers (HBB, CD93, AMICA1: blood; KRT4, SPRR2A, KRT13: mucosa; HTN3, STATH: saliva; SEMG1, PRM1: semen; HBD1, MUC4: vaginal mucosa; MMP7, MMP11: menstrual secretions; CDSN, LOR: skin) and three housekeeping genes (GAPDH, 18S-rRNA and ACTB) were simultaneously amplified by multiplex end-point PCR with a range of different cDNA inputs (7, 3.5, 1 μl) and detected by capillary electrophoresis on the ABI Prism 310 Genetic Analyzer (Life Technologies, Foster City, CA, USA), as described by Lindenbergh et al. [23].

2.4. Body fluid classification

On the basis of the mRNA profiling results, PHF samples were assigned to three broad tissue type categories: "skin", "other than skin", "indeterminate". Classification was based on the scoring guidelines introduced by Lindenbergh et al. in a previous study [24]. In brief, informative profiles (i.e., those showing the highest number of expressed housekeeping genes and no saturated peaks caused by overexpression of single tissue markers) were selected among the mRNA profiling results obtained with different cDNA inputs. Further RT-PCR reactions were then performed using the specific cDNA inputs that had previously generated an informative profile so as to obtain at least three informative mRNA profiling replicates for each sample. Moreover, for samples where the menstrual secretion and/or the vaginal mucosa markers were expressed, amplification of HBD1 was also performed in a singleplex reaction [23]. PHF samples were classified as containing "skin" if peaks at skin-specific marker positions (CDSN, LOR) were "observed", i.e., were present in at least 50% of the replicates. Since co-expression of general mucosa (KRT4, SPRR2A, KRT13) and skin