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Secondary DNA transfer of biological substances under varying test conditions

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ABSTRACT

This research investigates factors that may influence the secondary transfer of DNA. These include the type of biological substance deposited, the nature of the primary and secondary substrate, moisture content of the deposit and type of contact between the surfaces.

Results showed that secondary transfer is significantly affected by both the type of primary substrate and the moisture (wetness) of the biological sample. Porous substrates and/or dry samples diminished transfer (with on average only 0.36% of biological material being transferred from one site to another), whereas non-porous substrates and/or wet samples facilitated transfer events (approximately 50–95% of biological material was transferred from one site to another). Further, the type of secondary substrate also influenced transfer rate, with porous surfaces, absorbing transferred biological substances more readily than non-porous ones. No significant differences were observed among the biological substances tested (pure DNA, blood and saliva). Friction contact between the two substrates significantly enhanced secondary transfer compared to either passive or pressure contact.

These preliminary results will assist in developing general assumptions when estimating probability of a secondary DNA transfer event under simple conditions.

1. Introduction

Improvements in DNA technology over the last 20 years have lifted trace DNA to the forefront of forensic investigative research. Trace DNA can be defined from different perspectives. Initially, at the collection point, usually a crime scene, it is defined as the smallest amount, or volume, of biological material that may be successfully profiled, even though it may be impossible and/or impractical to identify its biological source. At the amplification stage, trace DNA is generally defined as less than 100 pg of template DNA [1,2]. Trace DNA can be recovered from many everyday objects, such as briefcases, car keys, telephone handsets [3], bed sheets [4], shoe insoles [5] and firearms [6]. Even a single cell has been shown to produce reliable and accurate multiplex profiles [7]. Whilst there are many advantages provided by trace DNA in forensic investigations there are some issues that are not well understood. One issue minimally addressed is the possibility of DNA transfer. Several authors have investigated primary transfer, defined as transfer of DNA from a person to an object or person [3–5,8–17], but research into secondary DNA transfer has been limited [8–12,18–19]. Secondary transfer occurs when DNA deposited on one item or person is, in turn, transferred to another item or person or onto a different place on the same item/person. There has been no physical contact between the original depositor and the final surface on which the DNA profile is located. Any biological substance such as blood, semen, hair, saliva and urine could be transferred like this.

A biological substance that has been transferred multiple times, if detectable, will often appear as components of complex DNA profiles. This is because the vectors aiding the transfer and/or the substrate from which it is ultimately collected also bear DNA [10–12]. Van Oorschot and Jones [3] found that plastic tubes held for short periods of time and then held by a second or third person usually provided the DNA profile of the last holder, but also provided the DNA profile, to varying extents, of the previous holders of the tubes. They also found that swabs of the hands that held the tubes regularly provided not just a DNA profile of the person whose hand was swabbed but also profiles of previous holders of the tube even though the individuals had not contacted each other. In contrast, Ladd et al. [19] found little evidence of secondary transfer in his experiments using coffee mugs and handshakes, and concluded that secondary transfer is irrelevant in a forensic casework setting.

Several factors may be relevant to understanding secondary transfer. Whilst the amount of DNA transferred may be independent of handling time, as much of the transfer of epithelial cells occurs upon initial contact [3], the type of substrate seems to be an important variable for secondary DNA transfer, with epithelial cells

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adhering to porous substrates more readily than non-porous substrates [8,20]. Dryness of the sample is also known to influences transfer, with wet and sticky materials being more transfer friendly [14].

Currently there is limited knowledge concerning conditions that may influence secondary DNA transfer. This ignorance not only limits sampling strategies, DNA profile interpretations, and case investigations in general, it could also be easily exploited by defence councils. In this paper, we investigate the occurrence of secondary DNA transfer of biological samples (pure DNA, blood and saliva) under a variety of conditions. The variables include different primary and secondary substrates (soft/porous (cotton) and hard/non-porous (plastic)) under different types of contact between surfaces (passive, pressure and friction) and different moisture content of samples (wet and dry). While other variables may influence secondary DNA transfer, our intent is to investigate those variables commonly encountered by forensic investigators. As we test the outer ends within the range of a given variable tested, others within the range that were not tested here could be expected to give values within our results range. Our results should, together with subsequent data, assist in providing guidelines for the interpretation of DNA evidence when secondary DNA transfer is proposed as the mechanism to explain the presence of a DNA profile at a crime scene.

2. Materials and methods

2.1. Biological samples

Pure DNA, blood and saliva were the three biological materials tested for transfer capability. Primary deposit volume differed according to sample type: 50 μl DNA (5 ng/μl), 50 μl saliva and 15 μl blood. These volumes were chosen to ensure that even minimal transfer (approx. 1%) would be detectable using Quantifiler™. According to the manufacturer Quantifiler™ accurately measures DNA to ≥0.023 ng/μl, but in some instances lower concentrations could be measured [21]. If one assumes that only 1% transfer occurs then if 50 μl DNA (5 ng/μl) or 50 μl of saliva (5 ng/μl) [22–24] is deposited on a surface, 2.5 ng of DNA will be transferred, or in the case of 15 μl of blood (20 ng/μl) [22,25] 3 ng of DNA will be transferred. These values lie well within the detection sensitivity of Quantifiler™.

2.2. Sample moisture

For wet samples the biological fluid was deposited onto the primary substrate and the secondary substrate applied within 10–60 s. For dry samples the biological fluid was similarly deposited on the primary substrate then allowed to dry for 18–24 h (at room temperature) before the secondary substrate was applied.

2.3. Type of substrate

The substrates were chosen to represent the outer spectra of surfaces regularly encountered in forensic case work. Three substrates were used: plastic (hard/non-porous), cotton and wool (soft/porous).

2.4. Experimental design

Fig. 1 represents the elements/processes involved in the preparation of the experiment. A grid stencil, divided into four squares of equal area with smaller 1 cm × 1 cm squares inside each large square, was used as a template. Two clear transparencies (A and B) were placed on top of the stencil; transparency A (closest to the stencil) was to prevent any contamination of transparency B by

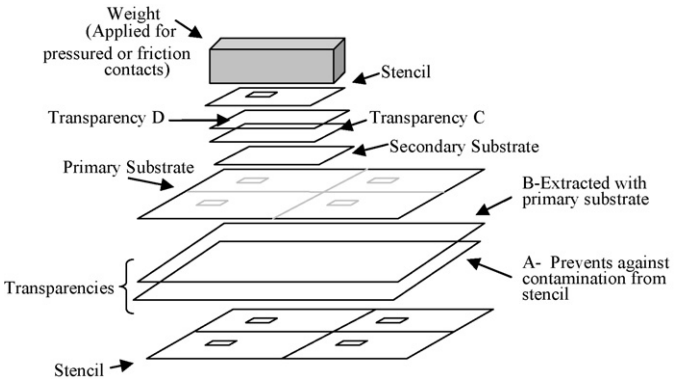

Fig. 1. DNA transfer experimental design.

the stencil that may have occurred while the stencil was prepared. Transparency B was co-extracted with the substrate, in case biological material had leaked through the substrate. The primary substrate (plastic, cotton or wool) of the same size as the transparency was placed on top of transparency B, and the respective biological sample spread over the whole area within each of the four small squares visible through the transparencies.

A 1 kg weight (9 cm × 8 cm footprint) was washed in 70% alcohol and wrapped in clean aluminium foil. A grid stencil, the area of the weight, was taped to the bottom of the weight, with two transparencies (C and D) placed under the stencil (D was to prevent possible contamination from the stencil and C was co-extracted, in case of sample leakage). The grid stencil had one small square on it. The secondary substrate (of an area equivalent to that of the weight) was placed under the transparencies and secured with sticky tape (tape had no contact with the biological samples). The small squares on both primary and secondary substrate were aligned and placed on top of each other for the duration of contact.

For each biological tissue (pure DNA, blood and saliva), all paired combinations of wool, cotton and plastic were used as both primary and secondary substrate. There were four replicates for each combination.

2.5. Contact type

Three types of contact were applied: passive, pressure and friction. Under passive contact, each biological material was deposited on the primary substrate and the secondary substrate was placed on top for 60 s (in four replicates), after which they were separated and samples processed. Pressure contact was identical to passive contact, except the 1 kg weight was added for that 60 s duration. Under friction contact, the experimental design was exactly as for pressure contact, but the weight was moved in every direction for the duration of the contact.

2.6. Sample processing

The 1 cm × 1 cm small squares (plus a surrounding margin of approximately 0.3 cm) were cut into smaller pieces and placed into 10 ml tubes. Separate tubes were used for the primary and secondary substrate squares. For the cotton and the wool substrate samples; the transparency C and B were also cut and pieces placed in separate tubes for extraction (DNA, saliva), or placed in the same tube as the respective substrate (blood).

To remove heme from blood samples a PBS wash was performed prior to extraction. DNA was extracted via 5% Chelex [26], and concentrated with a Centricon® YM-30 centrifugal filter (Millipore) (as per manufacturer's instructions) prior to amplification and quantified using Quantifiler™ Human DNA Quantification

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Kit and the ABI PRISM™ 7500 SDS Instrument [21,27]. Samples that contained inhibitors were subject to a further clean up using QIAquick PCR Purification Protocol (Qiagen) and re-quantified.

As a control to check that the DNA quantitated was indeed that which was deposited and transferred rather than contaminating DNA, a representative sample from each set of variable combinations tested, where typeable amounts of DNA was transferred, was also amplified and typed using AmpF*l*STR Profiler Plus™, ABI PRISM 3100® Genetic Analyser and GeneMapper™ ID software (Applied Biosystem), using standard procedures. All profiles generated from these samples matched those of the experimental samples used and no contaminating DNA was observed.

2.7. Data analysis

The percentages of transferred DNA were calculated in the following manner. Firstly the DNA amounts (ng) were determined by multiplying the volume of a given extract by its concentration as determined by Quantifiler (ng/μl). The percentage transfer is the amount of DNA extracted from the sample on the secondary substrate (plus transparency where applicable) divided by the sum of the extracts from the sample on the primary plus secondary substrates (plus their transparencies where applicable).

The Kruskal–Wallis one-way analysis of variance was used to test if the k independent samples are from the same or different populations [28]. Given the continuous nature of the variables examined, statistically significant groups (identified by Kruskal–Wallis analysis) were analysed with the Mann–Whitney U-test. Mann–Whitney U-test is a non-parametric significance test in which the null hypothesis is that two samples are drawn from the same distribution [29].

3. Results

3.1. Transfer of wet biological materials

The mean and standard deviation transfer percentage of each combination of primary and secondary substrate, and different contact methods (passive, pressure and friction) are given in Table 1. Table 1 illustrates that with wet samples the type of substrate has a major impact on the percentage of transfer. Plastic (non-porous) as a primary substrate facilitates greater transfer of DNA than cotton or wool, and this transfer is variable depending upon the secondary substrate. When the secondary substrate is non-porous, the transfer rate, on average, for all biological samples and contact types is 52.3% (max avg. of 64.1% for blood under pressure conditions). However, when the secondary substrate is porous, such as cotton or wool, the transfer rate increases to an average of 94.7% (max avg. of 100% for DNA under friction conditions). When the primary substrate is porous, such as cotton, only minimal transfer is observed across all combinations of secondary substrates, with an average transfer of 1.54% (max avg. of 18.8% for blood to wool under friction conditions). Similar values are seen for wool, with transfer rates only marginally higher than those for cotton, with an average of 3.46% (max avg. of 15.5% for blood to cotton under friction conditions).

Overall, wet biological samples show no statistically significant difference in transfer rate between passive (average of 28.6%; max avg. of 99.4% for saliva from plastic to cotton) and pressure (average of 29.7%; max avg. of 99.9% for DNA from plastic to cotton) contact types. Friction contact between the surfaces increases the transfer rates slightly (average of 32.1%; max avg. of 100% for DNA from plastic to cotton).

Statistical analysis demonstrated that in most cases, friction was responsible for the significance level identified (Table 2). Only significant types of contact are tabulated.

3.2. Transfer of dry biological materials

Irrespective of variables tested, the amount of transfer was significantly lower for dry samples than for wet samples. Combinations of primary and secondary substrates show different transfer rates for dry samples depending on the type of substrate (Table 3). Plastic as primary surface provided greater transfer, with an average of 4.2% (max avg. of 44.5% for blood transferred to plastic under friction conditions). Both cotton and wool behaved

Table 1
Mean % transfer (standard deviation) of DNA under experimental primary and secondary substrate combinations and different types of contact (60 s), with wet pure DNA, blood and saliva.

Primary substrate	Biological source	Secondary substrate								
		Plastic			Cotton			Wool		
		Passive	Pressure	Friction	Passive	Pressure	Friction	Passive	Pressure	Friction
Plastic	DNA	–	–	–	98.6 (1.5)	99.9 (0.05)	100 (0.02)	–	–	–
	Blood	48.6 (27.1)	64.1 (7.71)	44.3 (16.6)	98.2 (1.5)	90.2 (8.75)	97 (2.38)	81.5 (6.63)	87.5 (2.41)	88.1 (3.3)
	Saliva	–	–	–	99.4 (0.2)	96.7 (1.24)	99.6 (0.2)	–	–	–
Cotton	DNA	0.005 (0.009)	0.02 (0.01)	0.04 (0.04)	0.02 (0.03)	0.07 (0.05)	0.23 (0.07)	–	–	–
	Blood	0.425 (0.79)	0.28 (0.38)	3.05 (0.77)	0.23 (0.45)	0.98 (0.59)	1.05 (2.1)	0.15 (0.19)	1.7 (1.91)	18.8 (10.7)
	Saliva	0.03 (0.05)	0.11 (0.17)	0.1 (0.07)	0.05 (0.004)	0.58 (0.4)	4.33 (2.45)	–	–	–
Wool	DNA	–	–	–	–	–	–	–	–	–
	Blood	1.63 (0.78)	1.85 (1.74)	2.55 (0.57)	0.23 (0.29)	1.78 (0.79)	15.5 (5.8)	0 (0)	0.2 (0.22)	7.43 (7.45)
	Saliva	–	–	–	–	–	–	–	–	–

Table 2
Mann–Whitney post hoc comparison of differences between different manners of contact for wet pure DNA, blood and saliva of different combinations of primary and secondary surface contacts (only combinations with Kruskal-Wallis significant difference are listed; $^*p < 0.05$; ns = not significant).

Manner of contact	DNA		Blood				Saliva	
	Cotton/cotton	Plastic/cotton	Cotton/plastic	Cotton/wool	Wool/cotton	Wool/wool	Cotton/cotton	Plastic/cotton
Passive vs. pressured	ns	ns	ns	ns	*	ns	*	*
Passive vs. friction	ns	*	*	*	*	*	*	ns
Pressured vs. friction	*	ns	*	*	*	*	*	*

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Table 3
Mean % transfer (standard deviation) of DNA under experimental primary and secondary substrate combinations and different types of contact (60 s), with dry pure DNA, blood and saliva.

Primary substrate	Biological source	Secondary substrate								
		Plastic			Cotton			Wool		
		Passive	Pressure	Friction	Passive	Pressure	Friction	Passive	Pressure	Friction
Plastic	DNA	0	0.84 (0.78)	3.75 (1.83)	0.05 (0.01)	0.02 (0.02)	0.25 (0.14)	–	–	–
	Blood	1.45 (2.9)	0.25 (0.5)	44.5 (16.4)	0	3.4 (6.8)	16.1 (10.1)	0.4 (0.47)	0	16.8 (21.7)
	Saliva	0.005 (0.01)	0	0	0.006 (0.01)	0.002 (0.002)	0.27 (0.32)	–	–	–
Cotton	DNA	0	0.004 (0.005)	0.02 (0.03)	0.03 (0.02)	0.06 (0.04)	0.49 (0.47)	–	–	–
	Blood	0	0	0.05 (0.1)	0	0	0	0.08 (0.05)	0	1.43 (1.25)
	Saliva	0	0	0.006 (0.01)	0.01 (0.02)	0	0.57 (0.18)	–	–	–
Wool	DNA	–	–	–	–	–	–	–	–	–
	Blood	0	0.05 (0.01)	1.35 (1.05)	0.05 (0.1)	0.15 (0.1)	1.15 (0.61)	0	0.13 (0.19)	0.5 (0.49)
	Saliva	–	–	–	–	–	–	–	–	–

Table 4
Mann–Whitney post hoc comparison of differences between different manners of contact for dry pure DNA, blood and saliva of different combinations of primary and secondary surface contacts (only combinations with Kruskal-Wallis significant differences are listed; $^{*}p < 0.05$; ns = not significant).

Manner of contact	DNA			Blood						Saliva
	Cot/cot	Plas/plas	Plas/cot	Plas/cot	Plas/plas	Cot/wool	Wool/plas	Wool/cot	Wool/wool	Cot/cot
Passive vs. pressured	ns	*	ns	ns	ns	ns	ns	ns	ns	ns
Passive vs. friction	ns	*	*	*	*	*	*	*	*	*
Pressured vs. friction	*	*	*	ns	*	*	*	*	ns	*

similarly (to wet) as primary surfaces, with transfer rates of 0.13% (max avg. of 1.43% for blood transferred to wool under friction conditions) and 0.38% (max avg. of 1.35% for blood transferred to plastic under friction conditions) respectively.

The manner of contact has a major influence on the percentage of biological material that is transferred. Transfer approximately doubled between passive and pressure contact, with an average of 0.12% (max avg. of 1.45% for blood from plastic to plastic) and 0.29% (max avg. of 3.4% for blood from plastic to cotton) respectively. Transfer rates increase further, approximately 17-fold, between pressure and friction (average of 5.1%; max avg. of 44.5% for blood from plastic to plastic).

Analysis demonstrated that in most cases, friction was again responsible for the significance level identified (Table 4). Only significant types of contact are tabulated. Interestingly, some of the substrate combinations showing significant impacts of the manner of contact differ between the wet and dry contact.

3.3. Effect of moisture (dryness) of the biological samples on percent-transfer rates

The results of our experiments demonstrate that if all the other variables are kept constant, moisture is significant ($p < 0.03$) for the DNA transfer of all biological samples, with wet samples much more likely to be transferred than dry samples. The following conditions: cotton/wool (blood), wool/wool (blood), cotton/cotton (pure DNA) and cotton/plastic (pure DNA) did not show significant difference in transfer rates when moisture content was varied.

3.4. Effect of different biological samples on percent-transfer rates

Statistical analysis shows that there were no differences between biological samples in relation to transferability ($p = 0.319$). When the data for all three biological samples was combined and reanalysed, similar trends were again observed (data not shown).

4. Discussion

This investigation of secondary transfer of different biological sources of DNA has shown that the nature of the substrate and the moisture content of the sample play a crucial role. Clearly, the nature of contact between surfaces is also important in estimating the possibility of transfer under specific case scenarios. Whilst passive and pressure contact showed no difference in transfer rates between surfaces, the applying of friction increased transfer.

Whilst the three different biological sources tested here (pure DNA, saliva and blood) have different viscosities, no major differences in secondary transfer rates were observed among them. One could suggest that other biological samples, such as semen, tears, and urine would produce similar results with respect to transfer in conditions similar to those tested here. However, confirmation of this requires further investigation.

With moist samples (wet) and absorbent substrates, such as cotton or wool, minimal transfer rates, on average of 2.1%, are observed and this increases slightly, to 5.3%, when friction is applied. These observations are in contrast to transfer rates from a non-absorbent primary substrate, such as plastic, which can produce 50–95% transfer, depending on the secondary substrate, with the highest % found with an absorbent secondary surface.

Minimal transfer occurred under all variables tested for the dry samples. Both cotton and wool primary substrates allowed only minimally transfer of DNA (0.13% and 0.38% respectively) which was well below what was transferred from plastic (4.2%). An increase in the absorbance/capture of the biological fluid within the matrix of a porous substrate, rather than it remaining all on top of the matrix, relative to non-porous substrate, may explain this difference. Friction contact increased the transfer of dry biological materials relative to passive and pressured contact with the greatest transfer evident for samples deposited on plastic. This could in part be explained by the fact that a dry sample becomes more powdery after friction, and could thus be more easily dislodged from a non-porous substrate. Additionally, any of the

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powder transferred could then also be easily lost from the secondary substrate.

When faced with secondary DNA transfer scenarios investigators should consider several variables, such as the kind of substrate upon which the evidentiary sample was deposited, as well as the vector involved. For instance, depending on the type of substrate, moisture content of deposited sample, and type of contact, the likelihood of the postulated scenario can vary significantly. Utilising the above findings of the average percentage transfers one could extrapolate how much DNA needs to be present in the initial deposit for say 1 ng to be collected from the final surface after secondary, or additional, transfer under known conditions. One would also need to consider what the biological substance of the primary deposit comprises as different substances contain different amounts of DNA per volume of sample, i.e. liquid blood contains approximately 20,000 ng of DNA/ml and saliva 1000–10,000 ng of DNA/ml [22–25]. For example, using our findings (Tables 1 and 3) one could assume that if a moist blood sample (assuming 20,000 ng of DNA/ml) is present on a hard surface and comes into contact with another hard surface in a manner where there is some pressure, a minimum of 0.08 μl blood is required to have been deposited on the initial surface for 1 ng of DNA to be transferred to, as well as collected and extracted from, another surface (an amount that should be sufficient to generate a DNA profile). If the scenario assumes a further similar contact with another hard surface (tertiary transfer) then 0.12 μl of blood would need to have been deposited at the original site for 1 ng of DNA to be collected from the ultimate crime scene surface. Given a different scenario where a saliva sample (assuming 5000 ng of DNA/ml) was deposited on an absorbent material and allowed to dry prior to coming into passive contact with another absorbent material, then 2000 μl (or 2 ml) of saliva would need to have been initially deposited for 1 ng of DNA to be available after secondary transfer and 2×10^7 μl (or 20 l) if a further similar transfer had taken place.

Trace DNA is increasingly being employed as forensic evidence in casework, and many issues and questions, such as the possibility of secondary DNA transfer, are arising as a consequence. The lack of knowledge relating to secondary DNA transfer can limit the acquisition of useful genetic profiles and restrict the ability of investigators, as well as the judiciary, to make reasonable evaluations of the likelihood of alternative crime scene scenarios.

Gill [30] discussed some of the difficulties inherent when inferring from DNA profiles derived from trace or LCN DNA, compared to those obtained from conventional DNA. These difficulties arise from uncertainties relating to the method of transfer of DNA to a surface, the time of transfer and persistence of DNA. Gill suggested applying a 'hierarchy of propositions' when addressing the use of profiles obtained from trace DNA. The simplest proposition, that relating to the individual from whom the collected DNA originates, can be readily determined by comparing highly discriminating genetic profiles derived from the crime scene sample, the suspect and potential contaminants. However, the higher level propositions, such as how the person's DNA may have ended up on the surface from which the sample was collected, are more difficult to articulate. Good investigative skills may assist in identifying the possible modes of actions that took place for a given sample derived from person A to be found on surface B, but this will in many cases be difficult to determine. Our research detailed above is an attempt to give greater clarity as to both which conditions may well enhance the possibility of transfer of DNA onto a surface as well as those conditions that may decrease the likelihood of such an event. This increased knowledge surrounding transfer events can only make application of DNA profiles more robust in casework investigations.

This paper is a contribution to our better understanding of secondary DNA transfer. Having established some baseline information using easily manipulated relevant biological samples such as blood and saliva, one should consider investigating other biological substances, substrates and conditions. One area of special interest would be to explore the aspects of transfer relating to skin, for which the standardisation of variables may not be as straight forward, as both the biological sample and as the substrate on which a biological substance may be present on or transferred to.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.fsigen.2009.05.001.

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