Forensic Science International: Synergy 6 (2023) 100330

Contents lists available at ScienceDirect

Forensic Science International: Synergy

journal homepage: www.sciencedirect.com/journal/forensic-science-international-synergy

A synthetic fingerprint solution and its importance in DNA transfer, persistence and recovery studies

Hilary Arsenault, Niamh Nic Daeid, Alexander Gray *

Leverhulme Research Centre for Forensic Science, University of Dundee, Smalls Wynd, Dundee, DD1 4HN, UK

ARTICLE INFO

Keywords:
DNA
Transfer
Persistence
Recovery
Synthetic fingerprint solution
Cell free DNA
Cellular DNA

ABSTRACT

A review of the literature on DNA transfer and persistence highlights many difficulties that are encountered when conducting research of this nature. One of the main problems highlighted repeatedly in the literature is the prevalence of inherent uncontrolled variation that accompany these studies, and in turn, the results obtained. This work aims to decrease the amount of intrinsic variability associated with DNA transfer and persistence experiments using a realistic proxy solution which is adaptable, of known composition, reproducible, and capable of being standardised. This proxy is composed of three parts: a synthetic fingerprint solution, cellular DNA, and cell free DNA. In this proof-of-concept study the proxy was tested with a small-scale DNA transfer and recovery experiment and the data obtained suggests that the use of a solution that mimics real fingerprint secretions, over an alternative (such as buffer or a body fluid), is important when working with non-donor provided trace DNA samples. This is because the DNA deposit solution likely impacts the transfer of DNA from fingers/hands to a surface as well as the ability to recover the biological material once deposited.

1. Introduction

A recuring problem in DNA transfer and persistence (T&P) experiments is their highly variable nature, in particular the input DNA [1–10]. Due to the multiple complex factors that affect DNA T&P, experimental designs are open to many sources of uncontrolled variation [1,2,11,12]. Decreasing the inherent variability within these experiments would lead to better experimental designs for T&P studies [1,11,13,14]. The need for a standardised approach to DNA T&P experiments and reporting has been previously highlighted [11,13,15]. The aim of DNA T&P experimental designs should be to eliminate as much of the inherent intrinsic and extrinsic variability associated with these studies to allow the production of relevant empirical data which can be used to inform probabilities given case circumstances [13,16]. One area of the experimental design that is susceptible to a wide variation, and one of the easiest to find a solution for, is the sample DNA deposits themselves. We hope the data presented in this paper will show that there is a viable solution to eliminate variation caused by input DNA.

The term 'touch DNA' is often used when referring to a DNA profile obtained which cannot be attributed to a body fluid [8,17]. This term implies the DNA was deposited on an item through the activity of touching or handling, which can usually not be confirmed [18–20]. Therefore, the term 'trace DNA' has been proposed for a DNA profile obtained in the absence of a body fluid giving no inference to the mode of deposition of the DNA [2,19]. Thus, we will adopt the term trace DNA herein.

Historically, most DNA T&P research has been carried out using human volunteers who have been instructed to carry out a series of strictly prescribed [3,21] or leniently prescribed actions [18,22–24]. After completion of these actions the deposits left behind by the volunteers would be analysed and conclusions made regarding DNA T&P. An extensive review of the existing research has recently been provided by van Oorschot et al. [19]. As highlighted several times in previous research there are challenges when analysing results with uncontrolled volunteer made deposits [7–9]. One main issue encountered by these experiments is the inherent variability between deposits even from the same volunteer [5,6,9,10,25]. This variation results in deposits which are not reproducible making the quantity of input DNA in the deposits impossible to know and the comparison between replicates highly problematic if not impossible.

Some research has attempted to decrease the variability in the deposit DNA material within DNA T&P experiments by taking a different approach. These studies have not relied on natural deposits created from volunteers handling items but have instead used a semi-controlled DNA

* Corresponding author.
E-mail address: azgray@dundee.ac.uk (A. Gray).

https://doi.org/10.1016/j.fsisyn.2023.100330
Received 15 March 2023; Received in revised form 20 April 2023; Accepted 2 May 2023
Available online 15 May 2023

solution (purchased or created from volunteers) as the experimental deposits [7,9,10,17,26–28]. Some of the deposits in these studies consist of a body fluid which allow the target DNA to be deposited with ease onto the target item [9,26,28]. Though this goes some way to solving the variation problem associated with using human donor deposits, it creates another issue. Trace DNA cannot be attributed to a body fluid and thus using a body fluid as the vector for depositing control DNA is not representative of a real-life trace DNA sample. Some experiments avoid this problem by using a suspension buffer to deposit a controlled amount of target DNA [9,10,26]. This method avoids the use of body fluid and thus does a better job at re-creating a trace DNA deposit however, it still falls short of creating a deposit that accurately represents trace DNA found at crime scenes, as a buffer solution is not representative of the hands of people committing crimes.

A small group of DNA T&P studies have aimed to address this shortcoming by taking advantage of an advancement that, until recently, was predominately used to standardise the creation of latent fingerprints for testing novel development methods [7,17]. For simplicity, we will use the term fingerprint when referring to the field of fingermark and fingerprint examination. The chemical composition of fingerprints has been thoroughly documented in the literature and summarised in a review by Girod et al. [29] and more recently Steiner et al. [30], and is now considered well understood. In short, there are three types of secretory glands in the human body, only two of which can be found on the finger: the eccrine and sebaceous glands [29]. Eccrine glands are located all over the body and their excretion is made up of mostly water containing small amounts of organic and inorganic compounds with the major components being: inorganic salts, amino acids, and proteins [29–31]. Sebaceous glands are located all over the body excluding the palms of the hands and soles of the feet [29]. Sebaceous excretions consist of five major groups of organic compounds: glycerides and free fatty acids, wax esters, cholesterol, squalene and cholesterol esters [29–31]. For a detailed account of the literature on the composition of fingerprints and factors which influence their variability see the review by Girod et al. [29]. The field of fingerprint analysis (development and chemical analysis) has recently utilised the understanding of fingerprint chemistry paired with the accepted concept that donor produced latent fingerprints are inherently variable to justify the creation and use of artificial fingerprint materials [29–35]. The use of these materials has recently become more common in experimental designs within the field of fingerprint development aiming to eliminate the variability that is characteristic of real latent fingerprints [31–33,36]. This advancement is of significant importance for DNA T&P experiments as well because it has been previously reported that the sebaceous portion of human fingerprints is an important vector for DNA transfer [37,38]. Therefore, using a DNA deposit solution that is dissimilar to the makeup of normal donor fingerprints would not accurately represent real life scenarios and could adversely affect DNA T&P results.

Only two relevant studies have explored using this kind of artificial fingerprint material for DNA depositions, neither of which focus on DNA transfer or persistence directly [7,17]. These studies utilised a version of artificial fingerprint material to investigate the loss of DNA during forensic collection and extraction [17] and for validation purposes [7]. The current studies however, fall short in two main ways: 1) they only use a portion of the fingerprint material (eccrine or sebum portion) and are thus not adequately replicating the complexity of the biochemical composition of a natural fingerprints [17] or; 2) the experimental designs do not adequately address or explore/control for which biological material the DNA being recovered is coming from e.g. cellular material or cell free DNA (cfDNA) [7,17]. These studies did, however, show that the use of a synthetic fingerprint solution does create a suitable, controllable, adaptable, and reproducible, alternative to donor provided trace DNA samples for DNA T&P studies as fingerprint secretions are likely to aid in the transfer of DNA [37,38]. To the authors knowledge, there are no study's that utilise a synthetic fingerprint solution with the intention to eliminate the characteristic variability of donor provided trace DNA deposits in a study that investigates DNA T&P despite indications that the solution could easily be adapted for this kind of work [7,31].

In the past there was a widely held assumption that the DNA present in trace DNA deposits was from shed skin cells [2,14] being transferred to the surface of an object. Based on this misconception-that is still held by many-experiments focused on creating DNA deposits from shed human skin cells without the consideration of other kinds of biological material [8,39,40]. The focused use of solely cellular material as the origins of the DNA in these types of deposits may, however, be misguided as our current understanding of the DNA content of trace deposits, though growing, is still limited [10,41,42]. In contrast other experiments use whole hand washes as their DNA deposits which would include all possible DNA sources (which would be indistinguishable from each other) that are washed from the surface of the hand [7,43]. Recent research, though still limited at this time, has aimed to identify the biological and cellular origins of DNA recovered from trace DNA deposits [8,41,42,44–46]. This research has proposed that the DNA in trace deposits can be associated with one of three sources: shed keratinocytes, nucleated epithelial cells, and cfDNA and that its likely these sources represent the majority of the DNA obtained [42,44,45,47–49]. To the authors knowledge no DNA T&P experiments in the literature have taken into consideration assessing the ability of different sources of trace DNA to transfer and persist on handled/touched objects. A limited number of experiments have highlighted a relatively simple way to investigate this question and that is by using a cell culture model as the cellular DNA component of trace deposits [10,26]. Other experiments have used a control cfDNA in experimental deposits [9]. The use of cell culture and control cfDNA allows for a known and controllable amount of DNA, attributable to each biological material, to be deposited in each replicate thus avoiding unwanted variation within deposits and providing a better model to assess transfer, persistence and recovery of each cellular and cfDNA.

This work presents a standardised method of sample deposition for DNA T&P experiments in which the DNA type and amount are accurately known. This proxy trace DNA will allow for decreased variability between DNA deposits and increased repeatability within and between experiments and replicates. This allows a more accurate estimate of the effects of each individual factor influencing the transfer, persistence and recovery of the DNA. The need for this kind of data was highlighted by Taylor et al. [13] and more recently reiterated by van Oorschot et al. [11]. Additionally, this allows for the manipulation of cellular and cfDNA composition of the deposit which will allow for better determination of the behaviour of these separate components to the transfer and persistence of DNA.

To address these needs we created a proxy deposit that consists of three components: 1) a synthetic fingerprint solution that acts as the deposit medium as described by Sisco et al. [31]; 2) sonicated rainbow trout DNA which represents the cfDNA fraction of trace DNA deposits and, 3) mouse embryonic fibroblasts (MEF) which represent the cellular component of trace DNA deposits. We show that by using this proxy we can control the consistency of the deposit medium which allows us to accurately mimic real human fingerprints all the while controlling the origin and quantity of the input DNA. This will allow us to accurately assess and compare the transfer and recovery behaviours of cellular and cfDNA in the same deposit while avoiding concerns of contamination and profile interpretation issues which often accompany working with trace DNA samples. The utilisation of this kind of proxy means the empirical data produced by experiments will more accurately represent the variable being analysed (for instance the time variable in persistence experiments) without the external factor of uncontrolled sample variation impacting the results. In providing this proxy we hope to offer a solution which will aid in the reduction of variability in future DNA T&P experiments, as the need for this is highlighted repeatedly in the literature [1,8,13,19]. Thus, in this proof-of- concept study we present a proxy for creating trace DNA samples which we test using a small-scale

DNA transfer and recovery study. In doing so we hope to bring the benefits of this proxy solution to the attention of the wider scientific community. Helping improve future experimental designs by eliminating the variability of donor provided DNA deposits, for the widespread use in further DNA transfer, persistence, prevalence and recovery (TPPR) studies.

2. Materials and methods

2.1. Sample DNA

Rainbow trout were provided by a local fisherman. DNA was extracted from the livers using a standard phenol/chloroform extraction and stored frozen in 10 mM TrisHcl pH 8.0 (EB buffer) until required [50]. 500 μL of the extracted high molecular weight (HMW) DNA was then sonicated for 30 min to reduce the molecular weight of the DNA since cfDNA is of relatively low molecular weight or degraded [20,42,48,51] see Burrill et al. [8] for a detailed review. The size of the DNA fragments produced by sonication was assessed by electrophoresis on a 1% agarose gel. It was determined that the sonication had produced a stock of trout DNA containing fragment sizes between 400 and 600 bp in length.

MEFs were provided by Dr Simon Hawley (School of Life Sciences, University of Dundee). They were removed from tissue culture flasks by trypsinisation and washed 3 times in phosphate buffered saline (PBS) by centrifugation. Cell concentration was determined by staining with 4′,6-diamidino-2-phenylindole (DAPI) and counting nuclei in a haemocytometer. Cells were then re-suspended at a concentration of 1×10^6/mL in 20% glycerol phosphate buffered saline pH 8.0 and stored at −20 °C until required. Before use the cells were centrifuged and re-suspended in Tris-buffered saline pH 8.0 to the required concentration. All experiments were conducted with these DNA sources.

2.2. Synthetic fingerprint solution

The procedures for the creation of the synthetic eccrine solution, synthetic sebum solution and the final emulsion solution was as described by Sisco et al. [31] with some small alterations which are highlighted herein. The synthetic sweat solution and the synthetic sebum solution were prepared separately before being mixed to create the final synthetic emulsion.

Table 1
Components and the amounts used to create the eccrine portion of the synthetic fingerprint solution as seen in Sisco et al. [31]. Supplier for all chemicals was Sigma Aldrich (UK).

Class of chemical	Chemical	Amount (mg)
Inorganic Salts	Potassium Chloride	700
	Sodium Chloride	650
	Sodium Bicarbonate	125
	Ammonium Hydroxide	87.5
	Magnesium Chloride	20
Amino acids	Serine	137.5
	Glycine	67.5
	Ornithine Monohydrochloride (Ornithine)	55
	Alanine	40
	Aspartic acid	20
	Threonine	20
	Histidine	20
	Valine	15
	Leucine	15
Other	Lactic acid	950
	Urea	250
	Pyruvic acid	10
	Acetic acid	2.5
	Hexanoic acid	2.5

2.2.1. Synthetic eccrine (sweat) solution

The synthetic eccrine solution was prepared, from the components, listed in Table 1 which were weighed and placed into a 500 mL volumetric flask and dissolved in 495 mL of ultrapure (Type 1) water. The solution was then sonicated for 15 min. Following sonication, the solution was pH adjusted to 5.5 using 5 M NaOH. The solution was made up to 500 mL and filtered through a 0.22 μm filter using a vacuum filtration unit. The resulting eccrine solution was used immediately or portioned into 50 mL aliquots and stored at −20 °C for future use.

2.2.2. Synesthetic sebum solution

The synthetic sebum solution was prepared as described by Sisco et al. [31], with the omission of oleic acid. The components listed in Table 2 were weighed (solids) or pipetted (liquids) and mixed in a 10 mL clear glass vial. The mixture was then sonicated at 35 °C for 15 min. The resulting sebum solution was used immediately or stored at −20 °C for future use.

2.2.3. Synthetic emulsion

The synthetic emulsion was prepared using the freshly made eccrine and sebum solutions. This synthetic emulsion was comprised of equal parts eccrine solution to sebum solution, by weight, to create a 1:1 eccrine/sebum solution. The emulsifying agent Brij® S20 (Sigma Aldrich, UK) was added to the 1:1 solution at a concentration of 0.5% by mass to aid in the emulsification process [31]. The solution was then sonicated for 15 min to ensure a proper homogeneity throughout the solution. As indicated by Sisco et al. [31] and also observed by Steiner et al. [32] the solution was a thick white opaque paste at room temperature but after heating during the sonication process it became a transparent, colourless, highly viscous liquid. This 1:1 eccrine/sebum solution containing Brij® S20 will be referred to as the synthetic emulsion and all downstream dilutions will use this as the starting point. The preparation of the dilutions is described in detail in the results

Table 2
Components and the amounts used to create the sebum portion of the synthetic fingerprint solution as seen in Sisco et al. [31]. The volumes, for chemicals liquid at room temperature, was calculated based on their density and the amounts listed below. Supplier for all chemicals is Sigma Aldrich (UK) except for * which is Fisher Scientific (UK).

Class of chemical	Chemical	Amount (mg)	Volume (μL)
Free fatty acids	Hexanoic acid	250	270
	Heptanoic acid	250	272
	Octanoic acid	250	275
	Nonanoic acid	250	276
	Dodecanoic acid	250	
	Tridecanoic acid	250	
	Myristic acid	250	
	Pentadecanoic acid	250	
	Palmitic acid	275	
	Stearic acid	275	
	Arachidic acid	250	
	Linoleic acid	275	299
Triglycerides	Glyceryl Trioleate (Triolein)	1375	1511
	Glyceryl Trioctanoate (Tricaprylin)	100	104
	Glyceryl Tridecanoate (Tricaprin)	100	
	Glyceryl Tridodecanoate (Trilaurin)	100	
	Glyceryl Trimyristate (Trimyristin)	100	
	Glycerol Tripalmitate (Tripalmitin)	100	
Other	Squalene	600	700
	Cholesterol	150	
	Cholesterol n-Decanoate (cholesterol ester)	200	
	n-Hexadecyl Palmitate (Cetyl Palmitate)*	775	

section.

2.2.4. DNA deposit solutions

There were three different types of DNA deposits used, the first solution contained only trout cfDNA, the second solution contained only mouse cellular DNA and the third solution contained both trout cfDNA and mouse cellular DNA. These three DNA types were used at three different concentrations: 1 ng/μL, 5 ng/μL, and 10 ng/μL and were deposited in 5 μL aliquots. Thus, the total DNA deposit amounts were 5 ng, 25 ng, and 50 ng respectively (when trout and mouse DNA were present in the same sample the amount indicated is for each DNA type, not total). In addition, the samples, as described, were prepared in both EB buffer and 1:120 dilution of the synthetic emulsion (synthetic fingerprint solution) as illustrated in Fig. 1.

2.2.5. Sample deposits

In this study we tested five different experimental parameters.

1) Samples placed directly into the extraction tube (direct to tube) were used as positive controls. The direct to tube samples were created by taking 5 μL of each of the 18 solutions and pipetting them directly into separate empty 1.5 mL tubes (Sarstedt AG & Co. KG, Germany), in triplicate. The samples were immediately extracted (see 2.5 for extraction protocol) and stored at −20 °C until quantification.
2) Samples were deposited directly on to a swab (direct to swab) and used as an estimate of maximum possible extraction efficiency. The samples were pipetted directly onto a dry cotton tipped swab (Technical services consultants Ltd, UK). The swab tip was then cut directly into its own empty 1.5 mL tube and stored at −20 °C until extraction.
3) Test samples were deposited onto a glass slide (direct to glass) and dried. These samples aimed to estimate the recovery of DNA from a glass surface. Samples were deposited to the glass slide by pipetting directly onto the glass (Fisher Scientific, UK). The samples were allowed to sit at room temperature (~19 °C) until visually confirmed to have fully air dried (~30 min) and were then collected using a cotton swab.
4) Samples were deposited directly on to a volunteer's finger (direct to finger) and spread over the whole fingerprint (distal phalanx) using the tip of the pipette. Samples were allowed to air dry (~5 min) then the fingertip was swabbed for sample collection. This was designed to estimate the range of recoverable DNA from the finger surface.
5) These samples follow the finger deposits as described above in 4) however, once the sample had dried the volunteer was asked to create a fingerprint on a new glass slide (transfer from finger to glass). This fingerprint was then immediately swabbed for sample collection. These samples were used to estimate transfer efficiency.

All swabbed samples (3, 4, 5) were collected using a swab moistened with 50 μL of EB buffer, immediately cut into a 1.5 mL tube and stored at −20 °C until extraction.

Additionally, all the experiments were carried out in triplicate and the entire study was carried out twice on two different occasions. Negative control swabs were taken including blank swabs, glass slides, synthetic fingerprint solution, fingers, and extraction buffer where appropriate and all produced the expected negative result.

2.3. DNA extraction

Swabs in this experiment were extracted using our in-house extraction protocol as described by Gray et al. [52] as preliminary experiments showed this method maximised DNA recovery over the available commercial methods. Briefly, swabs were extracted using a polyvinylpyrrolidone (PVP)/tween 20 solution containing proteinase K followed by a heating step to kill proteinase K activity and then direct qPCR of the extract.

2.4. DNA quantification and data analysis

The trout and mouse DNA was quantified, in duplicate, using a StepOnePlus Real-Time PCR system (ThermoFisher Scientific, UK). Ten microliter reactions were created using 5 μL of the extracted DNA and 5 μL of Luna Universal qPCR master mix (New England Bio Labs,UK) and 0.5 μM of each primer. The trout specific primer pairs were; forward-TCAGCAATCAGATGGGGAGG and reverse-TTTCAATGATGGCCTAG TGGGT (Eurofins, Germany) producing a 110 bp amplicon. The mouse specific primer pairs were; forward- GACGAGGGGGAGCTTTACTTG and reverse- ATTGACTGTCTTGTGGACATGGG (Eurofins, Germany) producing a 231bp amplicon. Each estimate was carried out using in-house standard curves ranging from 10 ng to 0.0137 ng and a 0 ng blank. The

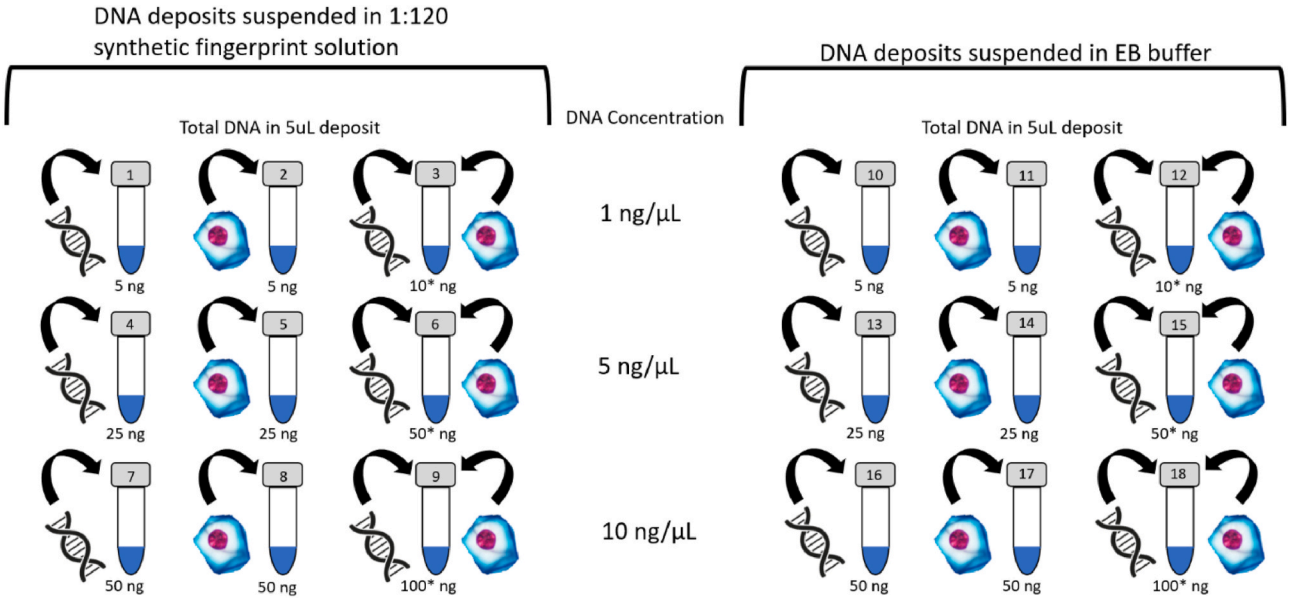

Fig. 1. Working solutions created for deposits. The * indicates that half of the total DNA was contributed by trout cfDNA and the other half was contributed by mouse cellular DNA.

cycling parameters were 95 °C for 2 min, 40 cycles of 95 °C for 30s, 65 °C for 30s, 70 °C for 30s. A melt curve was created with the parameters 95 °C for 15s, 75 °C for 1 min, 85 °C for 15s. The qPCR data was analysed using the StepOne Software (version 2.3, life technologies), Microsoft excel and SigmaPlot (version 14.5).

3. Results

3.1. Depletion series

The synthetic emulsion was highly viscous and a solid at room temperature meaning that it was impossible to dispense with a pipette (Fig. 2). In addition, the use of this mixture would not accurately reflect the amount of material one could reasonably expected to encounter on a normal finger or hand considering that hands and fingers are not normally covered in a paste of fingerprint secretions. Therefore, we aimed to create a workable dilute form of the solution that would still accurately represent donor provided trace DNA samples from fingerprints.

To determine an appropriate concentration of the synthetic emulsion to use, dilutions of the emulsion were prepared. The emulsion was heated to 56 °C to obtain a liquid, thoroughly mixed using sonication, and serially diluted in eccrine solution to the required concentrations. These dilutions were then tested in a fingerprint depletion series in comparison to natural and sebum loaded (neck/forehead rub) fingerprints. To create the natural fingerprints a volunteer was asked to wash and dry their hands before touching, using moderate force (about enough to flick a light switch), their index finger on a piece of glass consecutively five times to create a set of depletion fingerprints. To create the sebum loaded fingerprints, the volunteer was asked to follow the same procedure as the normal fingerprints however was asked to rub their index finger on their face and/or neck before touching the glass. One set of natural and two sets of sebum loaded fingerprints were created. Due to the COVID-19 restrictions in place at the time this work was completed access to only one fingerprint donor was permitted. Though this deviates from the guidelines for phase 1 studies provided by the International Fingerprint Research Group (IFRG) [53] we determined that since the donors hands were washed prior to creating samples, and the enhancement, collection and identification of fingerprints was not the aim of this research, the variation introduced by the volunteer would have a minimal effect on the results. The synthetic emulsion was diluted in the eccrine solution to create 5 test solutions: 1:20, 1:40, 1:80, 1:120, and 1:160. The volunteer was asked to wash and dry their hands after which 5 μL of the first dilute solution was spread evenly over the fingerprint and allowed to dry (~5 min). The volunteer was instructed to place their finger on the glass to create a set of 5 depletion fingerprints, as before. The volunteer then repeated the above procedure until all test solutions were deposited on the glass. The fingerprints were developed using Magneta Flake, black fingerprint powder (Crime Scene Investigations, UK) and imaged using a FujiFilm LAS-3000 luminescent image analyser (Raytek Scientific Limited, UK). The results of this experiment are shown in Fig. 3.

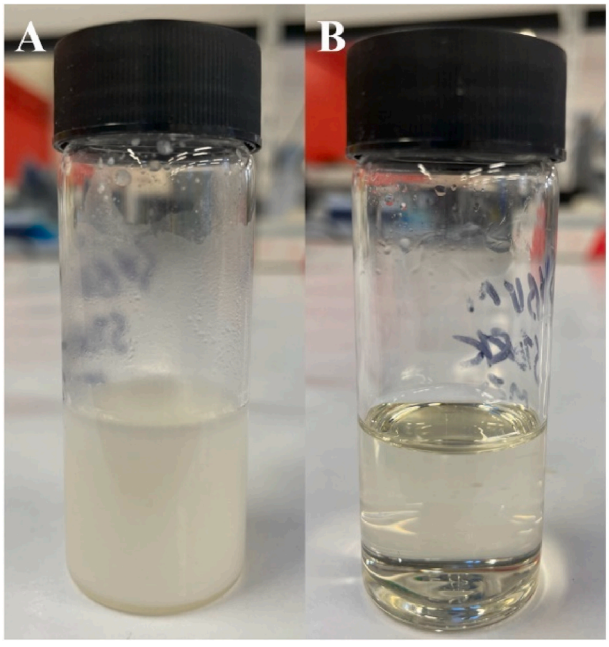

Fig. 2. The synthetic emulsion at room temperature (A) and heated to 56 °C (B).

The purpose of the depletion series was to identify a workable dilution of the synthetic emulsion for use in downstream processes, which would accurately represent a real fingerprint. The aim was to create a synthetic fingerprint ranging between the volunteer created natural and sebum loaded prints. It was determined that the 1:120 solution (Fig. 3) was the dilution that best represented this and thus would be used for all downstream processes.

The authors would like to reiterate that the sole purpose for this depletion series was to choose a dilution that was a reasonable representation of real fingerprints in which the working solution could easily be pipetted and therefore reliably used for downstream DNA deposits. The most important aspect of choosing a solution was that it could be easily manipulated and could remain consistent throughout all experiments with less emphasis put on having a solution exactly mid-way between a donor provided sebum and natural print. Since donor prints are known to be highly variable this proximation was deemed acceptable. As shown in Fig. 3 dilutions at 1:20, 1:40 and 1:80 all more closely resemble the sebum loaded fingerprints than the natural fingerprint. Additionally, at these dilutions the solution was still slightly more viscous than optimal for pipetting. This left the 1:120 and 1:160 dilutions to be considered. The 1:120 solution was selected for the DNA deposits based on the visual observation that it seemed to fall more evenly between the natural fingerprint and the sebum loaded fingerprint where the 1:160 more resembled the natural fingerprint (Fig. 4). Based on the results of the depletion series the decision was made to proceed with the synthetic emulsion at a 1:120 dilution. This 1:120 dilution will henceforth be referred to as the synthetic fingerprint solution.

The synthetic fingerprint solution used for DNA deposits was created in two steps. First, the synthetic emulsion was diluted, using the eccrine solution, 20-fold to create a 1:20 dilution. Second, the 1:20 solution was diluted, using the eccrine solution, 6-fold to create the 1:120 dilution. To create the 1:20 solution the synthetic emulsion was warmed (56 °C water bath) and sonicated for 5 min before use and was diluted 20-fold using warmed (56 °C water bath) eccrine solution. The 1:20 mixture was then sonicated for 5 min before the next dilution. The newly created 1:20 solution was warmed and diluted 6-fold using warmed eccrine solution and sonicated for a further 5 min. Working with warmed solutions made the pipetting and mixing of the solutions more manageable (Fig. 2). The 1:20 and 1:120 dilutions were created fresh for each downstream experiment.

3.2. DNA recovery

To assess whether the synthetic fingerprint solution, used to make the DNA deposits, has an impact on the recovery of trace DNA we compared the percent recovery of cfDNA and cellular DNA from the synthetic fingerprint solution and EB buffer under four different experimental parameters; direct to swab (DS), direct to glass (DG), direct to finger (DF), and transfer to glass (TG) (methods 2.3.3). For each of the of the experimental parameters (DS, DG, DF, TG) DNA was deposited in triplicate at 3 different concentrations as detailed in Fig. 1. For each sample the percentage of DNA recovered relative to the direct to tube samples (as described in 2.3.3 experimental parameter 1) was determined by duplicate qPCR reactions. The final percent recovery for each

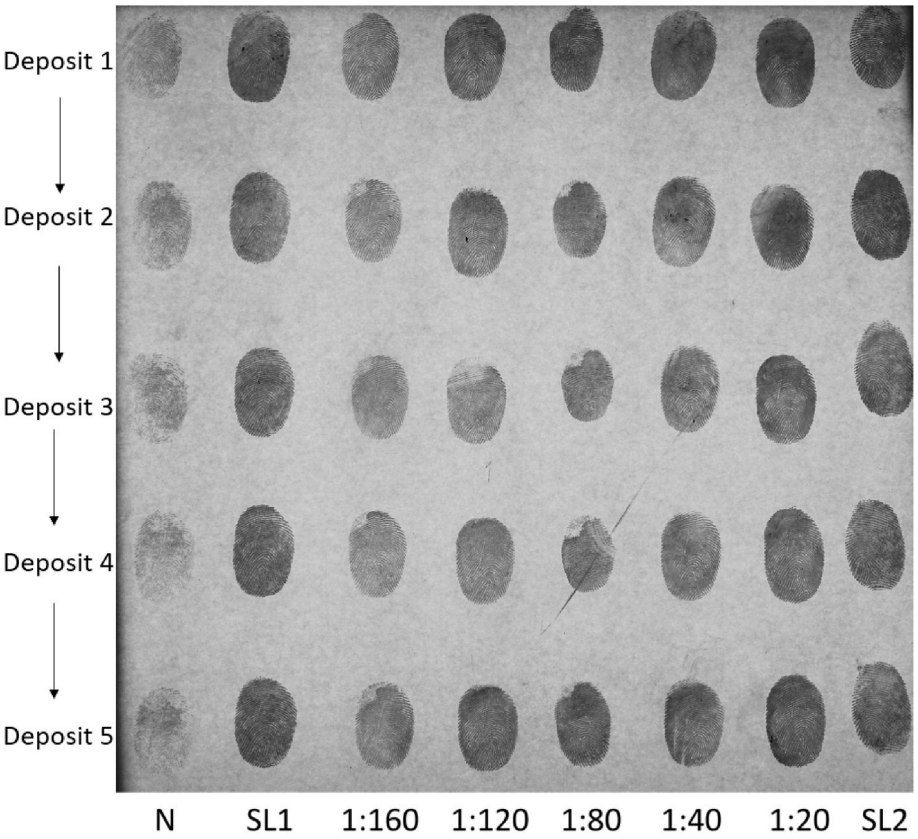

Fig. 3. Depletion series used for dilution determination. N column represents normal fingerprints. SL1 indicates one set of sebum loaded fingerprints and SL2 indicates a second set of sebum loaded fingerprints. The remaining columns represent the 5 dilute solutions of the synthetic emulsion.

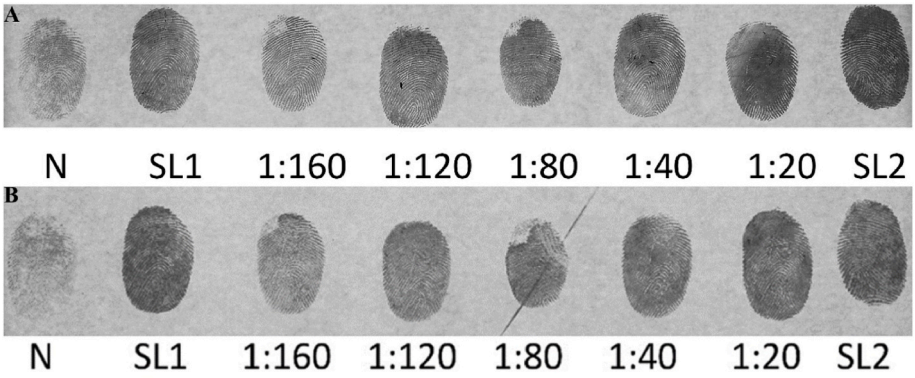

Fig. 4. Close up view from deposit rows 2 (A) and 4 (B) of Fig. 3.

experimental parameter and DNA type was calculated as the mean and standard deviation of the percentage recoveries at all concentrations. This results in a mean and standard deviation based on 18 qPCR determinations and 9 samples 3 for each DNA concentration. The data presented in Fig. 5 is the pooled data from two independent experiments and results in 36 qPCR determinations and 18 data points for each DNA type and experimental condition for samples suspended in both the synthetic fingrerprint solution and EB buffer.

Although the data set is small, we would like to highlight that the performance between the synthetic fingerprint solution and the EB buffer provides preliminary support that the synthetic fingerprint solution does not cause any unwanted adverse effects on DNA in solution when the solution is made fresh and used immediately. This is made evident as the recovery of DNA from samples where DNA is added direct to either EB or the synthetic fingerprint solution show no significant difference (see supplementary data). Considering samples added direct to swabs (Fig. 5A) there appears to be little difference between EB buffer and the synthetic fingerprint solution in most cases. In the direct to glass samples there appears to be a slight enhancement of recovery when using the synthetic sebum solution over the EB buffer in the case of cellular material (Fig. 5B). Additionally, there may be slight increase in recovery when the synthetic fingerprint solution is used to deposit DNA directly on fingers (Fig. 5C) and when it is used in the transfer from fingers to glass scenario (Fig. 5D). The small differences between the synthetic fingerprint solution and the EB buffer in the direct to glass experiment (Fig. 5B) and the slight increase in the recovery from fingers (Fig. 5C) when the synthetic fingerprint solution is used to deposit the DNA could account for the observed increase in the recovery in the transfer experiment (Fig. 5D) if the transfer is facilitated by the synthetic fingerprint solution. This has implications in the design of transfer

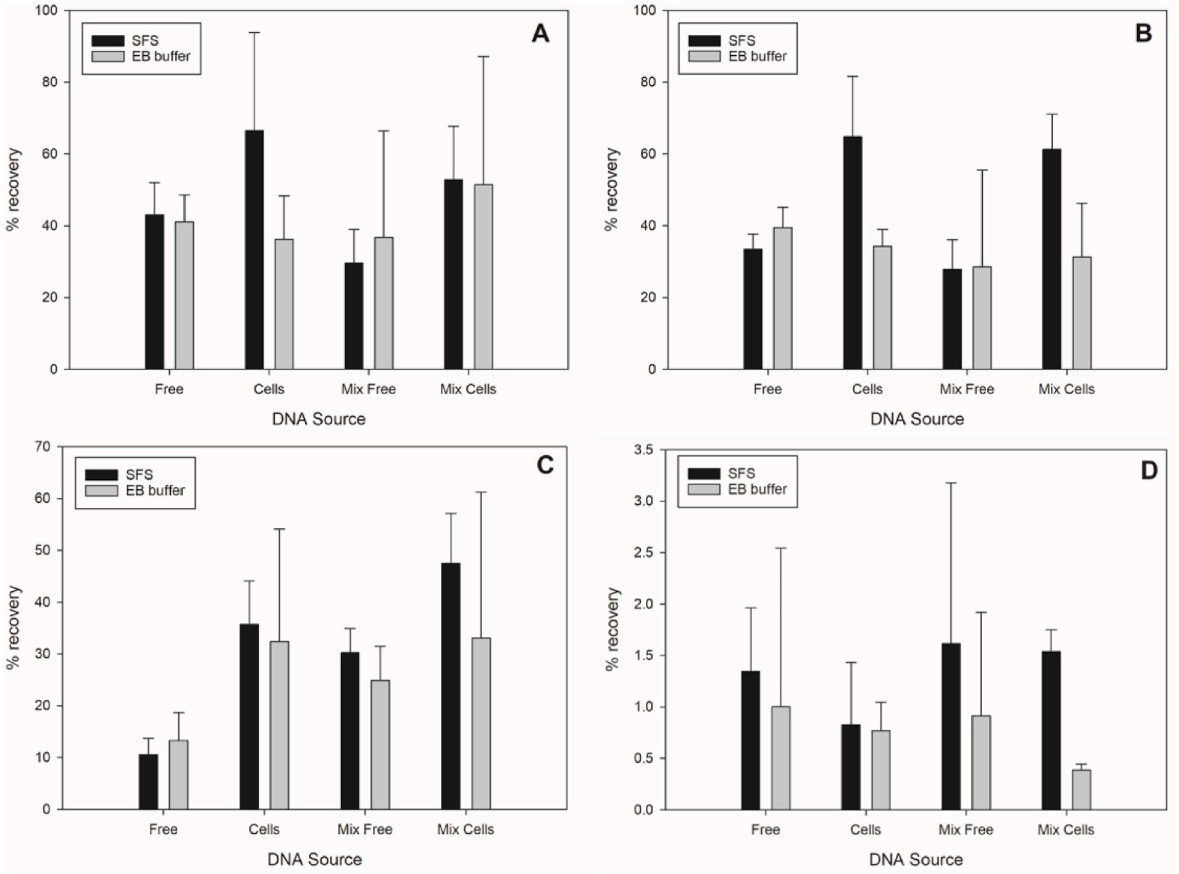

Fig. 5. Comparison of recovery of cfDNA and cellular DNA in EB buffer or synthetic fingerprint solution (SFS). The figure shows (A) direct to swab, (B) direct to glass, (C) direct to finger, (D) transfer from finger to glass. Where 'free' indicates samples containing only trout cfDNA and 'cells' indicates samples containing only mouse cellular DNA. Samples indicated as 'mix' specify the DNA analysed from a mixture of both trout cfDNA and mouse cellular DNA.

experiments using seeded DNA since, in reality, fingers and hands can normally be expected to contain fingerprint secretions.

4. Discussion

This preliminary study indicates that research in DNA T&P could benefit from the use of a synthetic fingerprint solution which is already well established in fingerprint research [31–33,35,36]. Future iterations of these experiments could consider implementing a fingerprint stamp, like the one used by Sisco et al. [31], for DNA deposition. This would add another level of control to DNA T&P experiments by eliminating possible unwanted background DNA effects unavoidable when using donor prints and/or allowing for the integration of known levels of background DNA into an experiment. Having this controlled method for investigating background DNA could lead to a better understanding of the role background DNA plays on transfer and persistence of self-DNA (DNA originating from a depositor/known contributor) and non-self DNA (DNA not originating from the depositor/known contributor). Further experiments concerning DNA T&P with the synthetic fingerprint solution will help determine the impact the solution has on the recovery of DNA. Although the DNA recovery using the solution was not substantially different than that of the samples deposited in EB buffer the data does show that there is a slight difference in terms of efficiency of transfer. This study aimed to illustrate that this proxy alternative (to water or buffer) could lead to more controlled DNA T&P experiments whilst not needing to compromise on the realism of the samples. However, regardless of the performance on DNA recovery between the two DNA solutions, the synthetic fingerprint solution is more representative of real-life circumstances where the EB buffer is not. We advocate that the synthetic fingerprint solution should be used regardless of its performance against the EB solution for experiments investigating DNA T&P. Using the solution that provides the best recovery to simply maximise recovery in an experiment is not appropriate, for example, if cells transfer poorly in the presence of fingerprint secretions that is exactly why experiments need to be using a synthetic fingerprint solution for transfer experiments as it will avoid over estimations of recovery efficiency from real scenarios. The alternative is also true, for example, if cells transfer well in fingerprint secretions and a synthetic fingerprint solution is not being used it could lead to underestimating the ability to recover cells in a crime scene setting. Thus, using the synthetic fingerprint solution allows more realistic transfer and recovery rates (either good or bad) which will allow experiments to provide data that could reasonably reflect real case circumstances.

In our experiments we elected to use non-human DNA which allowed us to avoid contamination issues and profile interpretation issues which are often encountered when dealing with human trace DNA deposits. This model provided us with a simplified method to do a preliminary investigation of the proxy deposits using cell free and cellular DNA in the context of DNA T&P while avoiding the increased complications using human DNA would have caused at this stage. However, this model could easily be adapted for use with human DNA. If using human DNA, it would be essential to ensure that the cellular and cfDNA have very distinct STR profiles as it is essential for the two biological materials to be easily distinguished from each other. Cellular DNA can be obtained using a cell culture method as proposed by Feine et al. [10] and cell free control DNA could be purchased from a supplier. This approach would ensure the biological material contained in the samples are an accurate representation of exclusively cellular and cell free samples. Once the

experiments using the synthetic fingerprint material with added human DNA are completed, creating a baseline, experiments with donor provided prints can then use this baseline to inform the design of more complex transfer and persistence experiments involving donor provided deposits.

Future work into the use of a proxy synthetic fingerprint solution for DNA T&P studies will focus on testing different surface types (porous and nonporous) and different concentrations of DNA/biological materials in solution. Additionally, tests using more complex transfer scenarios and an investigation into persistence will also be carried out. Most importantly for forensic science, these experiments (once a baseline is established using the proxy DNA) need to be transitioned into implementing the use of human DNA and analysed using STR profiling. This will increase the knowledge of how fingerprint secretions impact the transfer, persistence and recovery of biological material found on hands and will produce more empirical data that can be utilised in Bayesian networks when addressing activity level propositions [13,54].

5. Conclusions

The data in this paper shows support for the use of a synthetic fingerprint solution for DNA transfer, persistence and recovery studies as it is a reasonable alternative to volunteer-based deposits. Using these types of synthetic deposits also has many advantages over donor provided samples such as: eliminating contamination issues, allowing for a known amount of starting material and providing a diverse, reproducible, and standardisable starting point. This allows researchers to have complete control over the deposit material, while keeping the deposits closer to a realistic representation of true crime scene samples than has been presented in past research. These benefits will help decrease the inherent variability commonly encountered in DNA transfer and persistence experiments and allow for easier, more accurate, analysis of the experimental variable. It is our intention to use this proxy in a large-scale persistence experiment in hopes to add case realistic data to the existing pool of empirical data, and thus lead to a better understanding of the transfer and persistence of DNA from handled items.

CRediT authorship contribution statement

Hilary Arsenault: Conceptualization, Methodology, Validation, Investigation, Formal analysis, Visualization, Writing - original draft, Writing - review & editing. **Niamh Nic Daeid:** Funding acquisition, Writing - review & editing. **Alexander Gray:** Conceptualization, Methodology, Validation, Investigation, Formal analysis, Resources, Visualization, Supervision, Project administration, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work has been funded by Leverhulme Trust grant. RC-1015-01. The authors would also like to acknowledge the kind contribution of tissue culture cells from Dr Simon Hawley, University of Dundee School of Life Sciences and the donation of rainbow trout from fisherman Dr Grant Thomson.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsisyn.2023.100330.

References

[1] A. Gosch, C. Courts, On DNA transfer: the lack and difficulty of systematic research and how to do it better, Forensic Sci. Int.: Genetics 40 (2019) 24–36, https://doi.org/10.1016/j.fsigen.2019.01.012.
[2] G. Meakin, A. Jamieson, DNA transfer: review and implications for casework, Forensic Sci. Int.: Genetics 7 (2013) 434–443, https://doi.org/10.1016/j.fsigen.2013.03.013.
[3] M. Phipps, S. Petricevic, The tendency of individuals to transfer DNA to handled items, Forensic Sci. Int. 168 (2007) 162–168, https://doi.org/10.1016/j.forsciint.2006.07.010.
[4] I. Quinones, B. Daniel, Cell free DNA as a component of forensic evidence recovered from touched surfaces, Forensic Sci. Int.: Genetics 6 (2012) 26–30, https://doi.org/10.1016/j.fsigen.2011.01.004.
[5] R.A.H. van Oorschot, M.K. Jones, DNA fingerprints from fingerprints, Nature 387 (1997) 767, https://doi.org/10.1038/42838, 767.
[6] A. Lowe, C. Murray, J. Whitaker, G. Tully, P. Gill, The propensity of individuals to deposit DNA and secondary transfer of low level DNA from individuals to inert surfaces, Forensic Sci. Int. 129 (2002) 25–34, https://doi.org/10.1016/S0379-0738(02)00207-4.
[7] D.S. LeSassier, K.Q. Schulte, T.E. Manley, A.R. Smith, M.L. Powals, N.C. Albright, et al., Artificial fingerprints for cross-comparison of forensic DNA and protein recovery methods, PLoS One 14 (2019), e0223170, https://doi.org/10.1371/journal.pone.0223170.
[8] J. Burrill, B. Daniel, N. Frascione, A review of trace "Touch DNA" deposits: variability factors and an exploration of cellular composition, Forensic Sci. Int.: Genetics 39 (2019) 8–18, https://doi.org/10.1016/j.fsigen.2018.11.019.
[9] J.J. Raymond, R.A.H. van Oorschot, P.R. Gunn, S.J. Walsh, C. Roux, Trace evidence characteristics of DNA: a preliminary investigation of the persistence of DNA at crime scenes, Forensic Sci. Int.: Genetics 4 (2009) 26–33, https://doi.org/10.1016/j.fsigen.2009.04.002.
[10] I. Feine, M. Shpitzen, J. Roth, R. Gafny, A novel cell culture model as a tool for forensic biology experiments and validations, Forensic Sci. Int.: Genetics 24 (2016) 114–119, https://doi.org/10.1016/j.fsigen.2016.06.010.
[11] R.A.H. van Oorschot, G.E. Meakin, B. Kokshoorn, M. Goray, B. Szkuta, DNA transfer in forensic science: recent progress towards meeting challenges, Genes 12 (2021) 1766, https://doi.org/10.3390/genes12111766.
[12] R.A.H. van Oorschot, M. Goray, E. Eken, R.J. Mitchell, Impact of relevant variables on the transfer of biological substances, Forensic Sci. Int.: Genetics Supplement Series 2 (2009) 547–548, https://doi.org/10.1016/j.fsigss.2009.08.105.
[13] D. Taylor, A. Biedermann, L. Samie, K.-M. Pun, T. Hicks, C. Champod, Helping to distinguish primary from secondary transfer events for trace DNA, Forensic Sci. Int.: Genetics 28 (2017) 155–177, https://doi.org/10.1016/j.fsigen.2017.02.008.
[14] GE Meakin, B Kokshoorn, R.A.H. van Oorschot, B. Szkuta, Evaluating forensic DNA evidence: Connecting the dots, WIREs Forensic Science e1404 n.d.;n/a https://doi.org/10.1002/wfs2.1404.
[15] B. Kokshoorn, L.H.J. Aarts, R. Ansell, E. Connolly, W. Drotz, A.D. Kloosterman, et al., Sharing data on DNA transfer, persistence, prevalence and recovery: arguments for harmonization and standardization, Forensic Sci. Int.: Genetics 37 (2018) 260–269, https://doi.org/10.1016/j.fsigen.2018.09.006.
[16] D. Taylor, B. Kokshoorn, A. Biedermann, Evaluation of forensic genetics findings given activity level propositions: a review, Forensic Sci. Int.: Genetics 36 (2018) 34–49, https://doi.org/10.1016/j.fsigen.2018.06.001.
[17] J. Tang, J. Ostrander, R. Wickenheiser, A. Hall, Touch DNA in forensic science: the use of laboratory-created eccrine fingerprints to quantify DNA loss, Forensic Sci. Int.: Synergy 2 (2020) 1–16, https://doi.org/10.1016/j.fsisyn.2019.10.004.
[18] K. Atkinson, H. Arsenault, C. Taylor, L. Volgin, J. Millman, Transfer and persistence of DNA on items routinely encountered in forensic casework following habitual and short-duration one-time use, Forensic Sci. Int.: Genetics 60 (2022), 102737, https://doi.org/10.1016/j.fsigen.2022.102737.
[19] R.A.H. van Oorschot, B. Szkuta, G.E. Meakin, B. Kokshoorn, M. Goray, DNA transfer in forensic science: a review, Forensic Sci. Int.: Genetics 38 (2019) 140–166, https://doi.org/10.1016/j.fsigen.2018.10.014.
[20] E.K. Hanson, J. Ballantyne, "Getting blood from a stone": ultrasensitive forensic DNA profiling of microscopic bio-particles recovered from "touch DNA" evidence, in: D.M. Kolpashchikov, Y.V. Gerasimova (Eds.), Nucleic Acid Detection: Methods and Protocols, Humana Press, Totowa, NJ, 2013, pp. 3–17, https://doi.org/10.1007/978-1-62703-535-4_1.
[21] S. Jones, K. Scott, J. Lewis, G. Davidson, J.E. Allard, C. Lowrie, et al., DNA transfer through nonintimate social contact, Sci. Justice 56 (2016) 90–95, https://doi.org/10.1016/j.scijus.2015.10.004.
[22] E. Prasad, C. Hitchcock, J. Raymond, A. Cole, M. Barash, D. McNevin, et al., Touch DNA recovery from unfired and fired cartridges: comparison of swabbing, tape lifting and soaking, Forensic Sci. Int. 330 (2022), 111101, https://doi.org/10.1016/j.forsciint.2021.111101.
[23] M. Goray, R.A.H. van Oorschot, The complexities of DNA transfer during a social setting, Leg. Med. 17 (2015) 82–91, https://doi.org/10.1016/j.legalmed.2014.10.003.
[24] D.J. Daly, C. Murphy, S.D. McDermott, The transfer of touch DNA from hands to glass, fabric and wood, Forensic Sci. Int.: Genetics 6 (2012) 41–46, https://doi.org/10.1016/j.fsigen.2010.12.016.
[25] R.A.H. van Oorschot, D.G. Phelan, S. Furlong, G.M. Scarfo, N.L. Holding, M. J. Cummins, Are you collecting all the available DNA from touched objects? Int. Congr. 1239 (2003) 803–807, https://doi.org/10.1016/S0531-5131(02)00498-3.

[26] L.Y.C. Lee, H.Y. Wong, J.Y. Lee, Z.B.M. Waffa, Z.Q. Aw, S.N.A.B.M. Fauzi, et al., Persistence of DNA in the Singapore context, Int. J. Leg. Med. 133 (2019) 1341–1349, https://doi.org/10.1007/s00414-019-02077-2.
[27] J.J. Raymond, S.J. Walsh, R.A.H. van Oorschot, P.R. Gunn, L. Evans, C. Roux, Assessing trace DNA evidence from a residential burglary: abundance, transfer and persistence, Forensic Sci. Int.: Genetics Supplement Series 1 (2008) 442–443, https://doi.org/10.1016/j.fsigss.2007.10.040.
[28] M. Goray, E. Eken, R.J. Mitchell, R.A.H. van Oorschot, Secondary DNA transfer of biological substances under varying test conditions, Forensic Sci. Int.: Genetics 4 (2010) 62–67, https://doi.org/10.1016/j.fsigen.2009.05.001.
[29] A. Girod, R. Ramotowski, C. Weyermann, Composition of fingermark residue: a qualitative and quantitative review, Forensic Sci. Int. 223 (2012) 10–24, https://doi.org/10.1016/j.forsciint.2012.05.018.
[30] R. Steiner, C. Roux, S. Moret, Controlling fingermark variability for research purposes: a review, WIREs Forensic Science 1 (2019) e1338, https://doi.org/10.1002/wfs2.1338.
[31] E. Sisco, J. Staymates, K. Schilling, A chemically relevant artificial fingerprint material for the cross-comparison of mass spectrometry techniques, J. Can. Soc. Forensic. Sci. 48 (2015) 200–214, https://doi.org/10.1080/00085030.2015.1083167.
[32] R. Steiner, C. Roux, S. Moret, Production of artificial fingermarks. Part I – synthetic secretions formulation, Forensic Sci. Int. 331 (2022), 111166, https://doi.org/10.1016/j.forsciint.2021.111166.
[33] A. Jeanneret, A. Anthonioz, A. Bécue, Printed artificial sweat as replacement for natural fingermarks: qualitative and quantitative approach considering an amino acid reagent, Sci. Justice 61 (2021) 249–259, https://doi.org/10.1016/j.scijus.2021.01.004.
[34] L. Schwarz, An amino acid model for latent fingerprints on porous surfaces, J. Forensic Sci. 54 (2009) 1323–1326, https://doi.org/10.1111/j.1556-4029.2009.01168.x.
[35] M. de la Hunty, An Investigation of Latent Fingermark Residues and Their Development on Porous Substrates Using Physical Developer and Nile Red, PhD thesis. University of Technology Sydney, 2017.
[36] S. Hong, I. Hong, A. Han, J.Y. Seo, J. Namgung, A new method of artificial latent fingerprint creation using artificial sweat and inkjet printer, Forensic Sci. Int. 257 (2015) 403–408, https://doi.org/10.1016/j.forsciint.2015.10.005.
[37] S. Zoppis, B. Muciaccia, A. D'Alessio, E. Ziparo, C. Vecchiotti, A. Filippini, DNA fingerprinting secondary transfer from different skin areas: morphological and genetic studies, Forensic Sci. Int.: Genetics 11 (2014) 137–143, https://doi.org/10.1016/j.fsigen.2014.03.005.
[38] M. van den Berge, G. Ozcanhan, S. Zijlstra, A. Lindenbergh, T. Sijen, Prevalence of human cell material: DNA and RNA profiling of public and private objects and after activity scenarios, Forensic Sci. Int.: Genetics 21 (2016) 81–89, https://doi.org/10.1016/j.fsigen.2015.12.012.
[39] P. Kanokwongnuwut, B. Martin, K.P. Kirkbride, A. Linacre, Shedding light on shedders, Forensic Sci. Int.: Genetics 36 (2018) 20–25, https://doi.org/10.1016/j.fsigen.2018.06.004.
[40] M. Djuric, T. Varljen, A. Stanojevic, O. Stojkovic, DNA typing from handled items, Forensic Sci. Int.: Genetics Supplement Series 1 (2008) 411–412, https://doi.org/10.1016/j.fsigss.2007.10.161.
[41] J. Burrill, B. Daniel, N. Frascione, Illuminating touch deposits through cellular characterization of hand rinses and body fluids with nucleic acid fluorescence, Forensic Sci. Int.: Genetics 46 (2020), 102269, https://doi.org/10.1016/j.fsigen.2020.102269.
[42] J. Burrill, E. Rammenou, F. Alawar, B. Daniel, N. Frascione, Corneocyte lysis and fragmented DNA considerations for the cellular component of forensic touch DNA, Forensic Sci. Int.: Genetics 51 (2021), 102428, https://doi.org/10.1016/j.fsigen.2020.102428.
[43] M. Goray, R.J. Mitchell, R.A.H. van Oorschot, Investigation of secondary DNA transfer of skin cells under controlled test conditions, Leg. Med. 12 (2010) 117–120, https://doi.org/10.1016/j.legalmed.2010.01.003.
[44] J. Burrill, A. Kombara, B. Daniel, N. Frascione, Exploration of cell-free DNA (cfDNA) recovery for touch deposits, Forensic Sci. Int.: Genetics 51 (2021), 102431, https://doi.org/10.1016/j.fsigen.2020.102431.
[45] M. Vandewoestyne, D. Van Hoofstat, A. Franssen, F. Van Nieuwerburgh, D. Deforce, Presence and potential of cell free DNA in different types of forensic samples, Forensic Sci. Int.: Genetics 7 (2013) 316–320, https://doi.org/10.1016/j.fsigen.2012.12.005.
[46] C.E. Stanciu, M.K. Philpott, Y.J. Kwon, E.E. Bustamante, C.J. Ehrhardt, Optical characterization of epidermal cells and their relationship to DNA recovery from touch samples 4 (2015), https://doi.org/10.12688/f1000research.7385.1. F1000Res.
[47] M. Miller, M.K. Philpott, A. Olsen, M. Tootham, V.K. Yadavalli, C.J. Ehrhardt, Technical note: survey of extracellular and cell-pellet-associated DNA from 'touch'/trace samples, Forensic Sci. Int. 318 (2021), 110557, https://doi.org/10.1016/j.forsciint.2020.110557.
[48] T. Kita, H. Yamaguchi, M. Yokoyama, T. Tanaka, N. Tanaka, Morphological study of fragmented DNA on touched objects, Forensic Sci. Int.: Genetics 3 (2008) 32–36, https://doi.org/10.1016/j.fsigen.2008.09.002.
[49] E. Hanson, C. Haas, R. Jucker, J. Ballantyne, Specific and sensitive mRNA biomarkers for the identification of skin in 'touch DNA' evidence, Forensic Sci. Int.: Genetics 6 (2012) 548–558, https://doi.org/10.1016/j.fsigen.2012.01.004.
[50] J. Sambrook, D.W. Russell, Cold spring harbor, in: vol. 1. third ed.Molecular Cloning : a Laboratory Manual, Cold Spring Harbor Laboratory Press, N.Y, 2001.
[51] F. Oldoni, V. Castella, D. Hall, Shedding light on the relative DNA contribution of two persons handling the same object, Forensic Sci. Int.: Genetics 24 (2016) 148–157, https://doi.org/10.1016/j.fsigen.2016.07.002.
[52] A. Gray, A. Kuffel, N.N. Daeid, An improved rapid method for DNA recovery from cotton swabs, Forensic Sci. Int.: Genetics 64 (2023), 102848, https://doi.org/10.1016/j.fsigen.2023.102848.
[53] International Fingerprint Research Group (IFRG), Guidelines for the assessment of fingermark detection techniques, J. Forensic Ident. 64 (2014) 174–200.
[54] B. Szkuta, K.N. Ballantyne, B. Kokshoorn, R.A.H. van Oorschot, Transfer and persistence of non-self DNA on hands over time: using empirical data to evaluate DNA evidence given activity level propositions, Forensic Sci. Int.: Genetics 33 (2018) 84–97, https://doi.org/10.1016/j.fsigen.2017.11.017.