Accumulation of endogenous and exogenous nucleic acids in “Touch DNA” components on hands

Successful forensic DNA profiling from handled items is increasingly routine in casework. This “touch DNA” is thought to contain both cellular and acellular nucleic acid sources. However, there is little clarity on the origins or characteristics of this material. The cellular component consists of anucleate, terminally differentiated corneocytes (assumed to lack DNA), and the occasional nucleated cell. The acellular DNA source is fragmentary, presumably cell breakdown products. This study examines the relative contributions each component makes to the hand-secretions (endogenous) and hand-accumulations (exogenous) by recovering rinses from the inside and outside of worn gloves. Additionally, cellular and acellular DNA was measured at timepoints up to 2 h after hand washing, both with and without interim contact. Microscopic examination confirmed cell morphology and presence of nucleic acids. Following the novel application of a hair keratinocyte lysis method and plasma-DNA fragment purification to hand rinse samples, DNA profiles were generated from both fractions. Exogenous cell-free DNA is shown to be a significant source of touch DNA, which reaccumulates quickly, although its amplifiable nuclear alleles are limited. Endogenous DNA is mostly cellular in origin and provides more allelic information consistently over time.

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Additional supporting information may be found online in the Supporting Information section at the end of the article.

1 Introduction

It has been assumed that DNA deposited by handling, known as “touch DNA,” comes from hands shedding epithelial cells [1–3]. However, there is limited direct research into touch deposit constituents [4,5]. Touch DNA is widely used in forensic casework and observed to be of low quantity and degraded quality [6–8]. It is unclear whether this is due to environmental exposure or to the intrinsic nature of the DNA upon deposition. Increasing DNA profiling sensitivity has made touch DNA evidence more routine [9–11]. Understanding the evidential value of these samples depends on parsing the touch deposits’ components and their DNA origins.

It has been proposed that a combination of nucleated cells (secreted through pores), anucleate corneocytes (from the outermost skin layers), and possibly fragmented cell debris comprise touch deposits [12–14]. DNA profiles from bioparticles or skin flakes from clothing or tape-lifts have been demonstrated, although the aim is to simplify DNA mixtures rather than characterize cell content [4,15–18]. The vast majority of cells from touched objects appear microscopically to be corneocytes with few nucleated epithelia or free nuclei [19,20]. This content is also observed in deposited fingerprints [3,21], which have been shown to yield limited DNA even when recovered soon after deposition [22]. Cell counts do not correlate with quantified DNA recovery [14,23]. However, dead skin and corneocytes stain positively for nucleic acids [24–26], and some direct amplification results have indicated a correlation [3]. The shed corneocytes from handled objects may be sufficiently distinct to permit separation from other cell types or even by donor, using morphology and autofluorescence [27,28]. When hand
rinses were separated using flow cytometry, corneocyte cells indicated much higher levels of nucleic acid fluorescence than the subcellular fragment population [29]. Some studies report negligible DNA from these shed cells, while some yield useable DNA levels or full profiles, leaving ambiguity and variability [14,30,31].

Touch deposit research frequently overlooks the acellular fraction. However, cell-free (cfDNA) has been reported in body fluids, such as saliva [32], plasma [33–35], sweat [5], and hand rinses [38], and has been recovered from touched objects yielding useful STR alleles [14,39]. It has recently been more widely discussed as a majority contributor to touch DNA [40]. Profiles from cfDNA are often partial, which may result from degradation acquired by postdeposition exposure or may reflect its degraded in vivo condition [5,38,39]. It is unclear whether cfDNA is acquired from the environment or secreted.

Touch DNA research generally uses standard forensic lysis and recovery methods to directly reflect casework [41–43]. However, the cornified envelopes of terminally differentiated corneocytes contain outer proteins highly crosslinked by transglutaminases and coated in a lipid envelope of long-chain ω-hydroxyceramides, forming an insoluble protective barrier [44]. This structure and process is similar to terminal keratinization in hair shafts. Lysis buffers with reductants, such as DTT or β-mercaptoethanol, and longer incubations are used for hair shaft DNA extraction [45–48]. Similar lysis methods have been reported as beneficial for improving corneocyte DNA recovery from hand rinses [30]; both cfDNA and corneocyte DNA are likely highly fragmented, and thus, genomic DNA purification methods may limit their recovery [38] (see Supporting Information). Elevated DNA levels from hair keratinocytes and hand rinse cfDNA have been reported using fragment-targeting recovery methods [49,50]. Consequently, enhanced lysis and fragment-targeting DNA purification beads are used in these studies.

Although both corneocyte DNA and cfDNA may be important constituents of touch deposits, little is known about how they accumulate on people’s hands before being deposited. Transfer studies demonstrate that foreign, or nonself, DNA profiles are acquired through various physical interactions of people and objects [2,43,51–54]. Whether those profiles originate in cellular or acellular sources, and whether that impacts their ability to transfer cells or DNA is still unknown. Some studies have demonstrated that longer time periods or more interim interactions impact the reaccumulation of DNA on washed hands, and fingerprints have been shown to readily deposit detectable STR profiles within minutes of washing in individuals deemed “heavy shedders” [52–55]. However, the accumulation mechanism has not been closely investigated. Recent work has suggested anucleate cellular material correlating with profiling success is more often located near sweat pores when fingerprint ridge detail was examined, although cfDNA was not evaluated in that study [55]. Research collecting touch DNA following established activity often fails to consider resolving how much of one’s own DNA is accumulated through endogenous secretion versus exogenous handling. To better answer these fundamental questions, material from donors’ hands must be parsed appropriately into constituent parts and examined. We have isolated material accumulated from the hands themselves, versus external contact; we further separate cells from cfDNA and assess accumulation at timepoints after washing. This knowledge has implications for our fundamental understanding of transfer DNA. It may inform best practices for collection and recovery as well as aid DNA profile evaluation from touched samples.

2 Materials and methods

Volunteers were recruited as approved by King’s College London Biomedical and Health Sciences, Dentistry, Medicine and Natural and Mathematical Sciences (BDM) Research Ethics Subcommittee (HR-17/18-5500).

An overall schematic of the experimental design is provided in Figure 1.

2.1 Gloved hands collection

Ten volunteer donors (five females, five males) were asked to wash and air-dry their hands without interim contact as described previously [30]. UV-sterilized nitrile gloves were placed onto the donor’s hands and worn for 4 h outside the laboratory while conducting normal activities.

Upon return, exogenous rinses were collected as 8 mL autoclaved and UV-sterilized distilled water (SDW) was added to hands in 2 mL increments over a UV-sterilized petri dish as the donor lightly rubbed their hands together. Endogenous rinses were collected similarly from the hand surfaces and run over the inside-glove surfaces to make a combined 8 mL rinse. A 50 μL aliquot from the “outside” and “inside” collections was taken for microscopy; 10 μL was mixed with 0.4% trypan blue solution and cells in five nonadjacent squares of a hemocytometer were counted, allowing an extrapolated calculation of cells per milliliters.

A volume of 6 mL for each rinse was separated into 6 × 1 mL aliquots in 1.5 mL Eppendorf tubes. Three tubes were designated the “overall rinse” fraction (OV). The remaining three tubes were centrifuged (30 min, 8000 × g), to separate cells in the pellet (PL) from the cell-free supernatants (CF). All CF samples were stored at 4°C overnight and processed as described in section 2.4.

All OV samples were vacuum concentrated to 500 μL using the Savant™ Universal SpeedVac™ Plus system. PL samples were resuspended in 500 μL SDW. All PL and OV samples were combined with 500 μL keratinocyte lysis buffer (described previously [30,45]) and incubated at 56°C overnight. Samples were then centrifuged (5 min, 12 000 × g) and supernatants processed as described in section 2.4.

Reference buccal swabs from each donor were extracted using Chelex® 100 beads (Sigma-Aldrich) following the manufacturer’s protocol.
2.2 Accumulation timepoints collection

Ten volunteer donors (six females and four males) were asked to wash their hands as above. They then conducted one of the following “accumulation protocols” before returning for sample collection:

- 15 min/1 h/2 h of regular activity outside of the laboratory
- No activity prior to immediate sample collection
- 15 min/1 h/2 h seated in the laboratory without handling or touching any surface

Each donor followed all seven protocols once over the course of three appointments on different days.

After each accumulation protocol, hand rinse samples were collected as described above. Then, 6 mL run-off was separated into $6 \times 1$ mL samples after a 30 μL aliquot was taken for microscopy. These 1 mL samples were designated,
separated, and lysed as described above to make OV, PL, and CF fractions for each rinse.

Donor reference buccal swabs were collected and processed with Chelex® as above.

2.3 Microscopic examination for glove and timepoint samples

A sum of 5 μL of each collected rinse was spotted in triplicate onto a poly-

l-lysine coated slide and dried on a 56°C heat block. Each spot was covered with 5 μL Diamond™ Nucleic Acid Dye (DD) (Promega, 1:1000 in ethanol) and air dried. Samples were visualized with a Dino-Lite Edge Digital Microscope (Absolute Data Services Ltd.) at magnifications ranging from 50 to 200×, and images obtained with DinoCapture 2.0 software.

2.4 DNA recovery and processing of glove and timepoint samples

Each supernatant was purified using the cfPure® Cell Free DNA Extraction Kit (BioChain) following the manufacturer’s “Small volumes” protocol (25 μL beads/1 mL sample). Purified DNA from the same rinse sample was pooled following a 35 μL elution. DNA content was measured with Qubit dsDNA HS Assay and the Qubit 2.0 Fluorometer (In-vitrogen) per manufacturer’s protocol. DNA amplification of all rinse samples and references was performed using GlobalFiler™ PCR Amplification kit (ThermoFisher) on a ProFlex PCR thermocycler (Applied Biosystems). Reaction volumes of 12.5 μL for rinse samples and 7 μL for references were used. Target inputs were 2 and 1 ng, respectively because the former was expected to be degraded and overestimated by fluorescent quantification. CE was performed on a 3130xl Genetic Analyzer. Results were interpreted using GeneMapper® ID-X software. Extracts were stored at −20°C and subsequently measured with QuantiFiler Trio (ThermoFisher) per manufacturer’s protocol. Reaction volume was reduced to 10 μL per validated in-house casework protocol.

3 Results and discussion

3.1 Rinses from gloved hands

3.1.1 Cellular content

Aliquots from outside and inside glove rinses were stained with trypan blue for cell counting and with DD for microscopic examination. All inside samples had extremely high cell counts (66 2500-10 26 8700 cells/mL), while outside samples were less dense (0-18 700 cells/mL). Outside rinses were irregular in composition, featuring dust, fibers, and other noncellular particulates (Figure 2B).

Cells observed in both inside and outside rinses were almost exclusively corneocytes (Figure 2A), distinguishable from nucleated epithelia (Figure 2C) by their smaller size, lack of nucleus, and less intense fluorescence. Across triplicate aliquots from 10 donors, only five nucleated cells were noted, four in separate donor’s outside samples and one in an inside sample, suggesting that nucleated epithelia are not a major cellular constituent of touch deposits.

3.1.2 Relative DNA levels

In contrast to cell density, OV DNA levels from the outside (mean 49.3 ± 32.4 ng) were significantly higher than inside (11.0 ± 6.0 ng), indicated by a Student’s paired t-test, (α = 0.05, p = 0.006). This pattern was observed in PL and CF fractions (Figure 3A), although was only significant for CF fractions (p = 0.001 for CF, p = 0.1 for PL). These data suggest regular activity accumulates significantly more DNA exogenously than endogenously and that cfDNA on hands does not primarily originate from the hands themselves. That PL DNA content is consistent from the outside to the inside despite divergent cell densities suggest that the rare cells which are acquired exogenously (despite being observed rarely in our limited aliquots for microscopy) are likely nucleated epithelia providing high DNA content. Alternatively, it is possible that exogenous corneocytes have been exposed to the environment for longer, thus, are physically compromised, and so are better able to yield their DNA than freshly shed endogenous ones, even with enhanced lysis.

Figure 2. (A) DD-stained rinse from inside gloves. (B) DD-stained rinse from outside gloves. (C) DD-stained buccal cells for contrast.
Figure 3. (A) Bar chart representing mean DNA recovered in each fraction (left axis, blue), and scatter plot of corresponding mean profile completeness (right axis, red). Error bars indicate 1 SD. N = 10 donors. (B) CF and PL DNA levels shown as mean normalized percentages of OV DNA. (C) CF and PL allele counts shown as mean normalized percentages of OV alleles.

No linear correlation was observed between cell count and DNA level within any of the fractions either from the inside or outside ($R^2 < 0.4$, data not shown), which is consistent with previously published reports [14,23,30]. Rare, nucleated cells providing DNA levels disproportionate to their counts may obscure a linear relationship, but this likely reflects the ranging levels of DNA present in corneocytes at various stages of terminal differentiation.

Interestingly, the relationship of PL and CF DNA levels to each other differed between the inside and outside samples (Figure 3B). PL and CF DNA values were normalized as a percentage of OV DNA level for each donor. The PL comprised 36.2% of the outside OV DNA, while PL accounted for 76.2% of the inside OV DNA (mean normalized percent). Endogenous OV DNA consists of significantly higher proportion of cellular (PL) than does exogenous OV DNA ($p = 0.001$), whereas CF makes up a significantly larger percent of the exogenous OV DNA ($p = 0.04$). While this trend is clearly observable across donors, there are individual exceptions, that is, one donor had more CF than PL DNA from the inside sample, and two donors had more PL DNA than CF from the outside. Interindividual variability in relative cellular and cfDNA deposition patterns is still an important factor to consider.

### 3.1.3 DNA profile quality

All profiles displayed a “ski-slope” pattern typical of degraded template, with the larger STRs less successfully amplified than the shorter ones. A strong linear correlation was observed ($R = 0.85$) between amplification success (percent of samples with alleles detected above 200 relative fluorescence units [RFU]) and amplicon size, ranging from 42 to 72% success at the longest (TPOX) and shortest (D2S441) markers, respectively. The method was designed to eliminate environmental exposure of endogenous DNA, so the degradation observed is therefore likely to result from DNA fragmentation intrinsic to endogenous corneocytes and cfDNA from cellular breakdown.

Profile completeness (PC) was calculated for each sample (percent of the 40 expected GlobalFiler donor alleles observed). When outside profiles were multidonor DNA mixtures, PC was calculated in reference to the donor profile. As with DNA level, PC was significantly higher in PL than CF inside samples ($p = 0.008$), suggesting a distinction in amplification quality between endogenous cellular and cell-free DNA (Figure 3A). DNA may be less detrimentally fragmented in corneocytes than in cfDNA. While the outside OV DNA level may reflect predominantly cfDNA, the OV allele total is better accounted for by PL alleles than by CF in both inside and outside (Figure 3C), likely due to relative amplification dropout levels. This is in line with authors’ previous reports of fragmented DNA in anucleate corneocytes [30], although the mechanism remains obscure [56]. Mean PC was consistent across outside fractions. Better profile quality from outside CF than inside CF may reflect overlapping alleles from nonself sources or result from higher quality DNA that has become cell-free after deposition rather than existing in fragmented form upon secretion.

The inside and outside OV fractions of 90% of donors yielded some DNA typing results, with inside samples being primarily partial profiles of donor origin. One donor failed to yield any STR data. Sporadic nondonor peaks occurred at the shortest loci in 25% of inside samples, possibly representing nonspecific amplification products or low-level “contamination” from washed hands or gloves. They were easily resolved from the donor profile. The outside OV fractions contained all expected donor alleles at successfully amplified loci. Locus dropout occurred exclusively at the largest markers (FGA, D18S51, CSF1P0, TPOX, D7S820, and D2S1338). A sum of 56% of the successful outside...
Figure 4. Exemplar images from one donor collected on three separate days following each of seven timepoint protocols. Hand rinse aliquots have been stained with DiamondDye.

OV samples were mixtures, ranging from 2 to 5 minimum number of contributors (MNC). Samples with no profile results had DNA concentrations over 0.6 ng/μL, suggesting high degradation prevented amplification of the extant DNA.

Notably only five of nine outside OV profiles were complex mixtures, while four were effectively exogenously acquired single source (self-DNA) profiles. Mean DNA levels did not differ significantly between mixture and single-source subsets (p = 0.18) and CF accounted for more DNA but fewer alleles in both. More than half the donors acquired only their own DNA profile on gloves after regular activity, likely from a tendency to touch one’s own face, clothing, items, etc. Although it is an assumption that donors’ behavior would not alter as a result of the gloves, these data may reflect an underestimate of exogenously acquired DNA if self-grooming or face touching was reduced by their presence. The presence of nonself DNA was not associated with reported sex of the donor. DNA transfer levels may be even higher than previously thought if we consider that much of the observed self-DNA in touch deposits is transferred to begin with. DNA acquired by hands directly from one’s own saliva or mucus epithelia would be a rich source of genomic DNA showing more complete profiles.

3.2 Rinses from accumulation timepoints

Having examined the exogenously and endogenously acquired material, it was next queried how quickly each may reaccumulate after hand washing.

3.2.1 Cellular content

Rinses from each timepoint after washing (0 min, 15 min, 1 h, and 2 h, with and without contact) were stained with DD. Contactless timepoints showed less dense, more uniform cell collections with fewer irregular cell clumps and fibers/particulates than those following regular activity (Figure 4). All donors accumulated more cells after 15 min, while only 50% continued this pattern at each subsequent timepoint. The wide range of cell densities observed between donors accords with generally reported trends of variable shedding [3,52,57–59], that is, different donors deposit different numbers of cells under identical collection parameters.

Following collection, each hand rinse was split into OV, PL, and CF fractions for DNA recovery and processing. Cell count did not correlate with OV DNA levels (data not shown). Nucleated cells were rare, observed in only three donors, after either 1 or 2 h of regular activity. These limited observations do not permit quantitative conclusions about nucleated cell accumulation rates. However, these cells may contribute to interdonor variability in DNA levels, as they could increase DNA content with even sporadic presence.

3.2.2 Relative DNA levels

Rinses were not DNA free following hand washing (OV range: 1.97–29.4 ng total DNA), indicating that even clean hands contain endogenous DNA if targeted recovery methods (keratinocyte lysis and small-fragment bead purification) are used. Similar to inside glove samples, a majority of the endogenous DNA in contactless hand rinses appears accounted for the corneocyte pellet DNA (OV mean: 15.3 ± 8.5 ng, PL mean: 13.9 ± 9.6 ng) with CF making a baseline contribution (CF mean: 5.0 ± 6.8 ng). CF DNA levels were significantly lower than both OV and PL fractions (p = 0.0008 and p = 0.003), which were not significantly different from each other (p = 0.24) in paired Student’s t-tests. This relationship
Figure 5. Mean total DNA recovered (top, blue) and mean alleles counts (bottom, red) from 10 donors at timepoints following hand washing. Regular activity intervals shown in A and C (left), contactless intervals shown in B and D (right). T0 measurements are the same for interval sets. Error bars represent 1 SD. Time intervals with a significant increase indicated with a bracket and asterisk (*).
is consistent over time (Figure 5B); DNA levels at T₀ did not significantly differ from those at any subsequent timepoint for any fraction (T₁₅min: \(p ≥ 0.13\), T₁₀₈: \(p ≥ 0.15\), T₂₅₄: \(p ≥ 0.24\)). Only a limited amount of DNA is secreted by the hands and this does not increase without contact.

Following activity, OV DNA level increased significantly after 15 min (\(p = 0.04\)) and again between T₁₅min and T₁₈ (\(p = 0.004\)). The T₁₈ to T₂₅₄ increase was not significant (\(p = 0.7\)), suggesting a plateau as DNA is both accumulated and deposited during prolonged activity for a net neutral effect (Figure 5A). This pattern was mirrored in the CF fractions, with significant increases in total DNA between T₀ and T₁₅min (\(p = 0.009\)) and between T₁₅min and T₁₈ (\(p = 0.008\)), but not between T₁₈ and T₂₅₄ (\(p = 0.8\)). The PL fraction showed no significant changes in DNA over time. Although cellular DNA contributes consistently to overall hand rinse DNA, as with the outside gloves the observed accumulation is better explained by exogenous cell-free DNA accrual. These timepoint rinses represent both exogenous and endogenous material following regular activity, so the CF increase cannot explicitly be sourced to hand-exogenous contact. However, given how CF increase is absent without contact, it appears that either the exogenously acquired DNA is simply much greater or that activity levels induce endogenous DNA secretion.

### 3.2.3 DNA profile quality

As with gloved hands, most samples displayed descending peak heights with increasing amplicon length, clearly indicating DNA template degradation. Contactless samples were primarily single source, as expected, with a few observations of nondonor alleles at the shortest loci, D3S1358, D8S1179, D2S441, D22S1045, and D10S1248. These were possibly nonspecific amplification easily resolvable from the major donor profile, although the major profile was often partial as one or more of the larger loci dropped out in 50% of contactless OV samples. The mean number of observed alleles did not change significantly over time in any of the contactless samples, indicating a consistent rather than acquired contribution of endogenous allelic information (two-way ANOVA, \(F = 0.083, p = 0.97\)). However, the difference between fractions was significant (\(F = 19.8, p < 0.001\)), with the CF samples contributing fewer alleles than either PL or OV (paired \(t\)-test, \(p < 0.001\)). Allele count mirrored DNA level in contactless samples, that is, majority of both the overall DNA and allelic information is accounted for the PL contribution (Figure 5D). In contrast, regular activity samples displayed a significant difference in allele count between over time and between fractions (two-way ANOVA, \(F = 9.03, p < 0.001; F = 31.3, p < 0.001\), respectively). This reflected the significant increase in alleles observed at the first 15 min timepoint (paired \(t\)-tests, OV \(p = 0.03\), PL \(p = 0.03\), CF \(p = 0.02\)) and the higher allele contribution by the PL and OV fractions than the CF at each timepoint (\(p < 0.005\)) (Figure 5C).

Complete profiles occurred in only two contactless CF samples (6.7%) while observed in 10 contactless PL samples (33%), while no alleles at all were seen in 16.7% CF and 3.3% PL. While both CF and PL contain degraded DNA, the endogenous CF DNA fragment lengths have more serious implications for STR amplification. Regular activity samples contained all expected donor alleles, with exceptions at larger loci where dropout was widely seen. Most STR profiles were multidonor mixtures (86.7%), with MNC from 1 to 5 across regular activity timepoints. The 13.3% single source samples all came from two donors’ PL/OV samples (one female, one male) possibly suggesting individuals who shed and reaccumulate their own cells in elevated levels.

Neither allele count nor MNC correlated linearly with increasing amounts of DNA over time in any fraction (data not shown), indicating that most recovered genetic material may not be successfully amplified. This was particularly true in the CF samples, where acquired DNA level outpaced the PL DNA contribution over time, but the allelic content did not keep up. The OV and PL allele counts were similar at each timepoint (\(p > 0.05\)), suggesting a cellular origin. However, the overlap in contribution of the same shorter alleles from both CF and PL is widespread and, thus, the initial (within 15 min) contribution of CF alleles may be important. Allelic contribution of both PL and CF DNA could improve in the future if methods less dependent on intact target template were explored for this sample type.

### 3.3 Combined quantity and quality index from qPCR

All purified DNA samples from both the glove study and the timepoint study were additionally measured with qPCR. The QuantiFiler™ Trio (QT3) assay is widely used in forensic casework for the measurement of amplifiable human DNA. It contains a “Degradation Index” (DI) which is intended to yield an estimate of DNA quality. It is calculated as the ratio of short amplicon (SA) to long amplicon (LA) quantities, where the targets are 80 bp and 214 bp, respectively. A DI value of \(≤ 1\) indicates intact DNA due to the concentration of small and large fragments being approximately equal. A DI value > 1 indicates moderate degradation while a value > 10 suggests significant degradation. In samples, where the LA failed to amplify to a minimum threshold, but the SA was detected, the sample is likely extremely degraded in the absence of inhibition [60]. These samples were conservatively assigned a DI of 10. It should be noted that when reference swabs subjected to the same lysis and purification procedures were also quantified with QT3, their DI values were 0.73, 1.0, and 1.8 (mean 1.17 ± 0.55), which suggests that any preferential recovery of short fragments introduced by the purification protocol does not consistently elevate DI as an artefact. Further discussion of this possibility is present in the Supporting Information. The results from the gloves and timepoints accumulation samples are summarized in Figure 6.

It is immediately apparent that, as expected, Qubit values generally exceed QT3 values (light blue bars generally taller than dark blue in Figure 6). The former is likely to include both short DNA fragments which are of interest, as well as...
bacterial DNA, which is an unavoidable confounding factor. When donors are pooled as they were above, the same relative contribution patterns are present as were reported in Figure 3. The inside OV fraction (mean 4.21 ± 3.26 ng) consists primarily of PL DNA (mean 3.08 ± 2.60 ng) rather than CF DNA (mean 0.33 ± 0.53 ng). In contrast, the outside glove OV fractions (mean 38.16 ± 44.71 ng) contain a majority contribution by CF DNA (mean 23.63 ± 15.81 ng) and somewhat less from PL DNA (9.18 ± 11.23 ng). The QT3 values are greatly impacted by fragments length since short template and primer sequence interruption will prevent amplification, therefore, they are an underestimate of true total human DNA content. But likewise, Qubit values’ nonspecificity likely provides an overestimate, so having both may be advisable for estimating an accurate measure in the middle.

The presence of degraded human DNA previously presumed based on fluorescence and profiling data is confirmed by the DI values observed across samples in both studies (shown by red line graph in Figure 6). Degradation is consistently detected, as DI values are greater than 1. However, samples are significantly less degraded on the outside of gloves (Figure 6A) than the inside (Figure 6B). This is true for OV, PL, and CF fractions ($p = 0.0001$, $p = 0.007$, and $p = 0.003$, respectively). This suggests that the intact DNA is derived much more consistently from exogenous sources than from endogenous ones, which is consistent with greater PC. Interestingly, this degradation pattern is not observed in the regular activity timepoints (Figure 6C), which also contain higher levels of DNA than the contactless counterparts presumably acquired through contact (Figure 6D). Unlike the outside gloves though, these regular activity samples include a combination of endogenous and exogenous DNA, so while sufficient intact DNA is present to provide improved STR profile results, a great deal of secreted shorter fragments are also present in many samples, keeping the ratio of short to long elevated. These short fragments can alter the DI if they are 80 bp long, but they might only be providing very occasional STR allele data. The outside glove samples, which contain only exogenous DNA, are less degraded even in the CF samples, which indicates that the externally acquired DNA is in better condition than the secreted DNA. This may hint at a high-quality origin for this external DNA, since the endogenous touch deposits are unlikely to provide it. In other words, exogenous DNA may result from nucleated cells which become environmentally degraded rather than shed, intrinsically degraded material which is more severely compromised.

Although donor data have previously been pooled to detect overall trends, Figure 6 illustrates the wide range of interdonor variability which is consistently observed in this work. Both DNA level and DI vary widely between individuals in all three fractions, reflecting distinctive levels of both intrinsically shed and contact-acquired DNA. Not all human...
DNA fragments will be reflected in the QT3 measurement, as anything below 80 bp would fail to be detected or influence degradation assessment, meaning some samples could be even more fragmented than the DI value indicates. This level of degradation, whether it is occurring endogenously or exogenously, merits further exploration, ideally with some more sensitive tools which can assess fragment lengths, patterns, and sequences.

4 Concluding remarks

These data indicate that more DNA accumulates on our hands from their environmental contact than arises from the hands themselves. A large proportion of this externally derived DNA is one’s own DNA and is acellular, yet its provenance is not our hands themselves, underscoring the ubiquity of DNA transfer.

Corneocytes may be the primary DNA contributor in the endogenously accumulated deposits, when lysis and purification methods enhancing their DNA recovery are used. Despite the use of keratinocyte lysis buffer here, the disproportion of corneocytes to DNA levels on the inside glove samples suggests that more optimization of DNA recovery from these cells is advisable. Although not standard touch deposit case-work methods, keratinized cell lysis and small-fragment DNA recovery are worth investigating further.

DNA accumulates quickly on hands, within 15 min following washing, but as a result of regular activity rather than secretion. “Shedder status,” if assessed following contact, might reflect accumulation of one’s DNA from elsewhere on the body, possibly a behavioral variable as much as an intrinsic status; or a “good shedder” may reflect an individual with a higher secretion of the rarely observed but highly impactful nucleated cells. Although DNA levels further increase with regular activity, allele content plateaus, partially as a result of the additional material being one’s own alleles. Because it cannot be distinguished by DNA profile, self-DNA from acquired versus secreted sources had not previously been differentiated. Although one’s own cfDNA is accumulated immediately along with nonself cfDNA during regular activity, the allelic data rarely comprises a complete profile including the largest markers due to degradation. Alleles relevant to current STR typing methods may derive chiefly from the cellular component, and continue to do so over time, but such limitation may diminish in the future with adoption of shorter amplicons [46,61] including SNP panels [62,63] or other primer-ligation or shotgun-based amplification [49,64]. When DNA was measured with qPCR rather than Qubit, lower levels were generally detected due to the DNA degradation which was consistently elevated. The outside gloves acquired more intact DNA generally, in the CF fraction specifically, suggesting that both cellular and acellular DNA in the environment may be of better quality than what can be generated endogenously.

If DNA from the hands is minimal compared to DNA accumulated through contact, then most “touch DNA” may be “transfer DNA,” even if it is one’s own. Given the meaning attached to DNA profiles that have transferred onto incriminating items, it is significant to understand that transfer may be the origin of most touch DNA, occurring more frequently and at higher levels than was previously understood. This is critical as forensic scientists are increasingly asked to report and explain touch DNA and DNA transfer in a courtroom. Most key at this juncture is further exploration of touch deposit contents, whether cfDNA or cellular, whether endogenously or exogenously accumulated.

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The authors have declared no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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