



Praca przeglądowa
Review paper

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Recovery techniques for contact DNA traces

Techniki odzyskiwania śladów kontaktowych DNA

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Abstract

Donor DNA profiling can serve at least two purposes: 1) to enhance the evidential value of DNA deposited on garments/items and 2) to provide valuable tactical information during crime scene investigation. In this review, different types of methods for the recovery of the contact DNA traces have been summarized. Additionally, with the available techniques, the unique characteristics and limitations thereof have been overviewed. The aim of this paper is to review the techniques of touch traces collection.

Key words: contact DNA traces; shedder status; DNA recovery techniques

Streszczenie

Profilowanie DNA dawcy może służyć co najmniej dwóm celom: 1) zwiększeniu wartości dowodowej DNA zdeponowanego na odzieży/przedmiotach oraz 2) dostarczeniu cennych informacji taktycznych podczas badania miejsca przestępstwa. W niniejszym przeglądzie podsumowano różne rodzaje metod odzyskiwania śladów kontaktowych DNA. Dodatkowo, w odniesieniu do dostępnych technik, dokonano przeglądu ich unikalnych cech i ograniczeń. Celem niniejszej pracy jest przegląd technik pozyskiwania śladów dotykowych.

Słowa kluczowe: ślady kontaktowe DNA; predyspozycje do pozostawiania DNA kontaktowego; techniki pobierania DNA

1. Introduction

Increasingly, it is possible to obtain a person's DNA profile from traces left on objects which were touched. Deposition of DNA by wearing clothing or touching the surface may be sufficient to obtain a satisfactory result, i.e. procuring a suspect's profile [1]. The main purpose of this manuscript is to summarize various recovery techniques for contact DNA. The author focused on studying DNA research to optimize the location of contact traces left on everyday objects, e.g. to help verify the DNA profile of the person associated with the case. Moreover, the paper extensively describes and compares the so-called good and poor shedder.

2. Good shedders and poor shedders

2.1. Skin

The skin accounts for 15% of the total body weight, which makes it the largest human organ. An average human sheds approximately 400,000 epidermal cells daily. Prior to shedding, the human epidermal cells spend about one month on the epidermis. There are about 100 sweat glands and 10 oil glands per each square centimeter of the skin [1,2]. Excretions produced by these glands make their way through ducts and pores, hence exposing them to large numbers of DNA-bearing cells on the way to the skin surface. As a diploid human cell, an epidermal cell contains about 5 pg of nuclear DNA [2,3].

2.2. Shedder status in general

The propensity of an individual to leave behind genetic material on items and surfaces touched is referred to as shedder status [4]. Numerous studies claim that the ability to shed or deposit epidermal cell debris via direct contact may vary among individuals, hence two main types of shedders have been distinguished [5,6]. Good shedders tend to consistently deposit comparatively more DNA, whereas poor shedders leave small or undetectable quantities of DNA [7,8]. Tan et al. tried to categorize the standards for the shedder group. In their research study, in which the majority of samples (for example, ≥ 4 out of 6) gave reportable DNA profiles, the participants were considered as good shedders. If all six profiles were undetectable, then they were consid-

ered as poor shedders. Thus, a group that did not qualify for either a good or poor shedder, i.e. with one to three readable full DNA profiles, was referred to as a medium shedder [9]. Moreover, the category of intermediate/medium shedders, i.e. DNA deposited in the middle range, was described by the teams of Kanokwongnuwut et al. [10] and Goray et al. [7], because it had not been easy to find relative participants who would consistently have been either very good or very bad shedders. Lowe et al. claim that a result may depend on several factors, including the individuals themselves and the time interval after hand washing. If the individual touching an item has recently washed their hands, a full DNA profile can be recovered as long as that person is a good shedder. The importance of the shedder type decreases if the period after hand washing is between two and six hours [6]. Szkuta et al. [8] claimed that it is possible to detect a non-self-DNA acquired no later than within 15 minutes after a handshake, and the key factor in the transfer of foreign DNA through a handshake is the shedding ability of the pair. Moreover, a person rated as a good shedder covers traces of a poor shedder or a moderate one. The "DNA donor" during a handshake has been found to leave the same or more DNA than the handshaking partner on subsequent handshakes. More precisely, the amount of the DNA deposit will be comparable in both the first and the fourth handshake. The person may thus also be considered as a good shedder. Interestingly, the combination of handshakes from poor shedder or moderate shedder pairs will ensure that the shedders' DNAs are detected and will not be disturbed by either side [8].

Daly et al. studies have demonstrated that regarding shedding capacity, there was no significant difference between males and females [11]. Moreover, in determining the shedder status, no male versus female bias was observed by Lowe et al. [6] On the other hand, newer data obtained by Fonnelløp et al. indicate that males leave significantly more DNA than females [12]. Tan et al. supports the same position that males are more often than females categorized as good shedders [9]. Sessa and al. noticed that a "handler's" DNA profile (from a male) analyzed from garments (in this case brassieres) could overwrite a "wearer's" profile (from a female). The researchers' team analyzed if the handling time had

any impact on the detection of the “handler” and/or “wearer” profile. Their research revealed that in all mixed DNA profiles the “wearer” was the major contributor only in five out of 240 tests, which may indicate the advantage of leaving male DNA over female DNA. In the remaining mixed DNA profiles, the „handler” was the major contributor. The recovery percentage (alleles observed/expected) for “handler” profiles recovered related to the handling time was analyzed and complete „handler” profiles were found with a high percentage from 87.6% to 99.24% [13].

Lower amounts of foreign touch DNA are deposited on objects routinely used by one person, although there are exceptions. A higher amount of foreign DNA compared to that of self-DNA may suggest that the owner is a poor shedder. Lowe et al. found that a poor shedder leaves a partial profile only [6]. Certain activities such as washing hands or wearing gloves decrease DNA deposit levels [4].

2.3. Skin conditions

Lowe et al. suggested that good shedders tend to leave more epidermis than poor shedders due to drier skin on hands since DNA is found in flakes of dry skin [6]. The question is what could be the cause of dry skin? Skin conditions can promote DNA transfer, including sebum or sweat that contain cell-free nucleic acids [14]. The presence of sweat could have a similar effect as sebum but van den Berge et al. [14] noticed a less strong effect. Also, skin diseases such as psoriasis, dermatitis or skin ulcer before and after therapy, which increase skin cell turnover rates, may result in high touch DNA deposits [15,16]. According to the Polish Atopic Society (<https://www.ptca.pl/>), almost one million people suffer from atopic dermatitis in Poland. A high proliferation rate of the skin in these types of skin diseases results in a higher number of cells containing only slightly degraded DNA. It confirms the fact that different DNA quality and also quantity observed in epithelial abrasions or swabs from any handled material may be due to dermatopathies [16]. The examples described, concerning skin diseases, may be mentioned as possible reasons for major and apparently strange differences between amplification results of epithelial abrasions.

2.4. Biological and behavioral factors

The factors that influence DNA retention are presumed to be both biological and behavioral. The biological features include DNA more of which will be recovered from sweat. On the other hand, one of the strongest behavioral features affecting the amount of shedding of the epidermis is the habit of rubbing the eyes. The results obtained by Jansson et al. [4] suggest that the majority of DNA deposited on surfaces and items did not originate from the hands themselves. It may have been transferred to the hands by touching, scratching, or rubbing other body parts and/or handling personal objects [17].

2.5. Material and surface

The amount of DNA transferred to the contacted surface is highly dependent on the type of material and surface texture. Daly et al. [11] and Burrill et al. [18] reported an increased number of shed epithelial cells on rough and porous substrates, while non-porous substrates adhere less readily to genetic material. Notably, fabrics and cotton appear to be better DNA collectors than glass or plastic surfaces. It has also been proven that the consistent recovery of touch DNA from metal surfaces is more difficult [19].

2.6. Are the terms ‘good’ and ‘poor’ shedders correct?

Van Oorschot et al. considered replacing terms such as good and poor shedder, which can be confusing (due to the source of the DNA), with more neutral terms like „prevalence status/indicator”. Moreover, the authors of the aforementioned article suggest that the words ‘good’ and ‘poor’ which were used to indicate quantity, can be replaced with ‘high’ and ‘low’. Nevertheless, the same research team recommends further study of shedder status as a likely significant factor when interpreting profiles in assessing the level of activity [20].

2.7. Lateralization

A separate intriguing topic that is close to „shedder status” is lateralization. Goray et al. noticed that there is no difference in shedding more DNA regardless of laterality or right/left-handedness [7,21]. Phipps et al. [22] disagree with this conclusion as

their research has shown that the dominant hand is significant in the amount of shedding epidermis based on better results in DNA profiling (the unwashed hands shedding experiment). The latter authors have also revealed in their next experiment (where hand washing was performed) that a non-dominant hand can also exceptionally shed more DNA. It is possible that the non-dominant hand will tend to shed more DNA when the hands are clean, but the dominant hand begins to shed more as the hands get more dirty (the influence of the time interval from hand washing). Phipps et al. [22] showed approximately seven times higher coverage in allele number in DNA profiles from the dominant hand. This can be explained by the fact that the dominant hand tends to touch more objects than the non-dominant hand, which causes its epidermis to be more abraded. The dominant hand will also contact the face skin or the scalp hair where more epidermis is exfoliated. The habits such as regular touching the face and/or scalp can give an individual a higher DNA transfer rate. Repeated touching of frequently used objects such as a personal phone, also causes a more exfoliated epidermis on the dominant hand [22].

2.8. Is the shedder status determining easy?

An interesting article was written by the research team of Tan et al. [9] They describe the steps taken to validate the method for determining the shedder status. Touch DNA samples were collected to examine DNA recovery 15 min post-handwashing. The results of the research team study suggest that some individuals can simply be classified as either good or poor shedders, being consistent in their deposition of DNA at high or low levels. However, a considerable number of participants in this research were inconsistent in their DNA deposition [9].

2.9. Good and poor shedders – conclusion

As we can see, the findings were inconsistent, despite numerous studies on shedder status. Recent research shows that males shed more DNA than females and a hand washing activity can reduce the available quantity [9,10]. Whereas, physical activities (such as jogging or cycling) involving sweating lead to an increase in DNA transfer [14]. Body location impact results are closely related to this subject,

for example, dominant hand (vs. non-dominant) and sebaceous skin areas (vs. non-sebaceous) potentially facilitate DNA deposits [7,21,23]. Further research is needed to verify the influence of such characteristics as diet, temperature, and mental state on the resulting amount of contact DNA [17].

3. Recovery techniques

One of the crucial factors that affect successful DNA profiling is appropriate sampling. During the collection and analysis of touch DNA, small amounts of genetic material are expected. They can be difficult to analyze, but extremely valuable for research. Much of DNA evidence comes from an unknown biological source and is latent. Two steps of the strategy may be distinguished: precise and accurate determination of the sampling area, and the choice of the collection method [24]. The technique used to collect samples is of particular importance in criminal cases [13]. Since the DNA amount needed to obtain a full genetic profile decreased from nanograms to picograms, an interest in equipment implemented in biological trace recovery has increased [25]. According to Locard's exchange principle, contact between two objects always results in an exchange of material [2,26]. The sampling areas are determined by the information on a crime event and the knowledge and experience of the forensic expert investigating the evidence. Besides the number of DNA-bearing cells, factors that contribute to successful DNA typing include handling (contact) time and intensity, environmental factors such as moisture, and the type of surface [2]. That is why, it is of vital importance to choose an appropriate technique for given conditions of trace recovery.

3.1. Swabbing

A variety of swab sticks dedicated to the collection of biological evidence from crime scenes is available. A set of factors that influence the effectiveness of swabbing includes the material, length, and thickness of the swab head, the force with which the tip is wound and articulated with the swab shaft or bud, and the structure and shape of the transport/storage tube. The latter includes additional features that keep DNA intact, such as vents to improve air drying, antibacterial agents, and desiccants. The advantages of

swabs are low cost, simple use and transport, and the ability to use them on diverse biological traces. Based on extensive literature and our laboratory experience, the swab tip design and structure are the main factors affecting the effectiveness of the sample recovery and DNA extraction success [25]. Swab manufacturers ensure that the swabs are produced under dedicated controlled conditions for the complete reliability and integrity of forensic samples [27]. Currently, the swab heads that are commonly used in forensic cases are made from cotton, polyester, rayon swabs, FLOQSwabs – which are flocked swabs made of nylon, Dacron swabs, and BBL Culture Swabs, which are swabs composed of polyurethane foam [27,28]. One of the common methods of obtaining material for research is collecting cellular material with a sterile swab by double-swabbing either with a dry or a wet technique. This double-swab method involves using one wet swab and then one dry swab [27]. The dry swabbing uses a bare swab without a moistening agent. The wet swabbing uses a swab moistened with demineralized water, saline, or a buffer by wetting the cotton tip completely [13,29]. The maximum contact between the swab and the skin is ensured by rotating the swab on its long axis [29]. The double-swabbing may be used to collect saliva stains on human epidermal cells [30]. First, the wet swab is used, and then, the dry one with similar pressure and movements as with the first one. The dry swab is rotated over the skin to recover the most moisture remaining on the skin's surface from the wet one [29]. To preserve the DNA of the sample, it is important that the wet swab is air-dried at room temperature for several hours [30,31]. This method also has limitations, such as those related to the specific type of swab. Standard cotton swabs are traditionally preferred for the collection of biological fluids, but despite further research, they still tend to trap organic residues in the cotton fibers, reducing sample availability [32]. Another example, using the COPAN 4N6FLOQSwabs™ (single-swabbing) treated with an antimicrobial agent (crime scene variety – to get as close to natural conditions as possible), resulted in significant DNA degradation. In this case, after using the COPAN 4N6FLOQSwabs™, DNA remains unstable after the time intervals [33]. However, Giovanelli et al. noticed that the PurFlock® swab (single-swabbing) was more efficient for recovering donor alleles than the others [34].

3.2. Cutting-out

Cutting-out small areas is mainly used on textiles (garments). This approach is destructive to the evidence and its applicability is limited to absorbent substrates. Note that with cutting-out, both inner and outer surfaces are sampled at the same time. As a consequence, the sample may be contaminated with the wearer's DNA. The exception is clothes that contain a lining (several layers of fabric). Due to the separate fabric layer, the likelihood of DNA mixture is reduced [13]. A direct cutting can also involve traces of DNA left on the paper. It was found that certain paper types such as newspapers, magazines, and filter paper allowed good recovery and successful extraction of DNA. Conversely, others (such as office paper and white cards) allowed greater recovery of transferred (i.e. undesirable) DNA. What is more, the use of common office paper and white cards, resulted in poor-quality profiles, due to strongly interfering with the recovery of DNA [35].

3.3. Tape-lifting

The adhesive tape-lifting is considered to be effective, quick, and easy to lift traces deposited on porous surfaces, including textiles, contrary to swabbing, which proved to be more efficient for traces deposited on smooth surfaces [22,30]. Tape-lifting reduces challenges encountered in swabbed DNA profiling caused by PCR inhibitors, e.g. clothing dyes [36]. There are several types of tapes used to take a sample from a forensic trace. Among them, we find products such as Scenesafe FAST™ and Scotch® tape. Studies by Verdon et al. showed that Scenesafe FAST™ tape extracts significantly more DNA than Scotch® Magic™ tape. What is more, the same results were obtained in alleles detection – a higher proportion for Scenesafe FAST™ tape [37]. This method is suitable for recovering traces from fabrics and hairs [30]. Tape-lifting facilitates the collection of corneocytes, but they carry a lower amount of DNA. Like any of the presented methods, it also has its limitations. Tape-lifting is unsuitable for cell-free DNA collection from non-porous surfaces [38]. The difficulty in interpreting the results of the tape method is its stiffness, viscosity, and size of the tape [37,39].

3.4. 'Smart' enhanced analysis

Standard methods of collecting contact DNA are often embarked with contamination and/or mixture issues. In order to avoid admixed or non-specific DNA profiles, a novel sampling method has been reported. This method called 'smart' enhanced analysis involves the physical recovery of cell agglomerates deposited on the human skin and garments and further downstream procedures based on LT-DNA analysis. The 'smart' method is experimental and developed to be easily incorporated into forensic practice in the future. The 'smart' method is selectively focused on individual bio-particles, which is an advantage over standard blind swabbing. The cells analyzed in the 'smart' technique can be collected from various clothing and object surfaces touched [40]. Briefly describing the methodology, single and agglomerated putative bio-particles (i.e. putative cells as visualized under the light microscope) were viewed, imaged, and collected using a stereomicroscope. Bio-particles were collected from the GelPak® surface using the water-soluble 3M™ adhesive which was adhered to a clean glass microscope slide using double-sided tape, and next they were transferred to the tip of the tungsten needle by compression under the stereomicroscope. The collected bio-particles were transferred into a sterile PCR flat-cap tube and then they were going through the next standard stages of sample processing [40]. Perhaps in the future, this method will be used for mixed DNA profiles to distinguish a person of interest.

4. Recovery techniques of choice

In recent years, the sensitivity of DNA techniques has increased significantly and now it allows the analysis of minuscule quantities of evidence. Both the well-tested cotton swabs and more recent nylon flocked swabs may be used to recover the DNA traces with better precision [14]. Brownlow et al. indicate that due to the flexible nature of the plastic handle, which causes difficulties in the physical activity of collecting the trace, the nylon flocked swabs are not suitable for lifting dry samples [41]. Additionally, the nylon bud is hardly absorbing. Even so, the nylon swabs have been designed with a narrowing below the head that makes it easier to

break it off the stick, which is not entirely its advantage. Indeed, cutting off the intact swab head may cause DNA to be trapped in the cotton mesh, which reduces its content in the isolate. On the other hand, removing the cotton from the shaft by 'shaving off' enables a better access to cells by lysis [41]. Considering the studies by Wickenheiser et al., a higher proportion of DNA cells adhere to cotton swabs than the synthetic ones [2]. Thus, it can be concluded that cotton swabbing is more efficient than nylon flocked swabbing [41] for traces deposited on non-porous surfaces, while cutting out is more efficient than double swabbing for traces deposited on textiles and fabrics. Van Oorschot et al. indicated that hard non-porous objects (i.e. pen, lid) have less capacity to accumulate and retain deposited or transferred amount of DNA than a soft rough surfaced porous material (i.e. bracelet). It means that the transfer of touch DNA is mainly dependent on the substrate on which the biological material resides. Likewise, the freshness of the deposit and the manner of contact are also evaluated [42]. According to Hess and Haas, the success rate in trace DNA typing from traces on bright textiles is higher than from the dark ones [30]. Moreover, the indigo dye (an intense dark blue color) was found to be likely a PCR inhibitor [43]. Considering the brightness/color of the clothes, there is no difference between DNA yield collected by swabbing or cut-out method [44]. In the case where a victim's DNA is found in large excess and masks the perpetrator's DNA, the 'smart' enhanced analysis may be preferred since it allows the separation of the DNAs based on the recovery of single cell agglomerates and their meticulous analysis [40]. According to Hess et al., the adhesive tape lifting method is the best way to recover a DNA trace. Furthermore, the adhesive tape lifting is not substrate-dependent [30]. Verdon et al. noticed that using Scenasafe FAST™ tape lifts recovered a significantly higher percentage of DNA than that obtained using swabbing from cotton drill woven fabric and polyester/cotton plain woven fabric. However, this tape-lift method also has its limitations. Verdon et al. indicated that in comparison between the aforementioned modes of sampling from polyester strapping or cotton-flannelette, there was no significant difference [37]. Regarding the swabbing method, results of Hess et

al. showed that a higher level of DNA is recovered by taking the trace with the moistened swab than by using the dry swabbing method. The exception is a material with a non-porous surface such as a raincoat, in the case of which a better result is obtained when taking the dry swab. Nevertheless, the mini tape lifting method proved to be superior to a dry swab on the raincoat. Also, natural dark materials showed slightly more complete DNA profiles, correlated with synthetic materials [30].

It should be remembered that in addition to various DNA recovery techniques, the quality of the DNA profiles obtained is also significantly affected by the DNA extraction methodology, the amplification/profiling systems used, and the divergent interpretation and statistical methods used. Additional utilization of correction factors could be an avenue to allow more objective comparisons of data [20]. Van Oorschot et al. provide a valuable collection of information on factors that can potentially affect the level of transfer, persistence, prevalence, and recovery of DNA contributions (DNA-TPPR) [20,45].

5. Best location

The amount of the DNA deposited on clothes probably depends on many factors, including the category of DNA shedding already mentioned in one of the chapters of this article, as well as the way the clothes come into contact with the skin and the time of wearing them. DNA is also present in body materials, such as sebaceous fluid [46], sweat [17], and even dandruff [47], and these likely contribute to the formation of the wearer's DNA. The presence of underwear is expected to reduce DNA transfer, while clothing-to-skin contact due to the pressure of outerwear or its tight fit facilitates DNA transfer. In addition, friction between the wearer's clothing and the wearer's skin during daily activities may promote DNA transfer, as friction between the two surfaces has been shown to increase the amount of DNA transferred [48].

The study of Ruan et al. examined three areas located on the front, back, and shoulder of an individual's external clothing during regular daily activities by determining the amount of endogenous (self) and extraneous (foreign) DNA deposited. The most complex mixture samples in the study were obtained from the back of the shirt samples. Samples from the

front and shoulder areas of the shirt produced more single source profiles attributable to the wearer compared to the back shirt samples [49].

Van den Berge et al. [14] conducted research on four locations of clothing: winter gloves, trousers ankles, grabbed arms and armpits of shirts to recover the full DNA grabber profiles. The highest recovery of the full DNA profile was shown by winter gloves, as much as 80%, followed by armpits of shirts with a slightly lower result – 69%. The other two locations, trouser ankles and grabbed arms, showed only 40% full profile recovery each. A higher DNA transfer rate occurs in the case of habits such as regular touching the scalp or face. The presence of sebum on the skin also promotes DNA transfer. Van den Berge et al. [14] checked whether the presence of sweat on the skin has a similar result as sebum. The research team noticed a less strong effect of sweat than sebum regarding the quantity of DNA [14].

6. Conclusion

A greater amount of deposited trace DNA reveals behavioral habits, such as frequent touching of the face or scalp with hands. An additional factor is dandruff, increased secretion of sebum, and skin diseases such as psoriasis. For comparison, less touch DNA deposition occurs with frequent hand washing or wearing gloves. The article describes 4 main methods usually used in the forensic investigations, and outlines their advantages and disadvantages. Although the tape method of sampling is quick and simple, and tapes with better adhesion have been noticed to produce a higher yield of trace DNA than swabs, the viscosity, stiffness, and size of the tape make interpreting the results difficult. The single-swab method is an effective sampling technique and is extremely versatile. Another technique mentioned, the “cutting out” one, has certain critical constraints, for example, the material on which it is implemented (not every surface can be cut out) and its irreversibility. Whereas ‘smart’ enhanced analysis in the future, extended by further research, thanks to its precision, may answer the need for effective reading of obtained small amounts of touch DNA. The literature referred to in the present article will broaden the reader's knowledge of the discussed topic.

In conclusion, many factors influence the effectiveness of touch DNA as a forensic tool. To collect useful DNA evidence, the most appropriate technique for collecting trace material must be selected. However, there is no single best method that can be used in all cases without exception. Each technique described in this article has its limitations. The choice of method should be based on the background of collection, sample size, sample consistency, and circumstances of the scene. Particular attention should be paid to the location of the trace, shedder status (good or poor shedder), laterality, or possible skin disease. Highlighting once again, the

effectiveness of obtaining a DNA profile from contact traces largely depends on the choice of the appropriate method of recovering biological material and the method of its application. Knowledge and experience of the collector/forensic technician will also be necessary to select the best method. There is considerable variability in how results are presented in the current scientific articles and the type of data on which the comparison of techniques used in touch DNA scenarios is based. This topic needs further analysis. Nevertheless, its most important aspects have been presented in the article.

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