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Persistence of biological traces in gun barrels after fatal contact shots

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ABSTRACT

In the majority of cases suicidal shots are put to the head. Typically the gun's muzzle is held against the head.

The aim of the present prospective study was to investigate whether victim DNA could reliably be recovered from the inside of the barrels of firearms that were used in 20 cases of homicidal or suicidal close contact shots. Additionally, it was investigated whether such biological traces were eliminated by subsequent firing.

After autopsy sterile swabs were used to collect samples from the anterior part of the barrel thereby avoiding the muzzle. In some cases prior endoscopic inspection had revealed traces of blood and soft tissue in the barrel.

For 16 cases, another swab was used to also collect sample from the posterior part of the barrel entering from its rear end. Then one shot was fired through the weapon using the same ammunition as in the suicidal shot and the sampling procedure was repeated. DNA was extracted using a magnetic beads based protocol, quantified, and STR-systems were amplified using several commercially available multiplex-STR-PCR-kits.

For samples taken after the first shot DNA-analysis yielded STR profiles eligible for reliable individualization in 17 of 20 cases. After a second shot had been fired 8 or more STR systems were amplified successfully in 14 of 20 barrels.

1. Introduction

Statistically, the majority of fatalities in Europe caused by firearms are cases of suicide [1–7] which is reflected in forensic casework. Nevertheless, the differentiation between suicidal and homicidal gun shots has to reliably be based on forensic evidence by medical examiners and police investigators. Not only can an autopsy confirm the oftentimes obvious cause of death, but also document crucial findings. The morphology of entry wounds and soot cavities can indicate an exposure to muzzle gas pressure and thus point to an occurred close contact shot. Although contact shots cannot prove that suicide was indeed committed or attempted, they are characteristic for suicides [8,9]. Backspatter from the victims' hands represents valuable evidence to reconstruct the hands' position when the fatal shot was fired [8,10,11]. Finally topographic gunshot residue (GSR) collection from the victim's hands allows a mapping of GSR to determine whether or not the victim fired the gun [12].

Occasionally, e.g. after emergency care has been administered to persons surviving an attempt of suicidal shooting, traces of GSR cannot be analysed. In these cases inspection of the firearm may provide supplemental information. Ideally, typical traces of backspatter can be located on the outside of the weapon, but more often firearms used in suicidal shootings are covered in blood or characteristic stains are masked by splashes of blood. However, the inside of the gun barrel is generally not affected by such secondary staining.

As early as 1934, Brüning and Wiethold described the presence of traces of blood and tissue within gun barrels as a consequence of contact shots [13]. In another, preliminary study, 21 firearms used in suicidal shootings had been examined using a technical endoscope: 20 barrels showed visible biological traces. In eleven cases with positive morphological findings – from small calibre weapons up to 12/70 shotguns – STR systems could successfully be PCR-amplified and the resulting profiles matched with the respective victims' profiles [14,15]. However, in these studies only 8 STR systems were analysed and the procedures for sampling and DNA extraction were not standardized.

In a recent study we were first to present experimental models to emulate backspatter from contact shots and showed that profilable victim DNA may reproducibly be recovered from gun barrels even after a subsequent or "cleaning" shot had been put through the gun [16]. The aim of the present study of cases of suicide by firearm was to investigate if and to what extent biological traces and STR-profilable victim DNA in particular can be recovered from such firearms' barrels and can then be quantified

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Table 1
Description of the case collective.

Case	Age	Location of body	Weapon		Entry	Exit wound	Ammunition
			Origin/normal use	Legality			
1	47	Car		Illegal	Left temple	None	Lead round nose
2	71	Chair	Sport shooting	Legal	Mouth	None	Lead round nose
3	59	Bed	Heirloom	Illegal	Mouth	None	Lead round nose
4	69	Home	Heirloom	Illegal	Right temple	None	Lead round nose
5	88	Cellar	Unknown		Forehead	Vertex	Lead round nose
6	90	Cellar	Heirloom	Legal	Mouth	None	Lead round nose
7	72	Forest	Heirloom	Legal	Forehead	None	Lead round nose
8	53	Riverside		Illegal	Mouth	Vertex	Full metal jacket
9	60	Proximity of cemetery		Illegal	Right temple	Left temple	Full metal jacket
10	40	Forest	Duty pistol	Legal	Mouth	None	Action-4[a]
11	53	Bed	Sport shooting	legal	Right temple	Left temple	Full metal jacket
12	53	Couch	Hunting	Legal	Right temple	None	lead round nose
13	86	Car		Illegal	Left thorax	Left back	Semi jacketed
14	68	Car	Unknown		Forehead	None	Hollow-point
15	73	Living room	Sport shooting	Legal	Mouth	Vertex	Lead round nose
16	50	Home	Sport shooting	Legal	Mouth	Vertex	Full metal jacket
17	49	Hotel room	Hunting	Legal	Submental	None	Lead shot
18	60	Bureau	Hunting	Legal	Submental	None	Lead shot
19	46	Living room	Sport shooting	Legal	Mouth	Krönlein-shot[b]	Semi jacketed
20	77	Bed room	Free	Legal	Right temple	External soft tissue injury	Blank cartridge

[a] Action-4 is an expanding brass bullet (RUAG).
[b] Krönlein-shot: exenteration of the brain.

and used for forensic individualization. Moreover, we compared the performance of our experimental models to the extent of retention of victim DNA in gun barrels in real cases and we assayed whether real biological traces persist in gun barrels even after subsequent firing.

2. Materials and methods

2.1. Cases

Over a three year period 16 autopsy cases with fatal gunshot wounds and the respective firearms were prospectively collected. Two other cases in which the firearm was available passed an external post-mortem examination (cases no. 4 and 19). The oldest case dated from 2002, when the prosecution had ordered GSR-analysis. For comparison the Colt revolver had been confiscated, stored and forgotten (case no. 15). One weapon was examined while the victim was in clinical care (case no. 5). The characteristics of our case collective are displayed in Table 1.

2.2. Firearms

The characteristics of the firearms analysed in this study are summarized in Tables 1 and 2. In all cases except for case no. 1 sample collection was completed before the usual examinations by police firearms identification departments were performed. The two .357 Magnum calibre weapons – a revolver and a lever action rifle – had been loaded with .38 special ammunition. Two rifles had been sawed off long time ago. The origin or normal manner of use of the firearms, if known, is indicated in Table 1.

2.3. Sampling procedure

All samples were collected using sterile, DNA-free cotton swabs moistened with sterile, desalted water to wipe the inner surface of the firearms' barrels.

The swabbing procedure was refined during the study and a scheme for partial swabbing was established. As illustrated in Fig. 1 one half of the inner surface of the barrel was swabbed, both in the anterior and posterior part of the barrel. Then, a shot was fired using the same kind of ammunition that was originally used in the respective case.

Finally, second samples were collected by intensively swabbing the complete inner surface of the front and rear end of the barrel.

In some cases, the barrel was examined endoscopically using a 21.5 cm "Technoscope" (Karl Storz GmbH & Co., KG, Tuttlingen, Germany).

2.4. Means to avoid contamination

All work was conducted wearing gloves and an aerosolproof facemask.

Before collecting the samples, in all cases the muzzle was thoroughly cleaned with sterile water to prevent any contamination from introducing the swab into the barrel.

2.5. DNA extraction

After sampling, swabs dried for at least 2 h in a dark place at room temperature. DNA was extracted from all samples using the magnetic bead based PrepFiler Forensic DNA Extraction Kit (Applied Biosystems, Foster City, CA, USA), according to manufacturer's prescriptions.

2.6. DNA quantification and detection of PCR inhibitors

DNA concentration and the presence of PCR inhibitors were measured by quantitative PCR (qPCR) using the Quantifiler™ Human DNA Quantification Kit (Applied Biosystems). When a sample contained PCR inhibitors as indicated by an impaired amplification of an internal positive control, that sample was discarded.

Table 2
Weapons and calibres.

Type	Pistol	Revolver	Rifle	Shotgun
n	6	5	7	2
Calibre	.22 long rifle	.38/.357	9 mm	Other
n	7	5	3	5

Fig. 1. Partial swabbing procedure: for the sampling procedure only half the circumference is swabbed, samples are taken separately from the front and rear end of the barrel.

2.7. STR-multiplex-PCR and fragment detection

Four different forensic STR-multiplex-PCR-Kits dedicated to the profiling of challenging DNA samples were used in this study: AmpFlSTR® NGM SElect™ and MiniFiler™ PCR Amplification Kits (Applied Biosystems) and PowerPlex® ESX 17 and ESI 17 Systems (Promega, Madison, WI, USA). All kits were utilized following the instructions provided by the manufacturer. Summed up, the four kits cover 20 different STR-systems with multiple overlaps for several STR-systems as described previously [16]. Fragment detection was performed on a 310 Genetic Analyzer (Applied Biosystems), Data analysis was done using the GeneMapper software (v3.2) (Applied Biosystems).

3. Results

3.1. Characterization of the case collective

The collective of cases available for this study was very heterogeneous concerning weapon types and calibres (Table 2). In some cases weapons bore distinct blood stains on their surface, while others showed no visible traces. We observed no correlation between the stain pattern and weapon type, calibre or entry localization. In the majority of cases an autopsy was performed during which the typical morphology of an entry wound caused by a contact shot was established. In two cases the external examination of the deceased allowed ascertainment of a contact shot. In only one case the shot distance could not be established directly: an 83 year old man had shot himself to the forehead using a small calibre semi-automatic rifle and survived for several days in an emergency care unit. The endoscopic inspection of the barrel (Fig. 2) showed extended blood staining up to the whole reach of the endoscope (21 cm). For this case and two other cases in which rifles had been used (cases no. 4 and 19, see Table 1) no collection of GSR had been performed. For the remaining cases polyvinyl-alcohol gloves [12] or adhesive foils [17] were used to examine the shooters' hands. In all cases with the exception of one case (case no. 1) GSR with typical distribution as well as traces of backspatter were detected proving that the gunshot injury had been self-inflicted. Case no. 1 was discerned to be a case of homicide instead. Apparently, the offender held a small calibre pistol (Walther P 99) against the victim's left temple and killed him with a single shot.

The two shotguns analysed herein were double barrelled, however only one barrel had been fired in each case, respectively. For case no. 17 blood stains could be detected over the whole length of the upper barrel from which the shot was fired (Fig. 3). The distribution of the spatter was very discontinuous so that a secondary flow-in of blood could be excluded. The lower barrel was bloodstained only over the first few centimeters of the front end whereas the rear end proved to be free of blood.

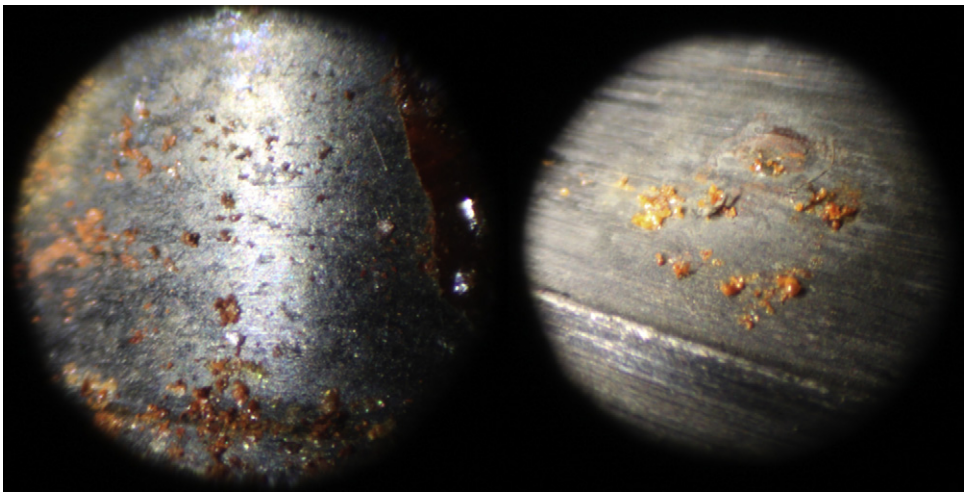

Fig. 2. Endoscopic view of the interior of the barrel of the small calibre rifle (case no. 5) showing intensive blood staining; left image: before sampling; right image: another location within the same barrel after a subsequent shot had been fired but before samples had been taken.

Fig. 3. Endoscopic view of the interior of the smooth barrel of the shotgun (case no. 17) rich with blood staining but without visible tissue.

3.2. Adaptive modifications of the sampling procedure

The present work was conducted as a prospective study. Analysing the first three cases (cases no. 12, 8 and 20) the inner surface of the whole barrel was thoroughly swabbed to maximize DNA yield. After a 'proof of principle' had been established by showing that successful STR-profiling in all these cases was possible, we modified the sampling procedure as described above and for the remaining cases only one half of the barrel was swabbed after the first shot (Fig. 1).

This procedure allowed for assaying the persistence of biological traces within the barrel even after subsequent firing. When endoscopic examination performed in several cases revealed that discontinuous biological traces were present even deep (>13 cm) within the barrel (Fig. 2), we again amended the sampling procedure to now also include sample collection from the rear end of the firearms. To minimize the disturbance of biological traces in the barrel, endoscopy was restricted to a short and careful inspection. Therefore a proper topographic analysis on neither the amount of blood or tissue nor of the ratio blood vs. tissue could be performed.

3.3. STR-typing results

The STR-typing results for all samples are summarized in Table 3. In all cases of suicide, a single STR-profile was obtained that fully matched the deceased's profile. Notably, even in case no. 11 (figure supplied as supplementary data) no mixed STR-profile was found. The victim in this case committed suicide by putting a shot through his right temple shortly after he had killed his wife by a short distance shot (2 cm) to her right temple using the same 9 mm Luger pistol.

Analysis after the second shot was performed to examine the persistence of the observed traces which were not completely

Table 3
Results.

Case	Calibre	Type	Barrel length (cm)	Area	Anterior part of the barrel				Posterior part of the barrel			
					First shot		Second shot		First shot		Second shot	
					DNA yield	STR typing	DNA yield	STR typing	DNA yield	STR typing	DNA yield	STR typing
1	.22 long rifle	Pistol	9	Temple	+	++	+	–	n.d.	n.d.	+	++
2	.22 long rifle	Pistol	11.5	Mouth	–	–	–	–	+	(+)	–	–
3	.22 long rifle	Rifle (sawed off)	20	Mouth	+	++	+	–	–	–	–	–
4	.22 long rifle	Rifle (sawed off)	25	Temple	+++	+++	(+)	–	–	–	(+)	–
5	.22 long rifle	Semi-automatic rifle	50	Front	+++	+++	+++	+++	+	++	+	++
6	.22 long rifle	Rifle	60	Mouth	+++	+++	++	++	n.d.	n.d.	n.d.	n.d.
7	.22 long rifle	Rifle	60	Front	+	(+)	+	++	++	++	+	++
8	7.65 mm	Pistol	10	Mouth	+++	+++	++	(+)	n.d.	n.d.	n.d.	n.d.
9	9 mm Makarov	Pistol	9	Temple	n.d.	n.d.	n.d.	n.d.	+	(+)	+	++
10	9 mm Luger	Pistol	10	Mouth	+++	+++	n.d.	n.d.	+	++	+	(+)
11	9 mm Luger	Pistol	11	Temple	+++	+++	++	++	+++	+++	(+)	–
12	.38 special	Revolver	5	Temple	++	+++	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
13	.38 special	Revolver	5	Left thorax	+++	+++	+	+++	(+)	++	+	–
14	.38 special	Revolver	6	Front	++	++	++	+++	+	++	(+)	++
15	.357 Magnum	Revolver	15	Mouth	n.d.	n.d.	+	++	n.d.	n.d.	+	++
16	.357 Magnum	Lever action rifle	61	Mouth	+	+++	+++	+++	–	–	–	–
17	12/70	Shotgun upper barrel	70	Submental	+++	++	++	++	+++	+++	++	+++
	12/70	Lower barrel	70	n.d.	+++	+++	+++	+++	–	–	+	–
18	12/70	Shotgun upper barrel	70	n.d.	++	+++	–	–	+++	+++	++	++
	12/70	Lower barrel	70	Submental	+++	+++	++	+++	+	++	++	+++
19	.30-06	Rifle	56	Mouth	++	+++	–	–	–	–	+	–
20	.380 blank	Revolver	6	Temple	++	++	n.d.	n.d.	–	–	n.d.	n.d.

Yield: n.d., not done; –, negative, (+) very low (<.001 ng/μl); +, low (.001–.01 ng/μl); ++, moderate (>.01–.05 ng/μl); +++, high (>.05 ng/μl).
STR-typing: n.d.: not done, – negative; (+) 1–7 STR-systems; ++, 8–16 STR-systems; +++, >16 systems.

removed or destroyed by this subsequent shot. In case no. 18 the lower barrel had been fired and examination of the barrel revealed an extended pattern of biological traces comparable to the one described for case no. 17. However, because this gun had been lying in a puddle of blood, a flow-in of blood was found in the rear end of the non-fired upper barrel. Also this gun showed macroscopically visible blood stains in both barrels even after subsequent firing.

4. Discussion

The investigation of cases of gun related death should typically be a collaborative effort of police and crime scene investigators, medical examiners and expert trace analytics [8]. This cooperation is well established in the medico-legal examination of crime scenes and corpses and also the weapons involved in such incidents should be an object of interdisciplinary examination. However, at present the main objectives of firearm investigation are to clarify the origin of a weapon, to characterize the projectile or cartridge case fired by it or to identify its handler by analysis of finger prints or DNA left on its surface. In contrast, there is no general awareness of the presence of biological traces *within* the firearm's barrel which, thus, is not explored by default in all cases of gun related deaths or injuries although it had already been reported early in the 20th century. For a case of homicide committed in 1922, Weimann described the identification of a revolver through evidential tissue particles found within its barrel [18]. Decades passed until Stone published a survey of 1200 firearms used in cases of suicide which had been probed for blood inside the barrel using leucomalachite green [19].

Ten years ago, we initiated systematic inspection of the barrels of firearms used in cases of suicide by utilizing technical endoscopes. While in most cases the morphological findings were positive, PCR amplification of short tandem repeats (STR) for identification purposes failed in about half of the cases. Samples of tissue or blood, retrieved from a firearm's barrel indeed present a challenge for molecular analysis. Such samples are most often of tiny size and have been exposed to the extreme temperature, pressure and friction associated with a gun shot. In addition, they are embedded in a sooty and oily matrix that contains high amounts of metallic particles. Dieltjes et al. already discussed the difficulties of extracting DNA from cartridges, bullets and casings [20].

By now, however, PCR based techniques outperform detection by eyesight since DNA extraction methods have been critically improved. Subsequent quantitative PCR analyses now enable a precise quantification of extracted DNA and can control for the presence of PCR inhibitors.

In the majority of our cases our DNA-extraction protocol yielded sufficient DNA resulting in successful amplification of all tested STR-systems. Only cases of shots to the mouth showed impaired STR-typing results. This may have a twofold explanation: firstly, in most cases, a shot into the mouth is not a proper contact shot to the palate because hardly can a firearm's muzzle be comfortably pressed against the palate (case no. 2). Secondly, our sampling procedure artificially reduced DNA yield per sample: as described above and illustrated in Fig. 1 the entirety of DNA recovered from a gun's barrel was deliberately divided in four portions (one swab per barrel half at the front and rear end of the barrel) thereby reducing the overall DNA yield per sample.

In some instances the firearm could not be examined immediately: in the case of homicide (no. 1) the pistol was placed at our disposal after test shooting. In the case of suicide committed by a police officer (case no. 10) we examined the inner surface of the firearm's barrel for biological traces. The inner surface of the weapon's front end was then thoroughly swabbed and the DNA yield was sufficient to generate a full STR-profile. Summarizing, the case parameters (type of gun and ammunition, entry wound location) as well as the sampling conditions are too heterogeneous to establish a correlation between calibre and DNA yield.

The presence of biological traces in gun barrels after delivering a contact shot is an established fact. Our results surpass the qualitative findings of Stone that were based on positive chemical evidence of blood [19]. In 1992, Stone found that 53% of revolvers and 57% of pistols were tested positive for blood after being used to deliver a contact shot. Thus, he concluded that a negative result does not necessarily indicate a shot having been fired from intermediate or distant range. On the other hand Stone cites MacDonell and Brooks [21] who claimed a distance of 76 mm or less to be sufficient for the formation of blood traces inside the barrel. This reasoning elicits two questions.

Can blood always be found inside the barrel after any contact shot? And up to how long a shot distance may blood still be found inside the weapon?

For all twenty cases direct muzzle contact with the body was proven to have occurred. With the above mentioned exceptions, full STR-profiles eligible for identification purposes were obtained in all cases, even in the case in which a revolver was used to deliver a shot to the heart through a sweat shirt (case no. 13) and in which endoscopic inspection of the short barrel had failed to reveal any visible biological traces.

However, the number of cases examined so far is too limited and the choice of weapons investigated herein is too heterogeneous to allow for any firm conclusions at present.

To evaluate the influence that calibre, gun and ammunition type have on amount, distribution and typability of DNA that can be recovered from a gun's barrel, we conducted another study, parallel to this work, comparing experimental contact shot scenarios over several self-devised ballistic models. We succeeded in emulating the backspatter that is generated by contact shots and showed that blood containing typable DNA can reproducibly be recovered from a firearm's barrel after a contact shot had been delivered [16]. However, much experimental investigation remains to be done, to better characterize and explain the heterogeneity of backspatter [10] and biological traces within the gun barrel.

Preliminary results of combined endoscopic examination and PCR-analysis [14,15] encouraged us to assay the persistence of DNA traces in gun barrels after a subsequent shot. Having refined a partial swabbing technique in our previous work [16], we applied it to the casework samples of this present study as described above and obtained successful STR-typing results in most cases even after a second shot had been fired through the barrel. This finding is of considerable practical importance because many incriminated weapons are tested by firearms identification service before biological traces have been secured.

Not only does DNA from biological traces in gun barrels exhibit a substantial resilience against the physical strain associated with gun blast, it also possesses a notable time-wise stability. This was documented by our successful STR-typing of DNA extracted from blood stains recovered from a revolver that had been kept in police custody for almost ten years (case no. 15).

Together, these findings suggest that even firearms from cold cases that had already been tested and fired by forensic ballistics long ago and that may have been stored away for several years now, may still contain vital forensic evidence in form of DNA persisting deep within their barrels.

Thus, our results underline the importance of consequent and comprehensive securing of evidence. The quantification of DNA extracted from the partial swabs documents that an artificial partitioning of a trace sample may result in partial STR-profiles (like case no. 2) which could foil an identification. Therefore, it is

advisable to collect as much trace material as possible and to pool all samples (anterior and posterior end of the barrel) as was recently proposed by Richert [22] to optimize the conditions for STR-typing.

Endoscopic inspection time and again showed a very discontinuous pattern of blood staining in gun barrels. The mechanism of how these traces get into the barrel has still not been elucidated yet, suction and negative pressure [13,18,23,24] have already been excluded [25]. Our reproducible observation that biological traces may be found even at the rear end of a firearm's barrel and up to 60 cm from the muzzle is a novel aspect that warrants further attention.

5. Conclusion

The inside of gun barrels is a contamination protected source of victim DNA whence it can be recovered after contact shots. A subsequent shot through the barrel does not necessarily destroy or remove biological traces and identification based on DNA from inside gun barrels may be possible even after several years of storage. We recommend to thoroughly swab the entire inner surface of the barrel after meticulous cleaning of the muzzle to prevent any contamination. The pooling of all samples taken from the barrel increases the chances of successful STR-typing.

Conflict of interest

None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fsigen.2012.05.008.

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