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ORIGINAL ARTICLE

Persistence of biological traces in gun barrels—an approach to an experimental model

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Abstract Traces of backspatter in gun barrels after homicidal or suicidal contact shots may be a valuable source of forensic evidence. Yet, a systematic investigation of the persistence and durability of DNA from biological traces in gun barrels is lacking. Our aim was to generate a realistic model to emulate blood and tissue spatters in gun barrels generated by contact gunshots at biological targets and to analyse the persistence and typability of DNA recovered from such stains. Herein, we devise and evaluate three different models for the emulation of backspatter from contact shots: a gelatine-based model with embedded blood bags, a model based on a spongious matrix soaked with blood and covered with a thin plastic membrane and a head model consisting of an acrylic half sphere filled with ballistic gelatine and with blood bags attached to the sphere under a 3-mm silicone layer. The sampling procedure for all three models: a first shot was fired with several types of guns at each model construction and subsequently a second shot was fired at a backstop. Blood samples were collected after each shot by probing the inner surface of the front and rear end of the respective gun barrel with a sterile swab. DNA was then extracted and quantified and up to 20 different short tandem repeat (STR) systems were amplified to generate DNA profiles. Although DNA quantity and STR typing results were heterogenous between the models, all models succeeded in delivering full STR profiles even after more than one shot. We conclude that biological traces in gun barrels are robust and accessible to forensic analysis and that systematic examination of the inside of gun barrels may be advisable for forensic casework.

Keywords Forensic genetics · Forensic ballistics · Victim DNA · Firearms · STR typing

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Introduction

Most forensic efforts to retrieve DNA from firearms are dedicated to the purpose of identifying the firearm's wielder. Therefore, those efforts are mainly focused on skin cells adherent to the weapon that have been shed by handling the object [1].

However, evidential DNA recovered from firearms that have been used to injure does not necessarily have to stem from the person who fired the gun. For instance, backspatter of blood and tissue originating from a wound caused by a contact shot in which a firearm's muzzle is held directly against a victim's body may be propelled into the firearm's barrel and persist there. Such traces may prove to be an important source of a victim's DNA. It is easily conceivable that the DNA-based connection of a gunshot victim to a particular weapon can be an invaluable cue for criminal proceedings. Not only may DNA retrieved from a firearm's barrel serve to identify a victim, its presence in the barrel may also help to discern how the victim was shot and it may even be possible, by determining the age of the blood stain, to estimate when the shooting took place.

In 1996, Karger et al. proved that PCR amplifiable DNA could be recovered from bullets that had been fired into calves. They hypothesised about the possibility of connecting

a bullet with an individual target that had been hit by it by analysing the individual target's DNA recovered from the bullet [2]. Also, in a 1997 study, Karger et al. were first to analyse human victim DNA retrieved from three penetrating bullets that had been recovered from a crime scene and they succeeded in identifying the victims based on short tandem repeat (STR) typing results from the collected DNA [3]. Two more recent studies introduce and analyse methods to recover DNA from firearms and ammunition to identify the individual that handled them [1, 4]. However, up to date, after thoroughly browsing the literature and to the best of our knowledge, there has been no effort to locate and characterise human victim DNA in firearms.

Thus, herein, we present the first systematic evaluation of integrity and profilability of victim DNA extracted from trace amounts of blood and/or tissue from backspatter generated by contact shots. To that aim, we devised, evaluated and compared three different models for the emulation of backspatter as caused by contact shots and analysed whether profilable DNA can be retrieved from different parts of the firearm's barrel. Additionally, we investigated if biological traces in gun barrels can endure subsequent shots fired through the weapon following the first and still yield typable DNA.

Material and methods

Blood samples

Blood samples were taken by venipuncture. EDTA (1.6 mg per ml blood) was added to prevent the blood from clotting. Blood was stored at 4°C until used. All blood samples used in this study were donated by adult, informed and consenting volunteers who had no physical contact whatsoever with any firearm and model construction used in this study.

Firearms

The characteristics of the firearms used in this study are summarised in Table 1.

Table 1 Characteristics of the firearms

Firearm			Ammunition	Length of barrel (cm)
Brand	Type	Calibre		
Franchi	R	.38 Spec.	CBC	15
Taurus	R	.22 LR	RWS	15
Astra	R	.38 Spec.	CBC	10
H&R	R	.22 LR	RWS	15
Beretta 76	P	.22 LR	RWS	15
Voere	Rf	.22 LR	RWS	55
Marlin	Rf	.22 Mag	RWS	55
Krico	Rf	.22 LR	RWS	55
Ruger	R	.357 Mag	CBC	15
S&W	R	.357 Mag	CBC	13.75

R revolver, *P* pistol, *Rf* rifle, *Spec.* special, *LR* long rifle, *Mag* magnum, *CBC* CBC Wadcutter .38 Special, *RWS* RWS lead round nose .22 long rifle

Sampling procedure

All samples were collected using sterile, DNA-free cotton swabs moistened with sterile, desalted water to wipe the inner surface of the barrel of the firearm. The first shot fired at each model construction was performed as a contact shot with the gun held directly to the model construction. The subsequent shot was not fired at the model construction but instead into a backstop.

Samples were collected from the inner surface of the front and rear end of the barrel immediately (models I and II) or 2 days after (model III) the first shot had been fired by the gun and again immediately (models I and II) or 2 days (model III) after the second shot had been fired. If the barrel length was 15 cm or less (Table 1), half of the barrel length was swabbed from the front end and the rear end, respectively. If the barrel length was 55 cm (Table 1), the barrel was swabbed up to a depth of 13 cm from both ends. In some cases, the sample's condition within the barrel was examined endoscopically using a 'Technoscope' (Karl Storz GmbH & Co. KG, Tuttlingen, Germany) before swabbing.

Also, after the first shot, only one half, e.g. the left half, of the inner surface of the barrel was swabbed, and after the second shot, the remaining half was swabbed. When the samples were taken from the front end of the barrel, any contact to the muzzle was carefully avoided to prevent contamination. After sampling, swabs were dried for at least 2 h in a dark place at room temperature.

DNA extraction

DNA was extracted from all blood samples using the magnetic bead-based PrepFiler™ Forensic DNA Extraction Kit (Applied Biosystems, Foster City, CA, USA), according to manufacturer's prescriptions.

DNA quantification and detection of PCR inhibitors

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

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 (Table 2). Fragment detection was performed on a 310 Genetic Analyzer (Applied Biosystems), and data analysis was done using the GeneMapper™ software (v3.2) (Applied Biosystems).

Three models

Model I Gelatine: This model consisted of a casted cuboid block made of ballistic gelatine 'Ballistic I' (Gelita, Eberbach, Germany) into which a foiled blood bag had been embedded during the casting process so that the blood bag was positioned in parallel to and several millimetres below the surface of the block.

Model II Spongious matrix: The second model was a spongious and absorbent matrix (e.g. a bath sponge) soaked with sample blood and then clad into a thin plastic membrane.

Model III Acrylic sphere: The third and most elaborate model is described elsewhere [5]. It was designed to mimic the physical properties of a human head. Briefly, the model comprised a roughly head sized acrylic half sphere filled with ballistic gelatine 'Ballistic I' to which two blood bags were attached and held in place by a 3-mm layer of silicone membrane (Fig. 1).

Ballistic gelatine for models I and III was cast from a 10% solution of gelatine in desalted water. After casting, the gelatine solidified for 48 h and was stored at 4°C.

Means to avoid contamination

All work was conducted wearing gloves and an aerosol-proof face mask. Weapons that were used in more than one model were thoroughly cleaned after having been used in one and before being used in another model. Briefly, a special cleaning rod with attached barrel cleaners of woollen felt (VFG, Giengen, Germany) that had been soaked with purpose-made ballistic oil 'Ballistol' (F.W. Klever GmbH, Aham, Germany) was used to soundly rub and wipe the barrel from the inside. This procedure was sufficient to quantitatively remove any traces of typable DNA from

Table 2 STR systems covered by four different STR Multiplex PCR kits

System / Kit	D3S1358	vWA	D16S539	D2S1338	D8S1179	D21S11	D18S51	D19S433	TH01	FGA	D10S1248	D22S1045	D2S441	D1S1656	D12S391	SE33	CSF1PO	D13S317	D7S820	Amelogenin
NGM-SE																				
MiniFiler																				
ESI	_a	_b	_a		_a	_b	_b	_b	_a	_b										
ESX	_a	_b			_b	_b		_b	_b		_a	_a	_a	_b	_b					

Grey shade: covered by the respective kit

[a] Mini-STR (<150 bp)

[b] Midi-STR (150–250 bp)

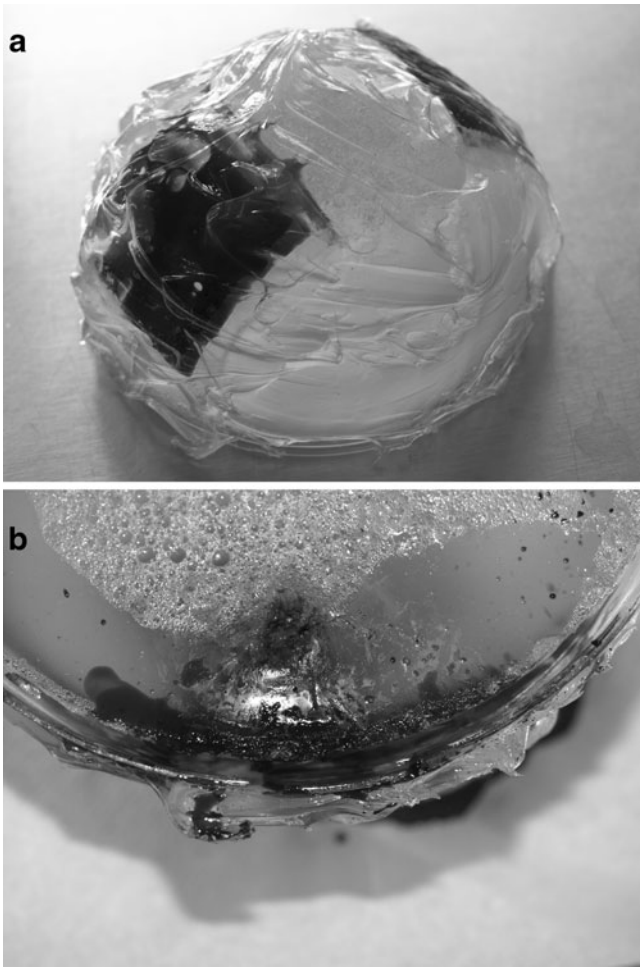

Fig. 1 Photography of model III (acryl half sphere head model). **a** A 'shot ready' model III construction. **b** Close-up of point of entry after the first shot had been fired at the model

the barrel (data not shown). To be able to detect cross-contamination in weapons used in more than one model, blood of a different person was used for each model.

Results

Optimization of sampling procedure

Sampling procedure had to be modified for model III because DNA quantity and consequently STR typing success were extremely low when sample was collected immediately after first and second shots (Table 3). For the modified procedure, we waited for 2 days before swabbing the barrel after the first and second shot to give the sample time to dry to a point that would mend reduction of DNA yield. However, endoscopic control revealed that even 2 days after the first shot, the samples had not dried completely. The reported results for model III thus have been obtained using the modified sampling procedure.

Table 3 Model III—comparison of swabbing immediately after versus 2 days after shot

Time (h)	Weapon	Calibre	STR typing results			
			First shot		Second shot	
			Front end	Rear end	Front end	Rear end
0	Franchi	.38 Spec.	−	−	−	−
	Taurus	.22 LR	−	−	−	−
	Astra	.38 Spec.	+++	−	−	(+)
	H&R	.22 LR	−	−	−	−
48	Franchi	.38 Spec.	+++	−	−	−
	Taurus	.22 LR	+++	−	−	−
	Astra	.38 Spec.	++	+++	−	−
	H&R	.22 LR	+++	−	−	−

Spec. special, *LR* long rifle

− negative, (+) 1–7 STR systems, ++ 8–16 STR systems, +++ all tested STR systems

DNA quantity

The results for DNA quantification for all samples across all models are summarised in Table 4. In general, more DNA could be obtained from swabbings of the front end and after the first shot as compared to swabbings from the rear end

Table 4 Results of DNA quantification

Model	Weapon	Calibre	First shot		Second shot	
			Front end	Rear end	Front end	Rear end
I	H&R	.22 LR	−	−	−	−
	Taurus	.22 LR	+	−	−	(+)
	Beretta 76	.22 LR	+	−	+	−
	Voere	.22 LR	+	+	(+)	−
II	Franchi	.38 Spec.	++	−	−	+
	Taurus	.22 LR	+	−	+	−
	Astra	.38 Spec.	+++	++	++	+++
	H&R	.22 LR	+	−	+	−
III	Franchi	.38 Spec.	+++	+	+	−
	Taurus	.22 LR	+	−	++	−
	Astra	.38 Spec.	+	+	+	−
	H&R	.22 LR	++	−	−	−
	Marlin	.22 Mag.	+++	+++	+++	+++
	Krico	.22 LR	+++	+	+++	−
	Ruger	.357 Mag.	++	−	+	−
	S&W	.357 Mag.	+++	+	+	+

Spec. special, *LR* long rifle, *Mag* magnum, − negative, (+) very low (<0.001 ng/μl), + low (0.001–0.01 ng/μl), ++ moderate (>0.01–0.05 ng/μl), +++ high (>0.05 ng/μl)

and after the second shot, respectively. DNA yields produced by model I were lowest while model II and model III produced comparable results for the weapons that have been used in both models, with model II doing even better for swabbings from the rear end after the second shot. However, averaged over all tested weapons, model III produced the highest DNA yield in three of four 'categories' (first and second shot from the front end, first shot from the rear end). Figure 2a shows a graphic comparison of average model performance.

STR typing results

The results for STR typing for all samples across all models are summarised in Table 5. In general, all models in at least two of four categories succeeded in producing blood traces sufficient for the generation of a full STR profile, i.e. consisting of eight or more STR systems. Notably, in no case, a partial profile with seven or less successfully typed STR systems has been obtained. Typing success for model I was lowest (4 of 16 possible STR profiles). Models II and III both achieved a 43% success rate with 7 of 16 and 14 of 32 possible STR profiles, respectively. However, model III showed a better average performance for swabbings after the first shot while model II did better on average for swabbings after the second shot. Figure 2b depicts a graphic comparison of average model performance. Also, as was assured by comparison with the respective blood donor's STR profile, contamination, indicated by a mixed profile, occurred in no case.

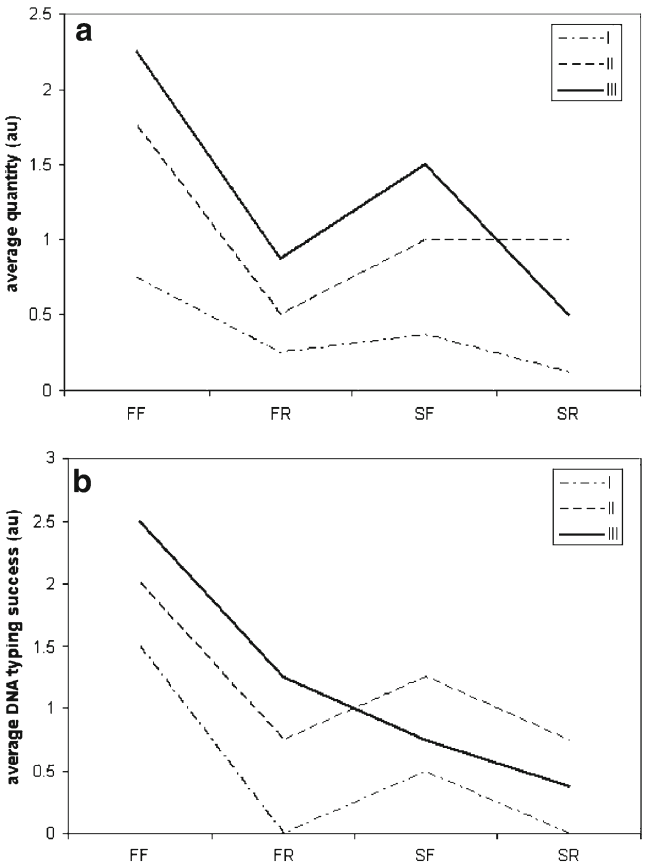

Fig. 2 Semiquantitative graphic representation of model performance. *au* arbitrary unit; *FF* first shot, front end; *FR* first shot, rear end; *SF* second shot, front end; *SR* second shot, rear end; *I* model I; *II* model II; *III* model III. **a** Results of DNA quantification; values were derived by adding 1 for each '+' and 0.5 for each (+) (Table 2) and division by the number of tested weapons per model. **b** Results of STR typing; values were derived by adding 1 for each '+' (Table 3) and division by the number of tested weapons per model

Table 5 Results of STR typing

Model	Weapon	Calibre	STR typing results			
			First shot		Second shot	
			Front end	Rear end	Front end	Rear end
I	H&R	.22 LR	−	−	−	−
	Taurus	.22 LR	++	−	−	−
	Beretta 76	.22 LR	++	−	−	−
	Voere	.22 LR	++	−	++	−
II	Franchi	.38 Spec.	++	−	−	−
	Taurus	.22 LR	+++	−	++	−
	Astra	.38 Spec.	+++	+++	+++	+++
	H&R	.22 LR	−	−	−	−
III	Franchi	.38 Spec.	+++	−	−	−
	Taurus	.22 LR	+++	−	−	−
	Astra	.38 Spec.	++	+++	−	−
	H&R	.22 LR	+++	−	−	−
	Marlin	.22 Mag.	+++	+++	+++	+++
	Krico	.22 LR	+++	++	+++	−
	Ruger	.357 Mag.	−	−	−	−
	S&W	.357 Mag.	+++	++	−	−

Spec. special, *LR* long rifle, *Mag.* magnum, − negative, (+) 1–7 STR systems, ++ 8–16 STR systems; +++ all tested STR systems (17–20)

Correlation of DNA quantity with successful STR typing

In any respective event over all models, a moderate (++) or higher DNA concentration was always associated with a result of eight or more typable STR systems (++ or +++). In the same manner, a negative DNA concentration (−−) always coincided with a negative typing result (−−).

Discussion

The detection, recovery and analysis of a victim's DNA from the barrel of firearms that have been used to deliver a contact shot to effect bodily injury such as in homicidal or

suicidal shootings may provide valuable forensic evidence. The most obvious avail of the analysis of biological traces rescued from a firearm's barrel is the possibility to establish a DNA-based connection between that specific firearm and the person that has been shot by it. This would be possible even if a ballistic analysis is not feasible due to absent, destroyed or severely deformed projectiles. Also, such analyses can be done for a long time after a person has been shot. We were able to generate full STR profiles from bloodstains that had persisted in barrels of guns that had been kept in police custody for several years (data not shown).

Secondly, the analysis of biological traces in the barrel of a firearm may also hint at the distance from which a person has been shot by it: if a minimally required shot distance for traces of backspatter in a gun's barrels to occur can be determined, the presence of such traces in a gun barrel may serve as an indicator for the maximal possible shot distance in a case of gunshot injury. Such can be crucial evidence for the reconstruction, ordering and interpretation of the events in a gunfight or shooting.

Thirdly, it is even conceivable to narrow down on a period of time during which the victim was shot by determining the age of the blood or tissue stains recovered from the barrel. This may for instance be done based on the assessment of the differential degradation processes for different RNA species in the blood or tissue samples [6–8].

However, before now, no systematic evaluation of profilability and persistency of victim DNA extracted from blood recovered from gun barrels has been undertaken. In this study, we present three self-devised models for the simulation of backspatter from a contact shot situation and demonstrate that recovery of typable DNA from bloodstains in gun barrels is feasible and not even barred by exposing such stains to the physical strain of a second shot fired through the barrel.

Although there was a somewhat heterogenous distribution of success rates for the production of sufficient DNA quantity and successful STR profiling between our models, all three models to a different extent admit of the conclusion that biological material containing DNA of sufficient quantity and quality for STR typing can be retrieved from a firearm's barrel after a contact shot and that the whole barrel may serve as a source of such material.

In addition, there is now evidence that typable DNA can well be recovered from a firearm's barrel even after a subsequent or 'cleaning' shot has been fired through the barrel proving that the physical and chemical conditions associated with a gun blast (heat, pressure and muzzle gases) do not necessarily destroy DNA beyond analyzability.

Clearly, the number of tested samples per model in this study was too small to warrant any conclusions regarding a significantly better performance of one model or the other. However, even from these limited data, it may be hypothesised that there is a large influence of weapon type, type and calibre of the projectile, type of model and even the combination of weapon type and model on DNA yield and rate of successful STR typing.

A considerable challenge for any effort of modelling backspatter and collecting samples in an experimental setting is to emulate blood clotting. As blood clotting does not normally occur in sample blood, splashes of such blood that have been propelled into a firearm's barrel by force of projectile impact take a comparably long period of time to dry. If this time is not conceded before sample collection, the still liquid droplets of blood may be blasted out of the barrel by force of subsequent shots and DNA yield for the successive swabbing can be critically reduced as was the case for our model III.

Thus, to decide which model makes for the closest approximation to reality and how to realistically simulate blood clotting, a direct and systematic comparison with real casework samples may be indispensable. From preliminary experiments it seems, though, that all our models in fact even underestimate volume and dispersion of traces by backspatter that can be found in gun barrels after 'real life' contact shots (data not shown). In any event, from the correlation data explained above, we can state that for practical reasons also in actual casework samples, execution of an upstream qPCR-based quantification of trace DNA seems to be advisable as a negative quantification result very probably indicates that any following STR typing effort for such sample will be futile.

We did not investigate whether DNA typability may be compromised solely by the material (mostly metal) the firearm is made of. This could be done by artificially depositing a defined volume of blood into the barrel of a 'cold' gun, i.e. that had not been and will not be fired afterwards. This situation may differ from collecting samples from a 'hot' weapon, like it was done in our experiments, as there possibly is an influence of muzzle gases on droplets of backspatter [9] and thus consequently on DNA quality; however, this has not been investigated, yet.

Conclusion

Herein, by simulating backspatter from contact shots and comparing three different self-devised models, we are first to show that recovery of typable victim DNA from bloodstains within gun barrels is possible and that fully typable DNA in a gun barrel may endure even the physical strain produced by subsequent shots. Therefore, we deem it to be highly advisable not to neglect biological traces within firearms that have been used to cause bodily injury.

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Declaration We hereby declare that all blood samples have been provided by consenting and informed volunteers and that all experiments performed in this study comply with the current German laws.

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