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ORIGINAL ARTICLE

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Trajectory reconstruction from trace evidence on spent bullets

II. Are tissue deposits eliminated by subsequent impacts?

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Abstract STR-based individualisation of biological deposits on bullets after perforation of tissue, can identify the person injured or killed by a particular bullet and comparison with the firearms used can identify the weapon and thus possibly the person who did the shooting. In this study, the effect of subsequent impacts on intermediate targets such as loss of cells was investigated by amplification of mitochondrial (mt) DNA. Bovine tissue was perforated and the 9 mm Luger FMJ bullets were recovered from the bullet collector. The mt cytochrome-b (cyt-b) gene could be amplified by the polymerase chain reaction (PCR) from 14 out of 15 bullets. Examination with a scanning electron microscope (SEM) and an energy-dispersive X-ray spectrometer (EDS) demonstrated the presence of minute dried tissue deposits on all bullets ($n = 10$) but was not able to establish preferential locations. In a series of 25 gunshots, various intermediate targets (glass, wood, car metal, gypsum board, asphalt) were perforated/impacted following perforation of tissue and the cyt-b gene could be typed from all bullets. It is concluded that subsequent impacts on intermediate targets do not eliminate enough biological deposits to render DNA analysis impossible and that the amplification of mtDNA is a useful additional method.

Keywords Trace evidence · Bullet · STR · PCR · Gunshot wound

Introduction

Human trace evidence on bullets has been investigated by routine cytological methods (Nichols and Sens 1990, 1991; Knudsen 1993) but individualisation is not normally possible with this method and cells are rarely found on FMJ bullets. However, individualisation of tissue deposits on FMJ bullets was successfully carried out by PCR typing of DNA in both experiments (Karger et al. 1996) and case work (Karger et al. 1997).

Increased experience with this method has directed attention to subsequent impacts which may cause loss of cells. The limited number of cells adhering to the smooth surface of FMJ bullets may be lost in violent impacts, in particular if the bullet hits hard intermediate targets (such as those used in part I of this paper). Also, various environmental factors such as rain, high temperature, animals or metal elements from the bullet can lead to a loss of cells and/or to degradation of DNA and thus to a further decrease in the amount of DNA available for analysis. The present study has investigated the effect of subsequent perforation of various intermediate targets on the recovery of DNA and the analysis of mitochondrial DNA was evaluated. Additionally, SEM/EDS was used to search for preferential locations of tissue deposits.

Materials and methods

Whole organs (heart, kidneys) and thoracic specimens (ribs, muscle, fat and skin) from cattle obtained from an industrial slaughterhouse, were combined in layers approximately 10 cm in thickness, which corresponded to the length of the missile channel. The ballistic set-up is described in detail in the first part of this paper: pistol SIG-Sauer P225, 9 mm × 19 Luger FMJ round nose bullets (8.1 g, 350 m/s; Geco), distance from muzzle to tissue 1 m, distance between two subsequent targets (if present) 50 cm, bullet collector from newspaper or cotton. The SEM analysis including evaporation with carbon (Stereoscan S180, Cambridge, UK) and the EDS analysis (beryllium window detector, PGT) are also described in part I.

After screening under a stereo-microscope, the anterior and posterior portion of the bullets (tip vs. base) were swabbed separately with the tip of a cotton wool swab soaked with 0.1 ml of sterile bidistilled water. In the case of asphalt, both the jacket and core were swabbed. The swabs were transferred to 1.5 ml screw-cap Eppendorf tubes. DNA extraction was carried out with Chelex 100 and proteinase K (Wiegand et al. 1993) with the modification that the extraction was carried out overnight at 37 °C. The extracts were ultrafiltered and concentrated to 10 µl with microcon-100 (Amicon). A portion of the mitochondrial cytochrome-b gene (cyt-b)

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Table 1 Summary of the 50 gunshots fired in this study

Number of gunshots	Target(s)	Method	Summary of results
15	Tissue	PCR (cyt-b)	Positive in 14 cases
10	Tissue	SEM/EDX	Positive in 10 cases, no preferential sites
5	Tissue + glass	PCR (cyt-b)	Positive in 5 cases
5	Tissue + pine	PCR (cyt-b)	Positive in 5 cases
5	Tissue + car wing	PCR (cyt-b)	Positive in 5 cases
5	Tissue + asphalt	PCR (cyt-b)	Positive in 5 cases
5	Tissue + gypsum board	PCR (cyt-b)	Positive in 5 cases

was amplified with the primers published by Parson et al. (2000). The PCR reactions were performed in a total volume of 25 µl with primers (1 µM each), $MgCl_2$ (1.5 mM), BSA (12 µM), 1 × reaction buffer (Eurogentec, Seraing, Belgium), dNTP (0.2 mM each) and Taq DNA polymerase (1 U, Eurogentec). PCR conditions were 30 cycles of 94 °C, 50 °C and 72 °C (1 min each), followed by a final extension step of 72 °C for 10 min. Amplifications were performed in a Trioblock thermocycler (Biometra, Göttingen, Germany). Non-denaturing horizontal polyacrylamide gel electrophoresis with subsequent silver staining was performed as described earlier (Karger et al. 1996, 1997).

In a first series of 15 gunshots fired into bovine tissue, the cyt-b gene was amplified and in a second series 10 bullets were examined by SEM/EDS (Table 1). In a third series of 25 gunshots, 2 targets were aligned in sequence and after perforation of tissue, various intermediate targets (described in detail in part I, e.g. glass, gypsum board, untreated pine wood, car wing) were perforated or a ricochet from asphalt was produced and the swabs from the bullets were typed for the cyt-b gene (Table 1).

Results

In the first series, the PCR reaction was positive in 14 of the 15 swabs taken from the tip of the bullet and in 7 of the 15 swabs from the base (Table 1). Most bands obtained from swabs originating from the tip were more intense than those from the base. No tissue remnants could be detected by direct light microscopy but SEM visualised typical dried tissue deposits on every bullet (Fig. 1). A preferential location of the small tissue deposits could not be ascertained. EDS analysis demonstrated a variety of elements and the major peak was frequently Ca. The subsequent perforation of tissue and of 5 different intermediate targets yielded positive results for the cyt-b system in all 25 bullets fired (Table 1). A clear difference between tip and base could not be observed. The strongest bands were present after ricochet from asphalt and the perforation of a car wing resulted in relatively weak bands. The detection limit of the cyt-b system was estimated by a dilution series to be ca. 10 templates.

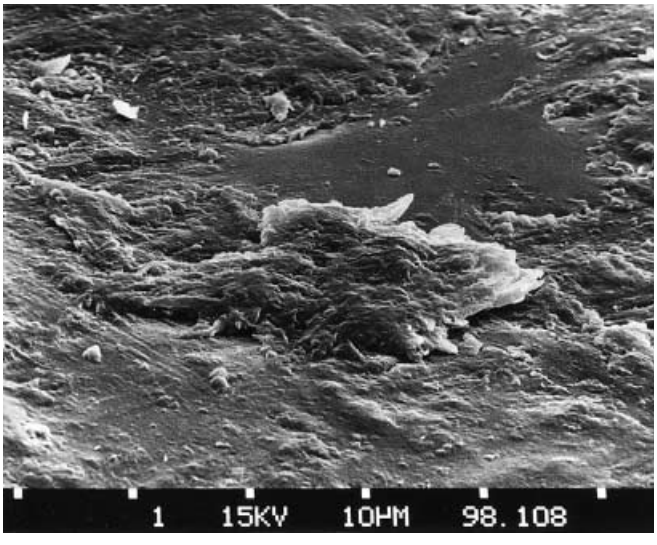

Fig 1 Scanning electron microphotograph of scaly tissue deposits on a bullet after a gunshot to bovine tissue. The tissue appears to be dried out

Discussion

The subsequent intermediate targets used in this study (and the bullet collector) caused violent secondary impacts involving considerable forces acting on the jacket and adhering tissue fragments. However, the cyt-b gene could be typed from all bullets fired. This clearly demonstrates that subsequent impacts do not eliminate enough cells to render DNA analysis impossible, not even in the case of FMJ bullets. Exsiccation of the tissue deposits may increase the adhesive forces to the jacket and also represents favourable conditions for the conservation of DNA. Exsiccation is caused by the hot surface of the bullet, which is thought to briefly reach temperatures of approximately 100–200 °C (Sellier 1982; Marty et al. 1994).

Typing of mtDNA has been shown to be successful in difficult cases such as ancient tissue (Handt et al. 1994), extremely charred corpses (Lutz et al. 1996) or hair shafts (Hühne et al. 1999; Pfeiffer et al. 1999). The high sensitivity of mtDNA may compensate to a certain extent for subsequent loss of cells and degradation of DNA, even if a limited number of cells was originally transferred to the bullet. It is therefore suggested that mtDNA should also be used for typing of human tissue traces on bullets as long as the victims are not maternally related.

Neither visualisation by SEM nor separate swabbing was successful in establishing preferential locations of cells on bullets. From this study it appears that cells are more likely to be found on the anterior portion but a pre-

liminary study (Karger et al. 1996) suggested that the base is the preferential location. In any case, "blind" swabbing of the entire FMJ bullet instead of a visual search for tissue is certainly the most promising way of collecting material for analysis.

DNA analysis of tissue deposits on spent bullets therefore represents a sensitive method capable of demonstrating and at the same time individualising minute tissue fragments transferred during perforation of a human body. This makes it possible to determine who was killed or injured by which bullet. Additional comparison of the bullet with the firearms involved can possibly identify the person who fired this particular bullet. The recovery of cellular material by swabbing the bullet can be easily and rapidly performed so that further examinations will not be delayed. Prior cleaning or contamination of the bullet of course is contra-indicated.

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