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ORIGINAL ARTICLE

MicroCT detection of gunshot residue in fresh and decomposed firearm wounds

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Abstract Gunshot residue (GSR) evidence may be altered or obscured by after-death events such as putrefaction, autolysis, and/or damage by animals. The present study aimed at evaluating and comparing the amount and differential distribution of GSR utilizing microcomputed tomography (microCT) analysis of fresh and decomposed gunshot wounds. A total of 60 experimental shootings at three different firing distances (5, 15, and 30 cm) were performed on human calves surgically amputated for medical reasons. Thirty specimens (10 for each tested distance) were immediately formalin-fixed, while the other 30 specimens were enclosed in a cowshed for 15 days, before formalin fixation (air temperature ranging from 11°C to 38°C). MicroCT analysis with three-dimensional image reconstruction detected GSR particles in all the investigated entrance wounds. In fresh specimens, GSR was concentrated on the skin surface around the entrance hole and in the epidermis and dermis layers around the cavity, while in decomposed specimens, the high density particles were detected only in the dermis layer. No GSR was detected in exit wounds of both fresh and decomposed specimens regardless of the tested firing distance. Statistical analysis demonstrated that also in decomposed wounds the amount of GSR roughly correlated with the distance from which the gun was fired, exhibiting, however, a higher variability than in fresh samples. The obtained results suggest that microCT analysis can be a valid screening tool for differentiating decomposed entrance and exit gunshot wounds.

Keywords Terminal ballistics · Gunshot residue · GSR · Putrefaction · MicroCT

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Introduction

Gunshot residue (GSR) consists of particles composed of antimony, barium and lead that arise from the condensation of primer vapors [1] and also sooting debris consisting of carbon and metallic fragments from the bullet and cartridge case [2]. When the reconstruction of gunshot fatalities is in question, the macroscopic examination of gunshot wounds [3–8] as well as the investigation of GSR particles gains extensive forensic significance [9–12]. Recently, microcomputed tomography (microCT), a radiological technique with a resolving power in the micrometer range [13], has been applied to the analysis of GSR for the determination of the shooting distance in intermediate range gunshot wounds [14].

Although it is well-known that after-death events (such as damage by animals, environmental effects, and putrefaction/autolysis changes) may alter or obscure gunshot evidence, only

a few experimental studies on the modifications of the GSR distribution after the body starts to decompose do exist [15–18].

The present work aims at evaluating and comparing the amount and differential distribution of GSR on fresh and decomposed samples utilizing microCT analysis on entry and exit gunshot wounds experimentally produced on human calf sections.

Materials and methods

Firing trials and sample collection

After approval of the Ethical Committee of the University Hospital of Padova (Protocol n. 2013P), 60 sections of approximately 6 cm in length were obtained from human calves, surgically amputated for medical reasons (circulatory disorders, road or workplace trauma). Inclusion criteria were male gender and age between 20 and 50 years. Exclusion criteria were neoplastic and/or inflammatory and/or infective diseases of the skin, scars, and traumatic skin wounds. Three hours after surgical amputation, each calf was washed to remove dirt, dried blood and any other contaminants from the skin surface, and kept frozen at −20°C until the day before the shooting experiments, when the samples were defrosted in open air for 12 h.

The study was conducted during the month of July, in the countryside around Padova (Veneto region, Italy). The shooting experiments were all carried out in the same day, using a .32 ACP pistol (Beretta Mod. 81) loaded with full-jacketed bullets (7.65×17 mm, Browning SR) and placed on a fixed stand with the muzzle perpendicular to the skin surface; all the ammunitions came from the same production lot. A total of 60 shots were performed, 20 at 5 cm, 20 at 15 cm, and 20 at 30 cm distance (Fig. 1a).

After the firing tests, 30 calf sections (10 for each tested firing distance) were immediately fixed in formalin (4%, pH 7.4) for 3 days, while the other 30 calf sections were enclosed in a wooden box covered by a mosquito net to avoid any maggot infestation and placed in a cowshed for 15 days before formalin fixation. The decomposing changes affecting the gunshot lesions were documented at 24-h intervals using a digital camera (Nikon D-90 equipped with AF-S Nikkor 18–105 mm ED, Nikon Corporation, Shinjuku, Japan). The ambient air temperature, recorded hourly, ranged from a high of 38°C to a low of 11°C (Fig. 1b). The relative humidity ranged from a high of 100% to a low of 25% (Fig. 1f).

Gunshot microCT analysis

Two specimens, comprising the epidermis, dermis and subcutaneous fat around the entry and exit holes were obtained from each calf section, cut into parallelepipeds (height 1 cm, side 1 cm) with the gunshot wound in the middle, and then located in a cylindrical polyethylene container (1.1 cm diameter). All specimens were scanned using a Skyscan 1172 High Resolution Micro-CT (Skyscan, Aartselaar, Belgium).

The amount of GSR and the 3D reconstruction of the gunshot wounds were calculated with the method previously reported by the authors [14]. Briefly, since GSR deposits are mainly formed of heavy metal particles (i.e., barium, antimony, lead, etc.), their percentage was calculated as the proportion of the volume of interest (VOI), occupied by binarized solid objects with a density higher than 1,000 HU [14]. After microCT analysis, each sample was embedded in paraffin, sectioned by microtome in 5-μm slices, deparaffined, hydrated by distilled water, stained with hematoxylin–eosin, and examined with a Leica DM-4000B optical microscope (Leica, Cambridge, UK).

Statistical analysis

For each type of sample (fresh and decomposed) and for each firing distance tested, the amount of GSR particles was expressed as mean±standard deviation (Table 1). Comparison between mean GSR values of fresh and decomposed samples was performed with the two-way ANOVA test. Subsequent analysis of mean GSR percentages at the three tested firing distances (5, 15, and 30 cm) between the two groups (fresh and decomposed samples) was conducted with the Bonferroni *t*-test.

Results

Visual inspection and histological investigation

The fresh entrance lesions exhibited the typical characteristics of intermediate range gunshot wounds [3]; at close examination, individual powder particles embedded in the skin were always evident and the intensity of the skin blackening decreased with the increase of the firing range as expected (Fig. 1d).

Over the course of the experimental period of observation, characterized by skin slippage (confirmed also by histological investigation that revealed an extensive detachment of the epidermis of the gunshot wounds, Fig. 1g), powder particles and blackening became less visible and, consequently, the entrance wounds inflicted at different firing distances (5, 15, and 30 cm) became very similar at inspection and difficult to be distinguished from the exit wounds (Fig. 1d).

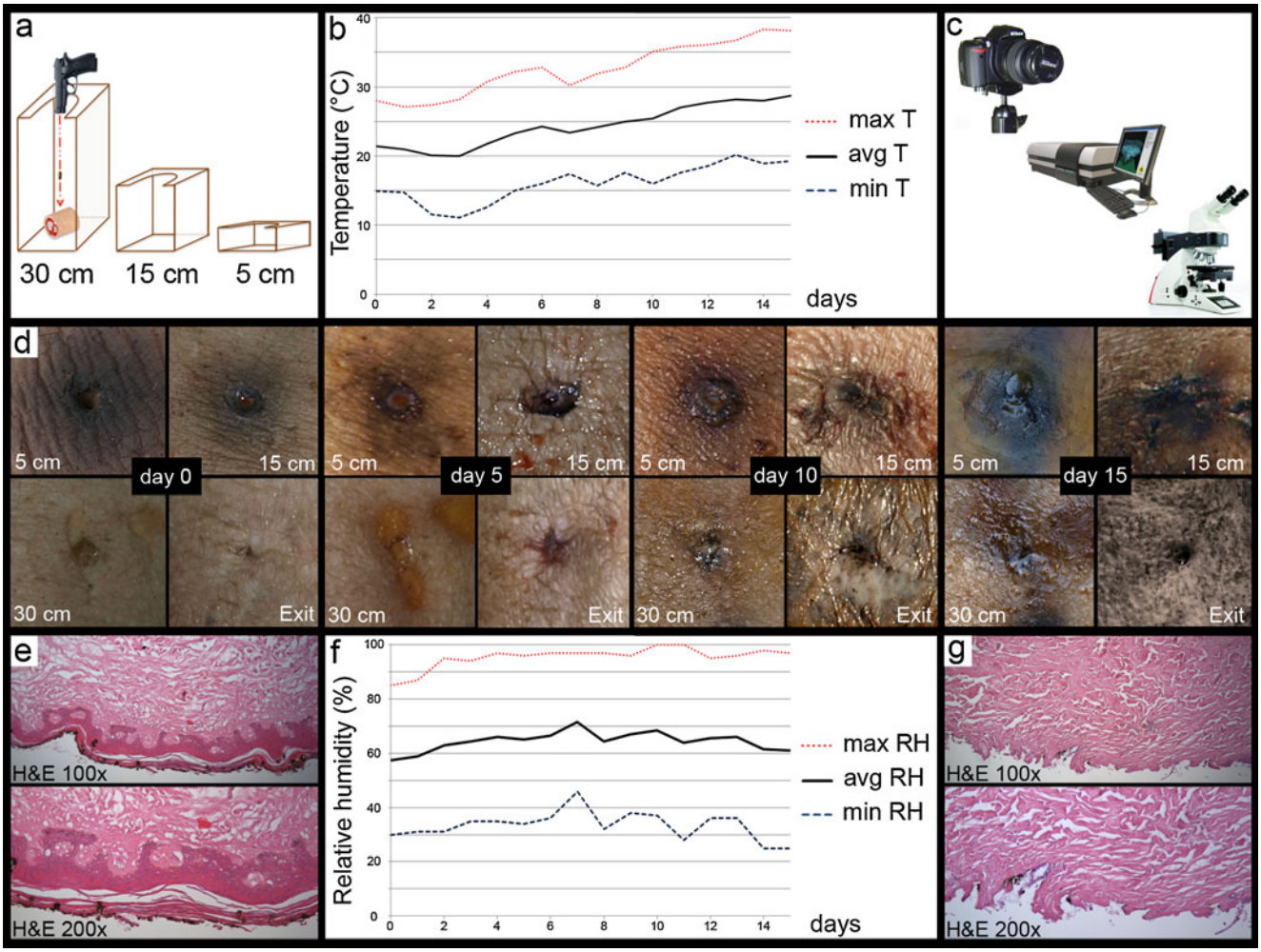

Fig. 1 Sketch of the stands used to perform the shooting trials (5, 15, and 30 cm) (**a**). Graphs showing the ambient air temperature (**b**) and relative humidity (**f**) during the experimental period of observation. Instruments used in the study (digital camera, microCT, and optical microscope) (**c**). Pictures of entrance and exit gunshot wounds (**d**); the fresh entrance lesions exhibited the typical characteristics of intermediate range gunshot wounds (day 0); at the end of the period of observation, the entrance wounds inflicted at different firing distances (5, 15, and 30 cm) became very similar at inspection and difficult to be distinguished from the exit wounds (day 15). Histological pictures of fresh (**e**) and decomposed (**g**) gunshot wounds inflicted at a distance of 5 cm. Smoke stain and gunpowder interspersions are well evident on the surface of the epidermis in the fresh wound (**e**), while the epidermis is absent and only a few gunpowder interspersions are present on the surface of the dermis layer in the decomposed lesion where fungal colonies with focal hyphae are also evident (**g**)

Table 1 Results of the shooting trials. Mean, standard deviation (SD), minimum and maximum values of GSR particles are reported for each tested firing distance (5, 15, and 30 cm). GSR particles are given in percentage, calculated as the proportion of the volume of interest (VOI) occupied by binarized solid objects with a density higher than 1,000 HU

Analysis variable: GSR percentage					
Firing range cm	Shots number	Mean %	SD %	Minimum %	Maximum %
Fresh samples					
5	10	0.420	0.038	0.370	0.470
15	10	0.211	0.021	0.180	0.250
30	10	0.028	0.018	0.010	0.050
Decomposed samples					
5	10	0.182	0.112	0.064	0.385
15	10	0.021	0.008	0.009	0.040
30	10	0.006	0.003	0.002	0.011

MicroCT analysis

The 3D reconstructions of entrance wounds revealed that in the fresh specimens GSR was concentrated on the skin surface around the hole and inside the cavity (epidermis and dermis layers) (Fig. 2a–c), while in decomposed samples, the high density particles (>1,000 HU) were detected only in the dermis layer (Fig. 2 e–g).

MicroCT analysis did not detect any GSR particles in the exit hole (both fresh and decomposed specimens) regardless of the tested firing distance (Fig. 2d and h).

Statistical analysis

The two-way ANOVA test showed that the mean percentage of GSR particles was significantly lower in decomposed than in fresh gunshot wounds ($p<0.0001$) as well as among the different firing distances ($p<0.0001$) (Fig. 3).

The post hoc test with Bonferroni correction revealed that the amount of powder particles in decomposed samples was significantly higher in the wounds inflicted from 5 cm than in those inflicted from 15 and 30 cm ($p<0.0001$) (Fig. 3).

Discussion

Decomposition and burial can obscure obvious GSR tattooing or stippling, while insect or other animal activity can create new tracts, obscure existing tracts, and, subsequently, change the morphology of the wound [19]. Hence, in cases of badly preserved corpses, the identification of gunshot wounds may be difficult to perform macroscopically.

Previous experimental studies on animals, in which intermediate range gunshot wounds were produced and macro/microscopically observed after a period of decomposition on air of several days have been reported [15–18].

MacAulay et al. have macroscopically examined putrefied gunshot wounds inflicted on pigs at three different ranges (contact, 2.5 cm, and 1.75 m), concluding that changes due to decomposition (tested conditions: 14 days of observation, temperature ranging between 7°C and 22°C) did not affect the collection and interpretation of gunshot wound evidence until the skin was degraded in the late active or advanced decay stage of decomposition [15].

Similar macroscopic results were obtained by Gibelli et al. with a 16-week period of observation; however, the authors detected lead and antimony in degraded tissues by means of histological analysis (sodium rhodizonate test) and radiochemical investigation (neutron activation analysis), respectively [16].

On the other hand, LaGoo et al. [17] observed that blackened wound edges and GSR particle deposition inside and around the entrance wound could not be macroscopically distinguished after a period of seven summer days (range of temperature: 1.8°C–31.4°C). Analyzing microwave-digested wound samples by inductively coupled plasma mass spectrometry (ICP-MS), the same authors detected antimony, barium, and lead particles at measurable levels throughout the entire sampling period (35 days), concluding that the presence and amount of GSR in tissue specimens was more dependent on the decomposition stage rather than on the time since the wound was inflicted. In a further study, it has been demonstrated that ICP-MS analysis of close-range gunshot wounds has the ability of differentiating GSR particles originating from shots fired with two different bullet types (jacketed vs. nonjacketed) in both fresh and decomposed samples [18].

Since all the above-mentioned experiments were conducted on animal models, the purpose of the present study was to assess the effects of early stages of decomposition on gunshot wounds produced on human samples.

In our experiment, fresh entrance wounds exhibited typical characteristics of intermediate gunshot wounds (i.e., powder particles and blackening), being easily distinguishable from the exit holes (Fig. 1d—day 0). After 15 days at open air with an average temperature of 24.5°C and an average humidity of 64.9%, the decomposition activities altered the skin surface, hampering the macroscopic differential diagnosis between entry and exit wounds (Fig. 1d—day 15).

In order to detect GSR, a microCT system coupled to an image analysis software was employed. This rapid, inexpensive and objective method permits scanning of the entire wound (epidermis and dermis around and inside the wound track), to perform tridimensional reconstructions, and to give a quantitative estimation of the amount of GSR particles in and around the gunshot wound [14].

Analyzing the 3D recostructions of both fresh and putrefied samples, GSR deposits were detected on the entrance gunshot wounds of all the tested firing distances (Fig. 2a–c and e–g), while the exit lesions did not present any particles with a density higher than 1,000 HU (Fig. 2d and h).

In a recent paper, Groβe Perdekamp et al. [20] have demonstrated that in contact shots fired against composite pig skin-gelatine models, GSR particles were detectable also in the distal sections of the missile track up to the exit hole, while in our study no GSR particles were visible within and around the exit holes. These apparent divergences might have been caused by the different experimental models used (composite pig skin-gelatine model vs. human calf sections) and by the longer muzzle-to-target distances tested in our

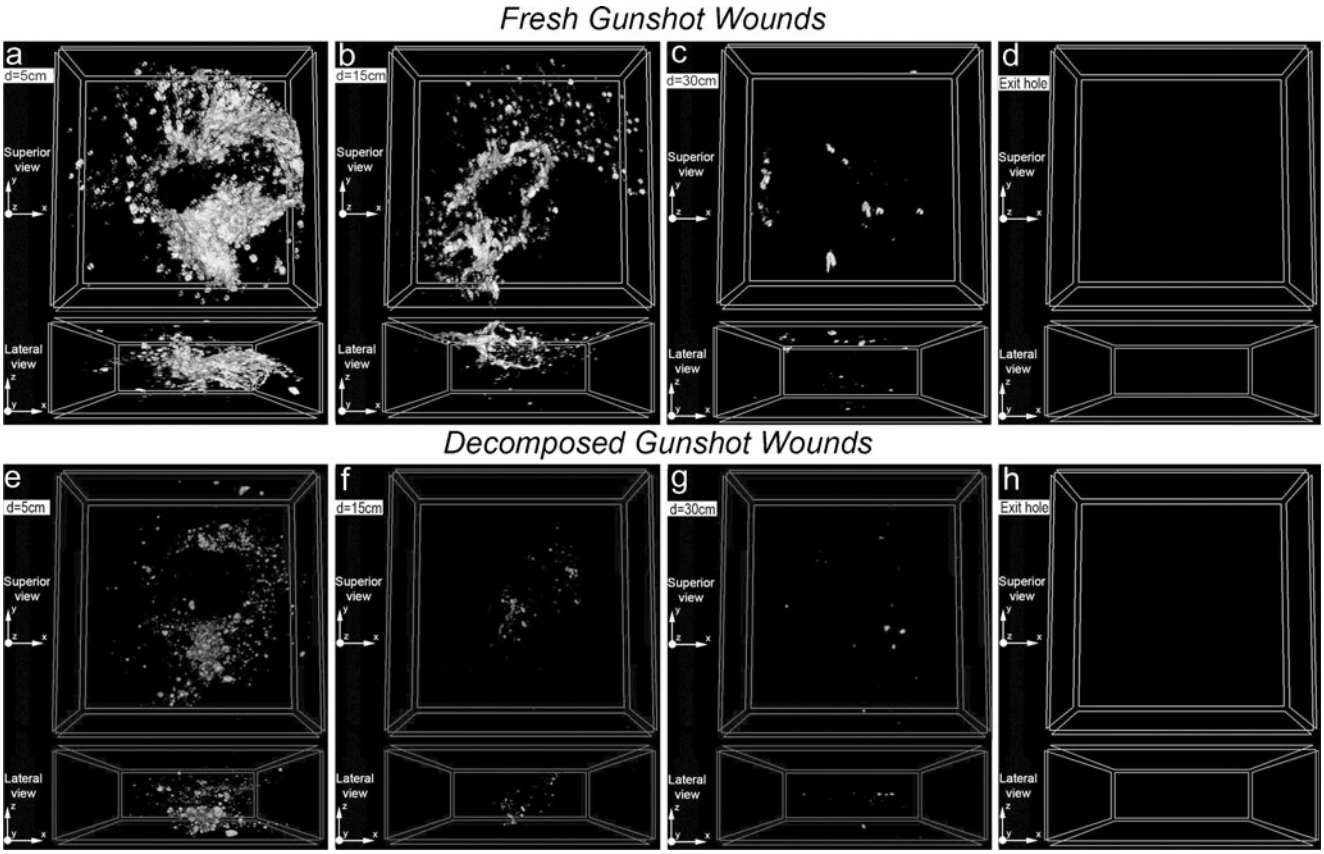

Fig. 2 Three-dimensional reconstructions of fresh and decomposed samples. Only particles of density higher than 1,000 HU are visualized. For the fresh entrance wounds inflicted from 5 cm (**a**) and 15 cm (**b**), GSR was concentrated on the skin surface around the hole, in the epidermis and dermis layers, and inside the cavity. For the fresh entrance wounds inflicted from 30 cm (**c**), the GSR was mainly deposited on the skin surface. For the decomposed entrance wounds shot from 5 cm (**e**), 15 cm (**f**), and 30 cm (**g**), GSR was evident only in the dermis layer around the bullet track. No GSR particles were detected in the exit holes of both fresh (**d**) and decomposed (**h**) samples

study (5, 15, and 30 cm), which probably avoided the massive invasion of combustion products along the wound track.

The ANOVA test confirmed the results of Lagoo et al. proving a statistically significant difference between the

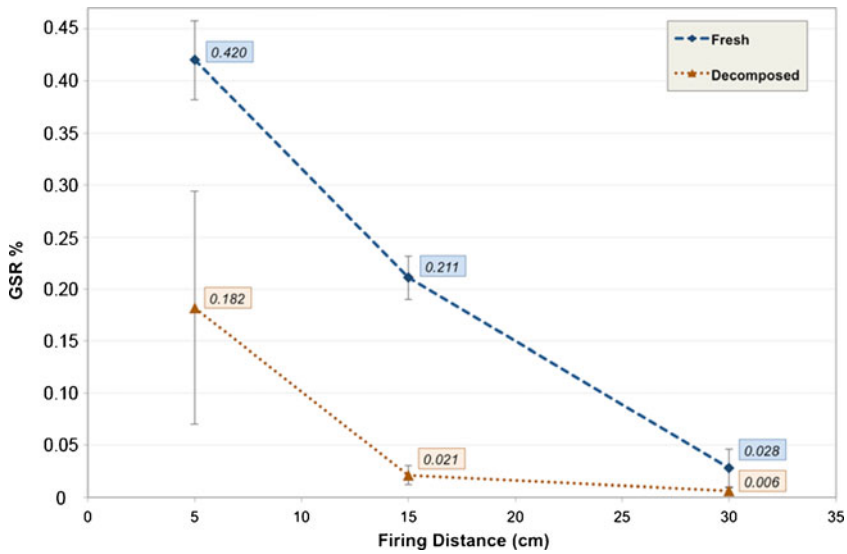

Fig. 3 Graphs showing the GSR concentrations estimated by microCT in fresh and decomposed gunshot wounds inflicted at different firing distances (5, 15, and 30 cm)

amount of powder particles detected in fresh and decomposed samples [16]. Additionally, in fresh entrance wounds, the 3D reconstructions (lateral view) revealed particles with a density higher than 1,000 HU in the epidermis and dermis layers of the skin around the hole (Fig. 1a–c), while in decomposed samples only the particles deeply penetrated into the dermis layer were evident (Fig. 1e–g). These findings may be explained by the postmortal detachment of the epidermis (Fig. 1g), which could have caused the loss of the GSR particles deposited on the skin surface.

The nature and amount of GSR reaching the target, in addition to its distribution around the entrance hole, are generally assumed to be related to the range of fire [21–23]. We have found that also in decomposed gunshot wounds, the amount of GSR roughly correlates in a nonlinear fashion with the distance from which the gun was fired. However, in contrast to fresh samples, a high variability of the GSR values estimated for shots repeatedly fired from the same distance was observed, particularly at very close range (5 cm) (Table 1 and Fig. 3). We believe that this variability could have been partly caused by the differences in the thickness and structure of the skin of the utilized human calves, which can significantly influence skin putrefaction and autolysis of the tissues [24].

It is well-known that decomposition is a progressive process affected by several intrinsic and extrinsic factors such as carrion insects abundance, temperature, humidity, rainfall, and exposure [25]. As reported in a previous article, microCT analysis might be of practical use for estimating the firing range, but a series of shooting trials should be carried out using human skin samples with the same, or at least an equivalent, type of weapon and ammunition in order to control the variables related to the type of firearm used [14]. Given the high variability of the results obtained on decomposed wounds even under standardized environmental conditions (i.e., site, time, temperature, and humidity), and considering that in forensic practice, it is generally very hard to determine the exact moment when the gunshot wound was inflicted, and to reproduce the same conditions of exposure from death until the retrieval of the corpse, we retain that the analysis of GSR through microCT, as well as other quantitative techniques [15–18], should not be used for estimating the firing range in decomposed wounds.

In conclusion, although chemical or histological analyses have been proposed for GSR detection on samples in advanced decomposition, microCT analysis could play an important role for studying intermediate gunshot wounds, particularly as a screening test for the differential diagnosis between entrance and exit holes. Obviously, considering that microCT is a nondestructive technique and that the proposed method allows only a presumptive identification of the GSR particles, positive results should be confirmed with a "gold standard" method such as environmental scanning electron microscopy coupled with an X-ray fluorescence energy dispersive spectrometry [26, 27], ICP-MS [15, 16] or neutron activation analysis [17].

In the future, further experiments will be performed for testing microCT analysis on samples altered by the effects of fire, water or covered by snow and ice, in order to estimate its diagnostic efficiency in extreme conditions often encountered in forensic practice.

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