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TECHNICAL NOTE

CRIMINALISTICS

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Differentiation of Bullet Type Based on the Analysis of Gunshot Residue Using Inductively Coupled Plasma Mass Spectrometry*

ABSTRACT: Porcine tissue samples shot with two different types of bullets, jacketed and nonjacketed, were collected in the fresh state and throughout moderate decomposition. Wound samples were microwave-digested and analyzed using inductively coupled plasma mass spectrometry (ICP-MS) to detect all elements present at measurable levels in gunshot residue (GSR). Elements detected included antimony (Sb), barium (Ba), and lead (Pb), which are considered characteristic of GSR, as well as iron (Fe) and copper (Cu). These five elements were used to differentiate shot tissue and unshot tissue, as well as tissue shot by the two different bullet types, both in the fresh state and throughout moderate decomposition. The concentrations of Cu, Sb, and Pb were able to distinguish the two bullet types in fresh tissue samples at the 95% confidence level. Cu and Pb were able to differentiate the bullet types throughout moderate decomposition at the 99% confidence level.

KEYWORDS: forensic science, gunshot residue, inductively coupled plasma mass spectrometry, firearms, microwave digestion, trace elements, decomposition

Conventionally, gunshot residue (GSR) determinations are made using gross injury examination at autopsy. However, interpretation of gunshot wounds can be difficult even when the deceased individual is well preserved, because the characteristics of gunshot wounds vary greatly based on the type of firearm and ammunition used, range of fire, and the location of the wound on the body. When postmortem factors such as decomposition, burial, and insect activity are present, the identification of gunshot wounds becomes even more challenging (1). Hence, the ability to chemically detect and identify GSR around a suspected gunshot wound is desirable.

Coroners, medical examiners, and forensic pathologists routinely use histology procedures to determine the presence of GSR in tissue sections from a suspected gunshot wound. Tissue sections cut from samples taken adjacent to the wound are stained using hematoxylin and eosin (H&E) and examined using light microscopy. Particles of burned gunpowder appear as fine, dark granules, and larger pieces of unburned gunpowder appear lighter in color. The lighter color is because of refraction of the incident light as it bends around the larger particles. However, as the stain does not react with the GSR particles themselves, only the tissue, any burned particles observed in the tissue sections cannot be definitively identified as GSR. Suspected particles of unburned gunpowder may be sampled for further analysis using both visual techniques and micro-Fourier transform infrared spectroscopy. However, tissue becomes more difficult to section and sample for analysis as decomposition progresses. These difficulties would be overcome by a chemical detection method suitable for use on tissue at any stage of decomposition.

GSR has been detected in gunshot wounds in fresh animal hide using Alizarin Red S staining and visual examination techniques (2,3). Human gunshot wound tissue has also been analyzed for GSR using staining and X-ray microfluorescence, but these studies focused on fresh, rather than decomposing, tissue and did not use the instrumental methods described later in this section (4,5).

Most crime laboratories today use scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM/EDS) to identify GSR on possible assailants or victims' skin or clothing to help confirm a victim's wound as a gunshot wound. Particles suspected to contain GSR are collected by dabbing an adhesive tab mounted on an aluminum stub across a surface. SEM/EDS analysis provides both particle morphology and elemental composition that are used to identify GSR particles. Wolten et al. described four elemental combinations that were observed in GSR particles and were therefore regarded as characteristic of GSR (6). The most prominent of these combinations was antimony (Sb), barium (Ba), and lead (Pb), which come from ammunition primers and bullets (7). However, SEM/EDS is limited by the ability to locate GSR particles on the adhesive tab, which becomes difficult if the particles are embedded in the adhesive or are obscured by other debris from the sample (8,9). While automated software programs are available, the analysis can still be time-consuming, taking up to several hours per cm^2 of sample (10). Furthermore, it becomes difficult to effectively collect particles from decomposing tissue using the adhesive stubs, especially when outdoors, as the tissue becomes oily (11).

Inductively coupled plasma mass spectrometry (ICP-MS) has also been used for the determination of Sb, Ba, and Pb in GSR, as well as in simulated samples of GSR (12–14). ICP-MS was

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successful in determining these three elements of interest from extracts of swabs spiked with the three elements (12), from extracts of swabs from shooter's hands (13), and from digests of shot cotton tissue (14). Koons described the analysis of cotton swabs spiked with Sb, Ba, and Pb and swabs taken from shooters' hands using ICP-MS (12). The limits of detection for the three elements were at least one order of magnitude less than levels typically observed in GSR (40–500 ng) and were lower than corresponding detection limits using graphite furnace atomic absorption spectroscopy and ICP-atomic emission spectroscopy. The use of ICP-MS to analyze digested bullet shavings to differentiate bullets according to elemental composition has also been described, but the resulting GSR was not analyzed to determine whether the residue could be used to differentiate the bullets as well (15–17).

Previous research in our laboratory has demonstrated the ability of ICP-MS to detect Sb, Ba, and Pb in porcine tissue subjected to gunshot wounds throughout decomposition (11). Analysis of the digested wound tissue allowed the detection of GSR at all stages during decomposition in both late summer and winter months. Wounds were also analyzed using SEM/EDS for comparison, and GSR was only identified on the first day of the study, after which rain washed away surface particles and prevented collection using adhesive tabs.

The purpose of this research was to identify additional elements characteristic of GSR to increase confidence in gunshot wound determination. The ability of ICP-MS to differentiate two bullet types, jacketed and nonjacketed, using their element profiles was also investigated. To make the results more applicable in a forensic setting, the effects of moderate decomposition on the ability to detect GSR and differentiate bullet types were also assessed.

Materials and Methods

Fresh Tissue Studies

Two studies were conducted using fresh tissue samples. The first study was used to identify elements that were potentially useful in differentiating the two bullet types, as well as to assess variation in GSR composition within a wound. The second study was used to quantitate the elements of interest to determine statistical differences in element concentrations between the two bullet types.

For the fresh tissue studies, two euthanized pigs (*c.* 150 lbs each) were obtained from the Michigan State University (MSU) Swine Research Facility in June 2009. All pigs used in this research were treated in accordance with the MSU Institutional Animal Care and Use Committee guidelines. A certified firearms instructor shot both pigs seven times using a Smith & Wesson® .357 Magnum revolver. One pig was shot using 158 grain copper-jacketed hollow point ammunition with a jacketed bullet base (Remington Arms Co. Inc., Lonoke, AR, Lot # B 17 HAS 502). The second pig was shot using 158 grain nonjacketed lead ammunition (Remington Arms Co. Inc., Lot # G 21 YB 6102). All shots were fired at a muzzle-to-target distance of 5 cm, and the wounds were spaced *c.* 10 cm apart to prevent cross-contamination between wounds. The gun barrel and chamber were also cleaned when the ammunition type was changed. Immediately after all shots were fired, the wounds were collected by excising the tissue and underlying fat in a *c.* 4 cm radius around each wound. Samples were also removed from these wounds for histology analysis. The tissue samples were wrapped loosely in waxed paper, sealed in plastic bags, and stored at −80°C until analysis.

In July 2009, one euthanized pig (*c.* 100 lbs) was obtained from the MSU Swine Facility to serve as a control. Four samples of unwounded skin and underlying fat (*c.* 20 cm × 20 cm each) were removed, packaged, and stored until analysis as described previously. As this pig was used as the control for all experiments, the natural element concentration variability among different pigs was not a feature of this study.

For the first fresh tissue study, two tissue samples with gunshot wounds (one from each bullet type) and one control sample were used. Three tissue sections were removed from each gunshot wound, and one section was removed from the control tissue sample. The tissue sections were microwave-digested and analyzed by ICP-MS, following procedures described later in this section. For the second fresh tissue study, 10 shot tissue samples (five from each bullet type) and one control sample were used. One tissue section was taken from each sample, microwave-digested, and analyzed using ICP-MS, quantitating the elements of interest.

Decomposition Study

In October 2009, three euthanized pigs (*c.* 150 lbs each) were obtained from the MSU Swine Research Facility. Control samples of tissue were removed from each pig before wounding. Two pigs were shot 12 times each with the same gun and ammunition types as the fresh tissue study. As before, one pig was shot with the jacketed ammunition and the second pig was shot with the nonjacketed ammunition. The gun barrel was cleaned after each shot, and the chamber was cleaned before reloading. All three pigs were then transported to a research field, where the control pig was stabbed 12 times to create open wounds to attract insect activity similar to the shot pigs. The pigs were placed inside separate wire cages to protect them from predators while still allowing exposure to the environment. The wounds and histology samples were collected over a period of 49 days, following procedures for excision, packaging, and storage described previously. One tissue section was removed from each tissue sample (gunshot wound and control) for microwave digestion and ICP-MS analysis to quantitate the elements of interest.

Histology

Tissue sections were taken from each wound in the area of highest soot density and placed in formalin fixative (10% buffered for the fresh tissue wounds [Fisher Diagnostics Co. LLC, Kalamazoo, MI]; 37% formaldehyde solution for the decomposed tissue wounds [Columbus Chemical Industries, Inc., Columbus, WI]). The tissue sections were processed for H&E staining by the Hurley Medical Center Pathology laboratory according to the established and standard staining procedures of that laboratory. Stained tissue sections were evaluated using a light microscope (Nikon Eclipse 50i equipped with a 2×–60× lens; Nikon Inc., Melville, NY).

Microwave Digestion of Tissue Samples

Tissue samples were prepared for ICP-MS analysis following microwave digestion procedures detailed by LaGoo et al. (11). Briefly, between 0.35 and 0.5 g of each tissue sample was placed into acid- and peroxide-washed quartz vessels (Milestone, Inc., Shelton, CT). A 1-mL aliquot of hydrogen peroxide (H_2O_2, 30%; Columbus Chemical Industries, Inc.) and 2 mL Optima grade nitric acid (HNO_3, 69%; Fisher Scientific, Pittsburgh, PA) were added to each vessel. The quartz vessels were then capped and placed into larger Teflon™ vessels (Milestone, Inc.) that contained 10 mL Milli-Q water (Milli-Q Academic; Millipore, Billerica, MA) and

2 mL 30% H_2O_2. The Teflon™ vessels were then securely closed and digested in a Milestone Ethos EX microwave digestion system (Milestone, Inc.). After digestion, the tissue digests were allowed to cool to room temperature and diluted with 2% HNO_3 by adding 0.5 mL of the digest solutions to 11.168 mL Milli-Q water in 15-mL polypropylene conical tubes (Corning, Inc., Corning, NY). The dilution was necessary to prevent the degradation of the polypropylene tubes and decrease the acid concentration for ICP-MS analysis. The diluted digest solutions were stored at 4°C until ICP-MS analysis.

ICP-MS Analysis of Fresh Tissue Samples

The seven tissue samples from the first fresh tissue study (three samples from one wound from each bullet type and one control sample) were analyzed using full mass scan mode to identify all elements present at significant levels. Prior to analysis, the diluted tissue digest solutions and procedural blanks were further diluted 1:10 (v/v) with 2% HNO_3 and spiked to yield 20 μg/L In and 20 μg/L Bi (SPEX CertiPrep, Inc., Metuchen, NJ) as internal standards. The solutions were analyzed by ICP-MS using a Micromass Platform quadrupole ICP-MS (now Thermo Fisher Scientific, Inc., Waltham, MA). The instrument was equipped with a CETAC ASX-500 autosampler (CETAC Technologies, Omaha, NE), and a Dynolite™ detector with a −15 kV conversion dynode and electron multiplier. Instrument operating parameters are given in Table 1. The instrument was operated in full mass scan mode using MassLynx software (version 3.4; Waters Corp., Milford, MA). Digest samples and procedural blanks were analyzed in triplicate in random order. After sample injection, the injector was rinsed for 90 sec with 2% HNO_3 to prevent sample carryover.

TABLE 1—*ICP-MS parameters for full mass scan and selected ion monitoring (SIM) analyses.*

ICP-MS operating parameters	
RF power (W)	1350
Argon flow rates (L/min)	
Outer	13
Intermediate	0.8–0.95
Nebulizer	0.67–0.7
Sampling cone	Ni with Cu core, 1.14-mm-diameter orifice
Cone voltage (V)	100–125
Skimmer cone	Ni, 0.89-mm-diameter orifice
MS resolution	Unit mass
Hexapole gas flow rates (mL/min)	
Helium	4–5
Hydrogen	2
Hex bias (V)	−1.0 to 1.0
Data collection parameters	
Mode	Full mass scan or selected ion recording (peak jumping)
Sample scan time (s)	75
Dwell time (s)	0.2 (SIM mode only)
Interchannel delay time (s)	0.020 (SIM mode only)
Autosampler parameters	
Sample read delay (s)	105
Rinse time (s)	90

ICP-MS, inductively coupled plasma mass spectrometry.

Instrument responses for elements below atomic mass 155 were normalized to ^{115}In, and elements with atomic weights above 155 were normalized to ^{209}Bi. Dilution factors were taken into account, and concentrations of all elements were expressed as μg element/g tissue for comparison.

Statistical Treatment of Fresh Tissue Data

All statistical analyses were performed using Microsoft Excel 2003 (Microsoft® Corp., Redmond, WA). The average elemental concentration (μg/g tissue) was calculated for each bullet type (three samples analyzed in triplicate from each wound, giving $n = 9$), the control sample (triplicate analyses, $n = 3$), and the procedural blank sample (triplicate analyses, $n = 3$). The instrument limit of detection, defined as three times the standard deviation of the procedural blank concentration, was calculated for each element, as well as the ratio of the average procedural blank concentration to the lowest concentration in a tissue sample with a gunshot wound from either bullet type. The relative standard deviation (RSD) was calculated for each element in each bullet type, and the Grubbs' test for statistical outliers was performed on all elements that had RSDs higher than 15% (18). For all elements, any data points determined to be outliers at the 95% confidence level were removed. Elements with an RSD >15% in both bullet types after removing statistical outliers were eliminated from the suite of potentially useful elements.

Analysis of variance (ANOVA) at the 90% confidence level ($p \leq 0.1$) was used to assess significant differences in element concentrations within and between wounds, using triplicate analysis of three tissue samples from each bullet type. The null hypotheses were that there was no difference in the elemental concentrations of replicates from the same sample and that there was no difference in the elemental concentrations of samples taken from the same wound. The alternate hypotheses were that the elemental concentrations of replicates and between samples taken from the same wound were not equal. Random effect corrections were applied to between-sample variances to correct for the random nature of sampling the tissue for analysis, where appropriate (19).

Student's *t*-tests were also performed to compare the average concentration of each element between the two bullet types. The calculated *t*-statistic for each element was compared with statistical tables of critical *t*-values at the 95% and 99% confidence limits (two-tail) to assess statistical differences in element concentrations.

ICP-MS Quantitation of Fresh and Decomposed Tissue Samples

The 11 tissue samples from the second fresh tissue study (one sample from five wounds from each bullet type and one control) and all tissue samples from the decomposition study ($n = 38$) were analyzed using selected ion monitoring (SIM) mode for the selected elements of interest to increase sensitivity. The instrument tune conditions were optimized daily using a 10 μg/L solution of Be, Co, In, Ce, Bi, and U (SPEX CertiPrep, Inc.) prepared in a 2% HNO_3 solution. Ten multielement external calibration standards containing the elements of interest were prepared from stock standard solutions of each element [1000 mg/L each; phosphorus (P), potassium (K), iron (Fe), copper (Cu), Sb, Ba, and Pb from SPEX CertiPrep, Inc.; magnesium (Mg) and zinc (Zn) from Thermo Fisher Scientific, Inc]. The stock solutions were diluted with 2% HNO_3 to concentrations ranging from 0.1 to 500 μg/L, with all elements having equal concentrations in each standard solution. Each standard solution was spiked to yield 20 μg/L In and 20 μg/L Bi as internal standards prior to ICP-MS analysis.

Calibration standards were analyzed in order from low to high concentration to minimize carryover effects.

Standard Reference Material (SRM) 1643e (Trace Elements in Water, National Institute of Standards and Technology, Gaithersburg, MD) was analyzed to assess instrument accuracy during sample analyses as the SRM contained known concentrations of all elements of interest. The SRM was diluted 1:10 (v/v) with 2% HNO_3 and spiked to yield 20 μg/L In and 20 μg/L Bi as internal standards prior to ICP-MS analysis.

Owing to the range in concentrations for the different elements in the shot tissue samples, it was necessary to prepare the tissue digest samples at two different dilution levels. After microwave digestion, the digest solutions (fresh tissue, decomposed tissue, control tissue, and procedural blank) were diluted with 2% HNO_3 as described previously. One sample set was not further diluted (hereafter referred to as "undiluted") prior to being spiked to yield 20 μg/L In and 20 μg/L Bi as internal standards. A second sample set was prepared by further diluting the digests 1:100 (v/v) with 2% HNO_3 (hereafter referred to as "diluted"). The diluted digests were then spiked to yield 20 μg/L In and 20 μg/L Bi as internal standards.

The undiluted and the diluted digests were analyzed by ICP-MS using the same instrument system described previously with additional parameters for SIM mode analysis (Table 1). Sets of calibration standards were analyzed after approximately every 30 samples to account for instrument drift. Samples were analyzed in the following order for both the fresh and decomposed tissue studies: calibration standard set, SRM, diluted digests and procedural blanks in random order, SRM, calibration standard set, SRM, undiluted digests and procedural blanks in random order, SRM, calibration standard set. After each sample injection, the injector was rinsed for 90 sec with 2% HNO_3, and two additional 2% HNO_3 rinses were performed between each group of samples.

The resulting instrument responses were quantitated using MassLynx software. Instrument responses for ^{24}Mg, ^{31}P, ^{39}K, ^{56}Fe, ^{63}Cu, and ^{64}Zn were normalized to ^{115}In, and ^{121}Sb, ^{138}Ba, and ^{208}Pb were normalized to ^{209}Bi, which was the standard procedure for the instrument. Element concentrations in the tissue samples were determined from the average of the linear calibration curves run immediately before and after the digest sample set to be quantitated. Element concentrations in each tissue digest were corrected for dilution factors and normalized to the original tissue mass to yield final element concentrations that were expressed as μg element/g tissue.

Results and Discussion

Fresh Tissue Sample Observations and Histology Results

Dense soot deposition was visible in the wound tract and around the immediate perimeter of wounds made by both the jacketed and nonjacketed bullets (Fig. 1). Wound edges were also darkened, exhibiting typical characteristics of a close-range gunshot wound. No stippling was observed because the pigs were euthanized prior to being shot. Microscopic examination of the wounds revealed soot staining in the epidermis. Histology results showed the expected thermal artifact associated with gunshot wounds (i.e., homogenization of the epidermis with streaming nuclei) in both bullet types, as well as dark granules of burned gunpowder and larger, refractive particles of unburned gunpowder (Fig. 1). There were slight differences in soot deposition patterns and histology results between the two ammunition types owing to different types and amounts of gunpowder, but this was not a feature of the study.

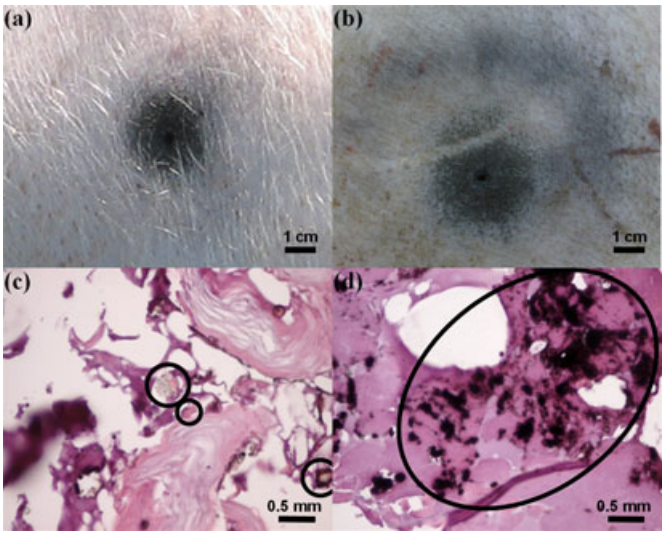

FIG. 1—*Gunshot wounds in fresh porcine tissue from (a) jacketed bullets and (b) nonjacketed bullets, along with corresponding micrographs of H&E stained histology results (c and d, respectively). Refractive particles of unburned gunpowder are circled in the jacketed bullet wound histology slide (c), and dark granules of burned gunpowder are circled on the nonjacketed bullet wound histology slide (d).*

Selection of Elements to Discriminate Bullet Types

The full mass scan ICP-MS analyses detected many potentially useful elements in the fresh tissue digest samples. Element concentrations from ICP-MS analyses were analyzed using a series of statistical procedures to select those elements most useful for differentiating shot from control tissue as well as differentiating the two bullet types. First, elements with average concentrations in tissue samples shot with both bullet types that were below the instrument detection limits for that element were excluded from further analyses. Next, if the ratio of the average procedural blank concentration to the lowest concentration in a shot tissue sample from either bullet type was >30%, the element was excluded from further consideration because more than one-third of the element signal was caused by contamination from the sample preparation procedure and/or the instrument. Finally, if the RSD for an element was >15% for both bullet types after elimination of statistical outliers, the element was excluded from further consideration.

The number of possible elements of interest was slightly reduced by excluding elements with concentrations in shot tissue samples below the limit of detection and below levels in procedural blanks. Many element candidates were also excluded because of high RSD levels, leaving the following elements as potentially useful for discrimination: ^{24}Mg, ^{31}P, ^{39}K, ^{56}Fe, ^{63}Cu, ^{66}Zn, ^{121}Sb, ^{138}Ba, and ^{208}Pb. It should be noted that the RSDs for ^{121}Sb, ^{138}Ba, and ^{208}Pb were higher than 15% in both bullet types owing to the high concentrations of those three elements in the samples. However, the three elements were included in subsequent analyses as these are the elements currently considered to be characteristic of GSR.

One-way ANOVA was applied to the three tissue samples analyzed in triplicate from each bullet type to identify those elements that showed significant differences within and between wounds at the 90% confidence level ($p \leq 0.1$). At this p-value, the probability that the elemental concentrations were the same was 10% or less. The variation was not significant among replicates of the same sample, as expected because of the high precision of ICP-MS. Furthermore, for both bullet types, there was no significant difference in concentrations for samples taken from the same wound for the

following elements: ^{24}Mg, ^{31}P, ^{39}K, ^{56}Fe, ^{63}Cu, and ^{66}Zn. There was significant between-sample variance in element concentration for ^{121}Sb, ^{138}Ba, and ^{208}Pb in samples from wounds made by jacketed bullets, and in ^{138}Ba and ^{208}Pb in wounds made by nonjacketed bullets. This variation was caused by high concentrations of those elements in the wound samples. In subsequent analyses, the digests were further diluted prior to quantitation.

Finally, to test whether the elements of interest could be used to differentiate the two bullet types, the Student's *t*-test was used to compare the mean element concentrations in each (Fig. 2). Elements with significant differences in concentration at the 95% confidence level and above were considered useful for differentiating the two bullet types. Concentrations of ^{24}Mg, ^{31}P, ^{39}K, ^{63}Cu, and ^{66}Zn were significantly higher in wounds shot with jacketed bullets, the elements ^{121}Sb and ^{208}Pb were significantly more concentrated in wounds shot with nonjacketed bullets, and ^{56}Fe and ^{138}Ba concentrations were not significantly different between the two bullet types. All elements were also confirmed to be useful for differentiating tissue with gunshot wounds from control tissue by comparison of the mean element concentrations in a similar manner.

Thus, the suite of elements considered potentially useful for differentiating shot from control tissue and between the two bullet types consisted of ^{24}Mg, ^{31}P, ^{39}K, ^{56}Fe, ^{63}Cu, ^{66}Zn, ^{121}Sb, ^{138}Ba, and ^{208}Pb. These metals could originate from the ammunition primer, gunpowder, cartridge case, or the bullet itself, as well as from the gun barrel (7). The three elements currently considered characteristic of GSR, Sb, Ba, and Pb, come from the primer, and Pb and Sb could also come from the bullet. Cu, Zn, and nickel (Ni) are known materials in bullet jackets and cartridge cases. However, as the sampling cone of the ICP-MS was composed of Ni, data for that element are not reliable in this study, and the element was not considered in subsequent analyses. The gun barrel used in these studies was made of stainless steel, which is mainly composed of Fe. Mg and P are likely oxidizers in the gunpowder, and K may come from the synthesis of the gunpowder. Fe, Mg, P, and K are also present in considerable concentrations in biological tissues.

Quantitation of Elements in Fresh Tissue Samples

At this stage, nine elements (^{24}Mg, ^{31}P, ^{39}K, ^{56}Fe, ^{63}Cu, ^{66}Zn, ^{121}Sb, ^{138}Ba, and ^{208}Pb) were identified as being potentially useful for differentiating tissue with gunshot wounds from control tissue as well as differentiating bullet type. The levels of these elements in the fresh tissue samples (one control, five shot with jacketed bullets, and five shot with nonjacketed bullets) were then quantitated by ICP-MS. However, the elements ^{24}Mg, ^{31}P, and ^{39}K were subsequently eliminated from consideration owing to nonlinear calibration curves as a result of high background levels in the instrument. Limits of quantitation for the remaining elements were determined as the concentration of the lowest linear point on the calibration curve or listed as less than the lowest standard concentration analyzed (Table 2).

Both the undiluted and diluted tissue digest sample sets were analyzed. The elements ^{56}Fe and ^{66}Zn were quantitated in the undiluted digests owing to the low concentrations of these elements observed in the first fresh tissue study. The remaining four elements (^{63}Cu, ^{121}Sb, ^{138}Ba, and ^{206}Pb) were one to three orders of magnitude more concentrated and were quantitated using the diluted digests. This was necessary to avoid detector saturation and to ensure that the instrument responses were within the linear range of the calibration curve for those elements.

The mean concentrations for all six elements of interest in the four SRM samples were calculated and compared with the reported concentrations in the material. The percentage error between the true and observed Zn concentrations was 25%, and hence Zn was excluded from further consideration. The percentage errors for the remaining five elements (Fe, Cu, Sb, Ba, and Pb) are shown in Table 3. Errors for these elements were all <10%, indicating that the ICP-MS was accurately quantitating the elements and that one analysis of each digest sample was sufficient.

A comparison of mean elemental concentrations in all tissue samples analyzed for each bullet type, as well as procedural blank and control tissue samples, is shown in Fig. 3. The mean element concentrations in tissue samples shot with either bullet type are higher than the corresponding blank and control concentrations for all five elements of interest, indicating that all elements can be used to differentiate shot from unshot tissue. However, these results are somewhat limited in that only one pig was used as a control. More work is needed to determine whether the background levels of the elements of interest fluctuate in different pigs. The Student's *t*-test was used to test for significant differences in the mean element concentrations between wounds inflicted by jacketed and nonjacketed bullets. There were significant differences between the two bullet types in the Sb and Pb concentrations at the 99% confidence level and in the Cu concentrations at the 95% confidence limit. Sb and Pb were both more concentrated in tissue shot with nonjacketed bullets, with mean concentrations of 5444 and 147,565 μg/g, respectively, compared with 1732 and 6567 μg/g in wounds shot with jacketed ammunition. Cu was more concentrated in tissue shot with jacketed ammunition, with a mean concentration of 2524 μg/g compared with 123 μg/g in wounds shot with nonjacketed bullets. These elements most likely originate from the bullets themselves, as the jacketed bullets have Cu jackets and the nonjacketed bullets are composed of Pb and possibly Sb. These three elements are therefore useful for differentiating the two bullet types in fresh gunshot wounds.

Mean Fe and Ba concentrations were not significantly different between the two bullet types, possibly due to the fact that these

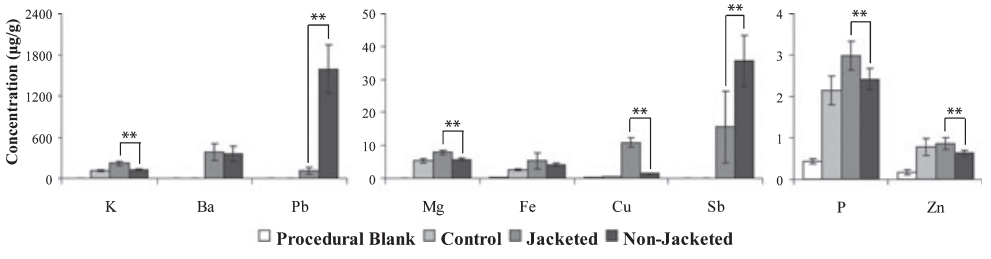

FIG. 2—*Average concentrations of elements of interest in the first fresh tissue study (procedural blank* n = *3, control* n = *3, jacketed* n = *9, and nonjacketed* n = *9). The error bars represent one standard deviation. Significant difference between element concentrations at 99% confidence level* ($p \leq 0.01$) *are indicated by* **.

TABLE 2—*Limits of quantitation (LOQs) for elements of interest in the fresh tissue and decomposition studies.*

	LOQ (μg/L)	
Element	Fresh Tissue Study	Decomposition Study
^{56}Fe	5	5
^{63}Cu	5	5
^{66}Zn	10	10
^{121}Sb	<0.1	<0.1
^{138}Ba	0.5	<0.1
^{208}Pb	0.25	0.25

TABLE 3—*Standard reference material recovery results for elements of interest in the fresh tissue and decomposition studies.*

	Average % Error	
Element	Fresh Tissue Study	Decomposition Study
^{56}Fe	7	0.5
^{63}Cu	1	6
^{121}Sb	2	4
^{138}Ba	6	7
^{208}Pb	3	5

elements originate from the interior of the gun barrel or the ammunition primers, which are more similar in elemental composition between the two ammunition types. Thus, all five elements of interest (Fe, Cu, Sb, Ba, and Pb) were successful in differentiating shot from control tissue, and three elements (Cu, Sb, and Pb) were capable of differentiating tissue samples shot with jacketed and nonjacketed bullets in the fresh state. While these results were promising, the effect of decomposition on element concentrations was investigated to evaluate the practicality of this method for forensic applications.

Decomposition Study Observations and Histology Results

Gunshot wounds collected on day 0 of the decomposition study were visually very similar in appearance to those from the fresh tissue study (Fig. 4). This observation was expected as wounds were inflicted using the same weapon and ammunition. During the 49-day sample collection period, temperatures ranged from a high of 70°F (day 37) to a low of 22°F (day 41). Appreciable rainfall occurred on days 0, 4, 7, 19, 20, 21, 28, 47, and 48. There was minimal insect activity during the first week because of cool temperatures and rain. Bloating was evident and wounds were seeping on all pigs by day 19. By day 24, the heads of all three pigs were beginning to turn black and skin slippage was apparent. The flesh of all three pigs continued to darken and show more slippage throughout the rest of the collection period. The carcasses did not reach the dessication/skeletonization stage of decomposition during this study owing to the cold temperatures experienced. The dense GSR deposition and blackening around the gunshot wounds persisted throughout the collection period. The stab wounds in the control pig widened and darkened over time, but stab wounds and gunshot wounds were visually distinguishable throughout this study.

Histology samples from all three pigs showed typical patterns of decomposition, including homogenization of the dermal collagen, separation of the epidermis from the dermis, loss of cellular detail, and bacterial and fungal overgrowth (Fig. 4). The sections at the wound edges showed the expected thermal artifact associated with gunshot wounds, consistent with the fresh tissue study. The GSR patterns observed in the fresh tissue persisted but decreased in quantity as moderate decomposition progressed in wounds inflicted using both bullet types.

Quantitation of Elements in Tissue Samples Throughout Decomposition

The five elements of interest (^{56}Fe, ^{63}Cu, ^{121}Sb, ^{138}Ba, and ^{208}Pb) were quantitated using ICP-MS, and the limits of quantitation are listed in Table 2. Because element concentrations were expected to decrease as decomposition progressed, all element signals were monitored in both the undiluted and diluted tissue sample sets. As ^{56}Fe and ^{63}Cu were present in relatively low levels throughout the study, these elements were quantitated using the undiluted digest samples so that signals remained within the linear range of the calibration curve. The elements ^{121}Sb, ^{138}Ba, and ^{208}Pb were more concentrated and were quantitated in the diluted samples to ensure that sample signals were within the linear calibration curve range and did not saturate the detector.

The mean concentrations of the five elements in the four SRM samples were calculated and compared with the reported concentrations, as described previously (Table 3). For all elements, errors were <10%, indicating that the instrument was accurately quantitating the elements in all samples.

Concentration ranges for each element of interest throughout moderate decomposition are listed in Table 4. The highest element concentrations did not necessarily occur on day 0, and the lowest concentrations did not always correspond to day 49. As element concentrations did not appreciably change as decomposition progressed, mean element concentrations were calculated for six tissue samples from each bullet type, collected throughout the study at different stages of decomposition on days 0, 5, 14, 24, 34, and 44

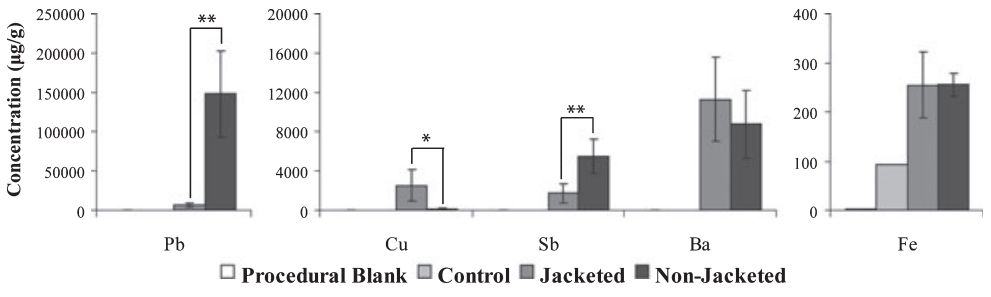

FIG. 3—*Average concentrations of elements of interest quantified in fresh tissue samples (procedural blank* n = *6, control* n = *1, jacketed* n = *5, and nonjacketed* n = *5). The error bars represent one standard deviation. Significant difference between element concentrations at 95% confidence level (*$p \leq 0.05$*) are indicated by * and significant difference between element concentrations at 99% confidence level (*$p \leq 0.01$*) are indicated by **.*

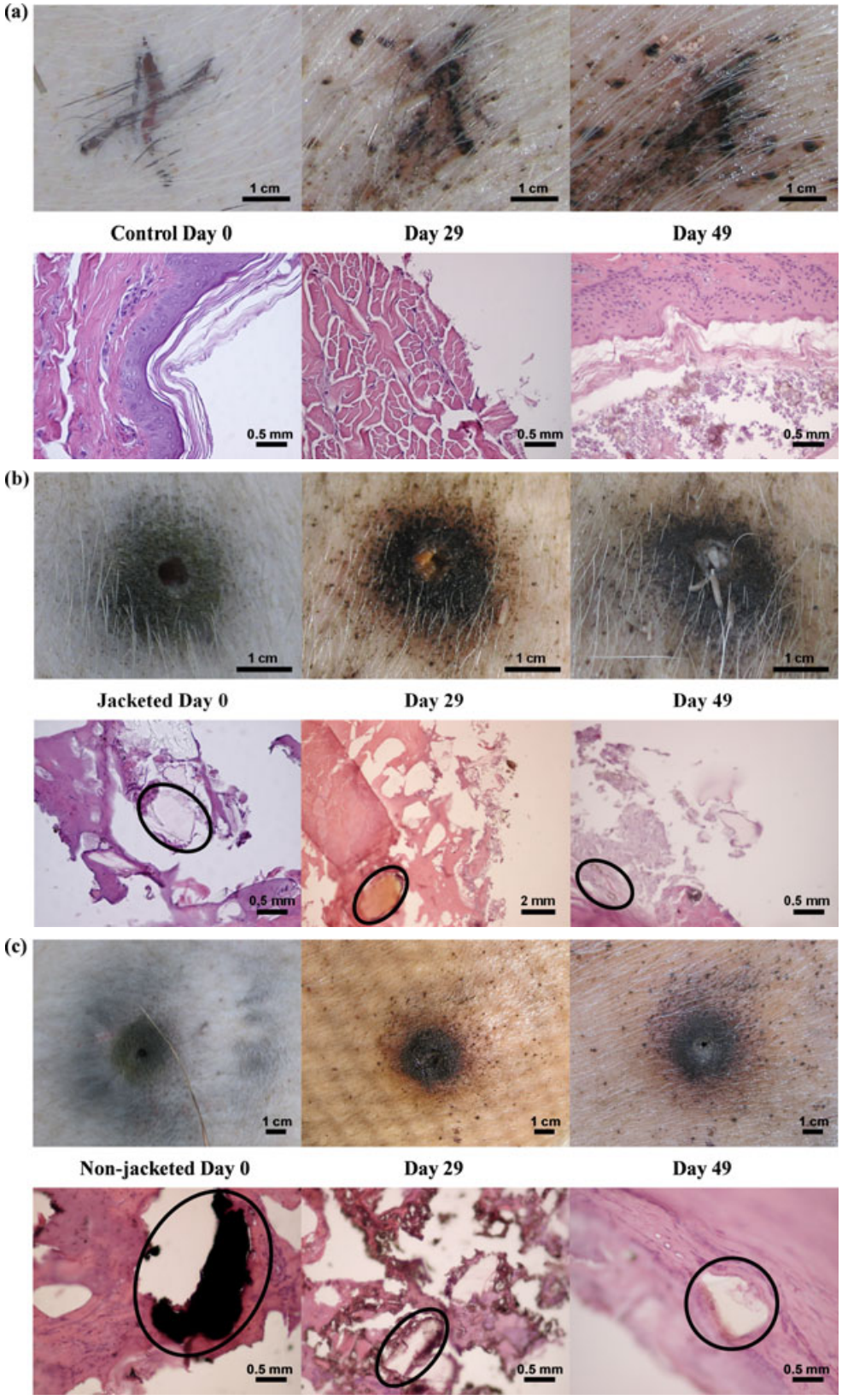

FIG. 4—*Wounds in porcine tissue on days 0, 29, and 49 during the decomposition study for (a) stab wounds, (b) wounds shot with jacketed bullets, and (c) wounds shot with nonjacketed bullets. Corresponding micrographs of H&E stained histology results are shown below the wounds, with particles consistent with gunshot residue circled.*

(Fig. 5). All elements of interest show higher concentrations in tissue with gunshot wounds relative to the control tissue and procedural blank samples, indicating that there was no significant contamination owing to the sample preparation method. Mean element concentrations for Cu and Ba (99% confidence level) and for Fe and Pb (95% confidence level) were significantly higher in

TABLE 4—*Element concentration ranges throughout moderate decomposition in each tissue sample type.*

Element	Range in Element Concentration (μg/g) Through Moderate Decomposition		
	Control	Jacketed	Nonjacketed
^{56}Fe	83–235	348–720	242–438
^{63}Cu	12–27	306–835	99–166
^{121}Sb	10–15	197–3677	1722–3923
^{138}Ba	ND*	1721–18159	5057–16599
^{208}Pb	0–11	702–11010	42753–131245

*ND indicates ''not detected.''

tissue shot with jacketed ammunition compared with the control tissue. For tissue samples shot with nonjacketed ammunition, all elements were present at significantly higher concentrations in the shot tissue compared with the control tissue at the 99% confidence level. Thus, all elements were suitable for differentiating tissue with gunshot wounds from control tissue throughout moderate decomposition.

When mean element concentrations were compared between the two bullet types, there were significant differences in the Cu and Pb concentrations at the 99% confidence level. Cu was more concentrated in wounds shot with jacketed bullets, and Pb was more concentrated in tissue shot with nonjacketed bullets, which is likely due to bullet composition, as previously observed in the fresh tissue study. Mean Sb concentrations were not significantly different between the two bullet types in the decomposed tissue samples because of the wide concentration range of this element in the tissue samples shot with jacketed bullets. As Sb possibly originates from both bullets and primers, successful differentiation of the two bullet types using Sb may be possible at longer firing distances where the GSR is mainly from the bullet alone.

Some of the variability in element concentrations in GSR was also because of the sample collection procedure. Tissue samples for digestion were cut directly adjacent to the entrance wound out to *c.* 1 cm, where the GSR was most concentrated. However, the GSR distribution was not uniform, which leads to differences in element concentrations in the samples, larger standard deviation values, and decreased confidence in element concentration differences between bullet types. Finally, similar to the fresh tissue study, mean Fe and Ba concentrations were not significantly different between the two bullet types and were therefore not useful for differentiation.

Overall, the element concentration results from the decomposed tissue study were similar to results obtained from the fresh tissue study owing to minimal decrease in element concentrations throughout moderate decomposition. All five elements of interest (Fe, Cu, Sb, Ba, and Pb) were able to differentiate tissue with gunshot wounds from unshot tissue, and Cu and Pb were able to discriminate tissue shot with the two different bullet types.

Conclusions

Chemical identification of GSR in tissue samples using ICP-MS is a promising option to supplement current gross and histologic examination procedures available to coroners, medical examiners, and forensic pathologists. Histology procedures indicated the presence of GSR, and ICP-MS was used to identify the presence of GSR in close-range gunshot wounds from two bullet types in both the fresh state and throughout moderate decomposition. However, GSR determination using histology became more difficult as decomposition progressed. Adding the elements Fe and Cu to the elements already considered characteristic of GSR (Sb, Ba, and Pb) increases confidence in gunshot wound determination. Using these element profiles, wounds shot with jacketed bullets were differentiated from wounds shot with nonjacketed bullets throughout moderate decomposition, providing a new level of information to investigators. While this research demonstrated success in differentiating bullet types from close-range gunshot wounds, more challenging tissue samples must be investigated in the future to determine the limitations of these methods.

The results of this research could benefit law enforcement agencies as well as coroners, medical examiners, and forensic pathologists. Law enforcement agencies may use the method to determine the type of bullet used, which can link a weapon and/or suspect to a crime scene. Coroners, medical examiners, and forensic pathologists could use the method to assist in gunshot wound identification and cause of death determination, even in corpses in a moderate state of decomposition.

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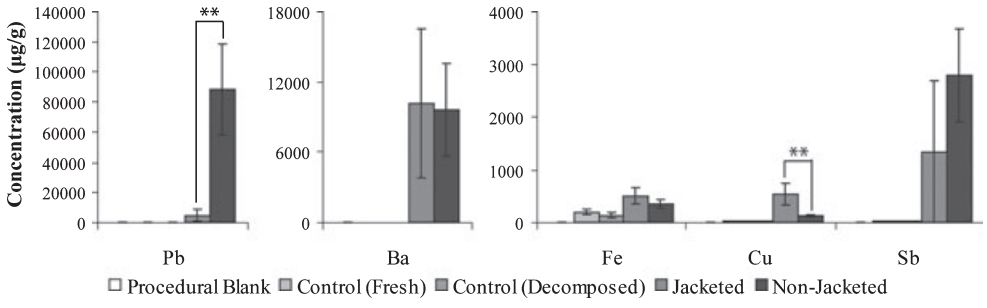

FIG. 5—*Mean concentrations of elements of interest quantified in samples collected throughout moderate decomposition (procedural blank* n = *5, control (fresh)* n = *3, control (decomposed)* n = *6, jacketed n = 6, and nonjacketed* n = *6). The error bars represent one standard deviation. Significant difference between element concentrations at 99% confidence level (*$p \leq 0.01$*) are indicated by **.*

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