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Non-invasive post-mortem interval diagnostics using a hand-held Raman spectrometer

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HIGHLIGHTS

- A hand-held Raman spectrometer can be used to predict PMI based on spectroscopic analysis incisors.
- Intensity of 1402 cm^{-1} band in the Raman spectra of teeth that can be used to differentiate days 22–42 and above day 42 PMI.
- Raman-based diagnostics of PMI is precise and non-invasive, which is critically valuable in forensic science.

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ABSTRACT

Reliable methods used to estimate the post-mortem interval (PMI) of skeletonized human remains are critically important for the accurate determination of time of death. During the early stages of decomposition (day ~ 1–25), PMI can be determined by visual observation, temperature analysis, and forensic entomology. On an annual timescale, luminol chemiluminescence and carbon isotope analysis can be used to determine the PMI. However, there is no reliable methodology that can be used for mid-term (day 22–300) timescale. In this study, we investigated the potential of Raman spectroscopy, a non-invasive and non-destructive analytical technique, for PMI analyses. The Raman-based diagnostics was achieved by spectroscopic analysis of human cadaver incisors. We observed gradual changes in the intensity of 1402 cm^{-1} band that can be used to differentiate days 22–42 and above day 42 PMI. These results demonstrate the great potential of Raman spectroscopy for precise and non-invasive analysis of decomposition processes in the human body which is critically valuable in forensic science.

1. Introduction

Forensic experts often analyze unburied human remains, typically due to murder, sudden death, or war-related issues. A challenging parameter in the analysis is the estimation of time since death, or post-mortem interval (PMI), of decomposing or skeletonized remains. Few dating techniques have shown to be reliable in estimating PMI of decomposing remains; however, these methods may be affected by various external and environmental factors, which are compounded if the remains are fragmentary or comingled. For this reason, researchers continue to work towards creating a reliable systematic dating method for skeletal remains at various stages of decomposition.

Estimations of the PMI prior to skeletonization are commonly performed by examination of morphological changes of the body, biochemical changes of bodily fluids, insects and microbial community assemblage/metabolic functions associated with the remains, or soil chemistry analysis of the cadaver's decomposition island [1–6]. However, these methods suffer from either imprecision, period limitation, or substantial variability depending on local climate, weather, body size, and scavenging amongst other factors [7–9]. Further challenges are faced when estimating the age of older remains. Precise estimation of a PMI beyond 28 days is difficult using entomological or luminol chemiluminescent data [3,10]. Additionally, carbon-14 isotope analysis and other methods are only reliable for the analysis of archaeological remains, which are inapplicable to modern or forensic cases [11]. These limitations demand a need for alternate to visual observation analyses of human remains, especially when associated with the time frame between 22 days and one year.

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Raman spectroscopy (RS) is a non-invasive analytical technique that provides information about molecular vibrations and consequently the structure of analyzed samples [12]. RS detects inelastically scattered photons by molecules that are being excited to higher vibrational or rotational states. RS has been used in forensic contexts for the identification of body fluids, drugs, explosives, and gunshot residues [13–16]. Specifically, RS was used to identify blood, saliva, semen, sweat, and vaginal fluids. Unique spectroscopic signatures of body fluids have also been used to determine the age, race, and sex of a suspect and the bloodstain age [17–20]. RS has also been used to estimate burial duration [21]. The Raman spectra of five ancient molar teeth were observed, with burial duration ranging from 150 to about 6000 years and compared to the spectrum of a modern human tooth. The bands related to organic components found on the enamel surface decreased in intensity with relation to burial duration, whereas the bands reflecting inorganic components remained constant [21]. Peak integration of Raman bands has further been explored to construct a preliminary model used for extrapolating burial duration [22]. This approach used Raman measurements of a segmented Turkey bone to correlate spectroscopic trends to burial duration. Mclaughlin and co-authors observed a consequent decrease in the intensity of amide I, CH_2 region, and NH bands with an increase in the burial time. No changes were observed for the PO_4^{3-} vibration. This study showed that RS can be used to detect chemical changes in bone tissues during short-time burial intervals (day 12–62) and predict PMI based on these spectroscopic changes.

Our laboratory develops the use of hand-held Raman spectrometers, which provide an opportunity to perform forensic analyses both in field and in the laboratory. The exhumation and recovery of skeletal remains impose great risks to the preservation of bone, however, the use of an in-field technique allows for *in-situ* analysis. This advantage enables analyses prior to skeletal processing and prevents any further contamination. Since RS is a non-destructive technique, destruction of skeletal elements will be avoided which allows for additional analyses of the observed elements. Additionally, measurements collected by a hand-held Raman spectrometer are taken in short intervals of 30 s, which allows for a vast amount of data collection in a short amount of time. The Raman spectrometer, equipped with a small, non-destructive laser beam, also enables investigators to easily sample comingled or fragmentary remains in the field.

The current study reports the potential use of a hand-held Raman spectrometer for PMI determination based on changes in spectroscopic signatures of incisors' dental enamel surface. Although surrounding periodontal ligament decomposes at a similar rate to the rest of the body, dental enamel is the most resistant skeletal element to environmental decay [23]. Additionally, if the body is lying face up, incisors are typically easily accessible, which makes their spectroscopic analysis minimally distortive to the rest of the body. As anterior dentition is commonly the first to detach from alveolar bone and is commonly lost during exhumation, this study may not be applicable to all archaeological contexts. The demonstrated method is ideal in forensic related circumstances, or with isolated dentition due to detachment. Skeletal elements are frequently dispersed due to criminal activity, scavenging, and natural factors such as bodies of water. These factors impose a greater need for diverse analyses of isolated elements.

2. Materials and methods

Research was conducted at the Forensic Anthropology Research Facility (FARF), a component of the Forensic Anthropology Center at Texas State University (FACTS). The 26-acre outdoor human decomposition research laboratory is located on Freeman Ranch, San Marcos, Texas and was opened in 2008. The training/research facility facilitates research on human decomposition, the postmortem interval, and other taphonomy related issues. Vegetation is perennial grassland which has been invaded by Ashe juniper (*Juniperus ashei*), and soil is characterized as shallow stony clay which has weathered from dolomite limestone. Annual precipitation is 857 mm and mean annual temperature is 19.4 °C [24].

Raman spectra were collected with a hand-held portable Agilent Resolve spectrometer, equipped with an 830 nm laser. The following experimental parameters were used for all collected Raman spectra: 1 s acquisition time, 495 mW power, surface scanning mode, and baseline spectral subtraction by device software. Measurements of the mandibular and maxillary central incisors' tooth enamel were collected from eight cadavers (6 males, 2 females, mean age = 64 years), with a total sample size of 10 incisors. Three data sets were collected over a time span of three months in intervals of about seven weeks (12/13/2018, 02/01/2018, and 03/19/2019). Each tooth was sampled three times for each data set. The cadavers' postmortem intervals ranged over approximately four months with the oldest placement date of June 27, 2018 and the most recent placement date of November 16, 2018. This enabled a PMI observation of 27 days to 266 days (421 to 4993 accumulated degree days), and thus data were collected during active and post- decomposition. Spectroscopic analysis was performed directly on the human remains after sunset to avoid possible interference of the sunlight with the spectrometer. It is imperative to note that cadavers observed were in-field, albeit not buried.

For analysis of variance (ANOVA), spectra were imported into MATLAB. The area under the 1402 cm^{-1} band was integrated for each spectrum, then these values were used to conduct the ANOVA. The anova1 function was used to conduct the initial ANOVA. The ANOVA was found to be statistically significant ($F = 16.69$; $P = 5 \times 10^{-14}$) at the $\alpha = 0.05$ level. The multcompare function was then used to conduct Tukey HSD tests to identify the PMIs with significantly different integrated intensities. The 95% confidence intervals (Fig. 2) were automatically constructed by the multcompare function while conducting the Tukey HSD tests.

Various factors affect the rate of decomposition such as temperature, insect activity, humidity, and embalming [25]. Donations in this collection are not embalmed prior to field placement. Although, the environment in the facility remains a constant to all individuals, the initial season of placement differs between subjects. Metal cages wrapped in chicken wire were placed on top of the human cadavers to prevent animal scavenging.

Stages of decomposition were described using a total body score and accumulated degree-days (ADD). Decomposition scores of the head and neck, trunk, and limbs were recorded daily and pertain to four stages of decomposition: fresh, early decomposition, advanced decomposition, and skeletonization [26]. Accumulated degree days (ADD) were calculated using an onsite datalogger following Megyesi et al. (2005) from date of placement to date of observation [26]. Incorporating time and temperatures allows researchers to use the scale across different seasons [27].

3. Results and discussion

Raman spectrum of the teeth exhibit vibrational bands at 433, 450, 587, 609 and 960 cm^{-1}, which can be assigned to PO_4^{3-} vibrations. We also observed vibrations that can be assigned to amide III (1243 cm^{-1}), and N–H and C–H vibrations (1402 and 1454 cm^{-1}). Lastly, we observed two bands at 1042 and 1070 cm^{-1} which can be assigned to CO_3^{2-} vibration, Fig. 1.

We found that most of the described above bands in the spectrum collected from D 22–42 teeth exhibited weaker intensities comparing to the corresponding bands in the spectra of D 64–84, D 106–126 and D 127–147. However, the amide III (1243 cm^{-1}) was found to be more intense in the spectra collected from D 22–42 teeth, which suggests an increase in the protein content on the surface of teeth. In the spectrum collected from D 22–42 teeth, we also observed vibrations at 1100, 1134, 1180, 1353, 1513, 1532 and 1558 cm^{-1} that were not evident in later PMI spectra. Using ANOVA, we identified a statistically significant

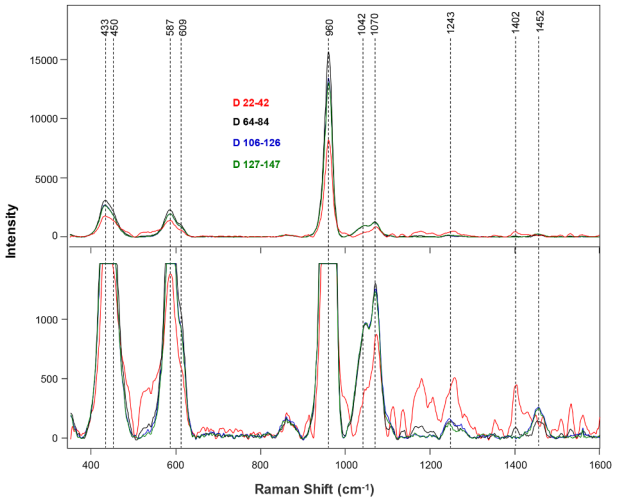

Fig. 1. Raman spectra collected from teeth of human cadavers at early PMIs.

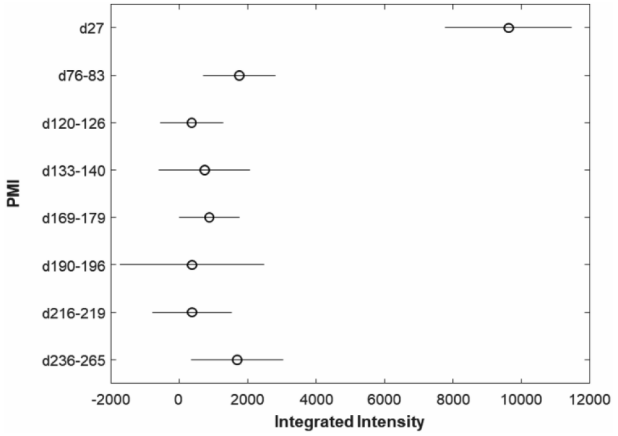

Fig. 2. 95% confidence intervals for the true mean integrated intensity of the 1402 band at each PMI bracket.

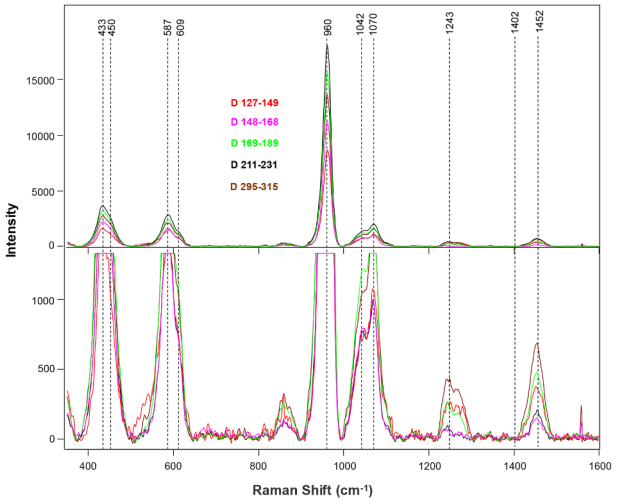

Fig. 3. Raman spectra collected from teeth of human cadavers at late PMIs.

difference in the integrated intensity of the 1402 cm^{-1} band across the PMIs, Fig. 2. The Tukey HSD revealed that D 22-42 was significantly different from all other timepoints with P-values below 6×10^{-8}. These spectroscopic changes suggest that D 22-42 teeth have a unique vibrational fingerprint, which makes them easily distinguishable from later DPIs.

At the time of observation, the subjects were in either Stage I (early decomposition), which would correspond to D 22–42, or stage II (advanced decomposition) (above D42). Based on this evidence, we can conclude that RS can be used for accurate identification of Stage I vs Stage II body decomposition based on the spectroscopic signatures of incisors.

Although the spectrum collected from D 64–84 has similar profile with the spectra collected from D 106–126 and D 127–147, we observed an increase in the intensity of bands at 1134, 1180 cm^{-1}. This observation suggests that D 64-84, like D 22–42, also has unique vibrational fingerprint.

We found changes in the intensities of the discussed above vibrational bands in the later > 84 days PMIs do not have any correlation with PMIs, Fig. 3. For instance, we observed an increase in the intensity of 433–960 cm^{-1} bands from D 127–149 to D 211–231, however, in the spectrum of D 295-315 this band appeared to be lower than in the spectrum of D 211–231. Similar changes have been observed for other bands. For instance, amide III first decreased in the intensity (D127-149 to D 148–168) and then increased in the intensity (D 148–168 to D 169–189).

4. Conclusion

Our findings indicate that RS can be used to predict early stages of PMI. Specifically, using RS, PMI D 22-42 can be distinguished from all later PMI based on the intensity of 1402 cm^{-1} band. Similar to D 22-42, we also found that D 64–84 has unique vibrational fingerprint that enables its identification from later PMIs. Our results also show that vibrational bands in the spectra collected from teeth at above 84 PMI have neither direct nor indirect correlation with PMIs.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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