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RESEARCH ARTICLE

Burning by numbers: A pilot study using quantitative petrography in the analysis of heat-induced alteration in burned bone

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Abstract

In the past, experimental research into the histomorphological examination of burned human bone has led to the creation of a criterion for assessing burning intensity, which can be used to infer firing conditions in both archaeological and forensic contexts. Current methods visually compare the microscopic alterations in burned bone with modern bone samples fired at known temperatures and durations. Despite the benefits of this approach, it is hindered by the use of qualitative analysis, which is subject to the expertise of the examiner. This paper reviews previous histomorphological studies of burned bone and presents a new protocol for producing burned bone thin sections. It also introduces quantitative petrography as an alternative statistical method for categorising burning intensity. Four categories of burning intensity were calculated based on the quantified heat-induced changes identified in a pilot study examining burned porcine bone. These categories were consistent with those produced using more traditional qualitative methods, demonstrating that the results produced in this pilot study are reliable. An interobserver study showed the repeatability of this new method by both anthropologists and non-anthropologists.

KEYWORDS

bioarchaeology, burned bone, forensic anthropology, histomorphometry, quantitative petrography

1 | INTRODUCTION

Burned human bone is frequently found in forensic and archaeological contexts. The analysis of these remains is imperative to any investigation as they can assist in building a biological profile (e.g. age, sex, ancestry and stature) of the deceased, the cause of death, as well as burning conditions. Studies have experimented with the use of histomorphometry and its value in analysing the microscopic heat-induced (H-I) alterations in burned bone (Castillo, Ubelaker, Acosta, de la Rosa, & Garcia, 2013; Gonçalves, 2012; Hanson & Cain, 2007; Squires, Thompson, Islam, & Chamberlain, 2011). As a result, there is now a considerable body of literature available that describes the stages of thermal decomposition in burned bone. It is firmly established that the bone undergoes four transitional phases of alteration when burned (Mayne Correia, 1997; Thompson, 2004; Ellingham, Thompson, Islam, & Taylor, 2015). These are (a) dehydration—the breaking of hydroxyl bonds and loss of water bound to the bone matrix; (b) decomposition—the organic component of the bone

is removed by pyrolysis; (c) inversion—the elimination of bone carbonate; and (d) fusion—the coalescing and melting of the crystal matrix (Ellingham et al., 2015).

Histomorphological research into cremated bone stems back to the 1940s with Forbes' (1941) experimental study that examined the effect of increased temperature on bone's microstructure. Later, Herrmann (1977) observed that between 700°C and 800°C, the organic component of the bone became fully cremated, and the hydroxyapatite fused, which caused the bone to shrink. This stage of the burning process was identified as the 'critical level' (Herrmann, 1977, p. 101). At this time, it was believed that incompletely burned bone displayed no morphological differences at the microscopic level; histomorphological examinations could only be achieved once the bone was fully cremated (Bradtmiller & Buikstra, 1984). It is now firmly established in the academic literature through extensive experimental research that this is not the case and that the bone exhibits microscopic H-I alterations before the 'critical level'. This misconception is most likely a result of the sampling and experimental methods employed in the past. In other words, the observations made in Herrmann's (1977) paper come from archaeological burned bone samples. The temperatures reached are therefore unknown and inferred in this study from macroscopic bone colour alone.

These initial histomorphological studies have developed greatly, thanks to the application of strict laboratory procedures and thorough methodological approaches. Researchers now have a better understanding of the nature of H-I changes in burned bone at the microscopic level, although there are some discrepancies between a number of studies (Ellingham et al., 2015). Table 1 summarises the histological observations of burned bone published over the last 80 years. It is clear from Table 1 that Forbes (1941), Nelson (1992), Hummel and Schutkowski (1993) and Cattaneo et al. (1999) found that the size of osteons decreased when exposed to increased temperatures; however, this was contradicted by Bradtmiller and Buikstra (1984), who found that osteon size grew when subject to higher burning temperatures. It is unclear what caused this difference in observations. Like Nelson, Bradtmiller and Buikstra also experimentally heated femoral sections to 600°C, suggesting similar experimental conditions (Gonçalves, 2011). It could possibly be a result of differences in the preservation of samples, for instance, archaeological versus modern bone sections or defleshed versus fleshed remains.

With regard to the preservation of histological microstructure, Brain (1993), Squires et al. (2011) and Absolonová et al. (2013) report that structures are still visible at 600°C. However, Hanson and Cain (2007) observed that histological structure disappeared between 482°C and 620°C. This discrepancy is most probably a reflection of the different experimental designs employed. Hanson and Cain's study involved burning sheep bone on a campfire, unlike the other studies that were conducted under laboratory conditions with greater temperature control. In addition, Hanson and Cain's temperature ranges overlap considerably, meaning that opposing microscopic changes are reported within the same temperature scale.

Although there are clearly some differences in the microscopic alterations in burned bone reported by experimental research, Table 1 also highlights several consistencies. Of the studies that discuss both histomorphology and burned bone colouration, the majority concur that bone carbonisation is present at temperatures between 300°C and 800°C (Herrmann, 1977; Squires et al., 2011). In Brain's (1993) study, it was observed that this change occurs at 500°C, at which point the carbon component of the bone oxidises, inducing a pale pigmentation. More recently, Squires et al. (2011) and Wolf et al. (2017) have noted that between 600–900°C and 750–850°C, respectively, bone microstructure is still preserved and can be used to conduct age assessment. The majority of studies also agree that by 800°C, the homogeneity of bone's matrix has largely disappeared (Brain, 1993; Castillo et al., 2013; Herrmann, 1977).

One prominent limitation of the literature is the overall lack of standardisation in experimental design and sampling methods. The microscopic observations recorded in Table 1 are based on both laboratory (Castillo et al., 2013) and field (Hanson & Cain, 2007) experimentations. This has resulted in some difference in temperature increments, which makes the direct comparison of results difficult (Gonçalves, 2011). Studies have also used a mix of human and animal bone samples, ranging in size from 10-cm sections (Bradtmiller & Buikstra, 1984) to 60-μ thin sections (Squires et al., 2011). In addition, different modes of analysis have been employed to conduct qualitative assessment; Herrmann (1977), for instance, examined images produced by a scanning electron microscope (SEM), and Castillo et al. (2013) used a Leica DMLB microscope. This variation in technology may reduce the replicability of results obtained.

Within the last 10 years, research has grouped the microscopic alterations associated with the four transitional phases (as outlined earlier in this paper) into three or four categories. Squires et al. (2011) provided a three-stage classification of cremated bone. The first category described the preservation of bone microstructure when heated between 300°C and 600°C, with the visibility of many Haverisian systems, Volkmann's canals and organic material. Less than half of the bone's microstructure remained (with the exception of canaliculi) when exposed to temperatures between 600°C and 900°C resulted in less than half of bone's microstructure remained; at this stage, many hydroxyapatite crystals had fused, resulting in a disorganised arrangement. At temperatures in excess of 900°C, no Haversian systems, Volkmann's canals or canaliculi survived. Instead, hydroxyapatite crystals had entirely fused. These categories of microscopic alteration were furthered by Castillo et al. (2013) who described four stages of thermal decomposition. These groupings shared several parallels with the Squires et al. (2011) categorisation system, which is attributed to their comparable sampling methods and experimental design. The only difference was that Castillo et al. employed a wider temperature range that started at 100°C. At this initial phase, the authors reported the formation or longitudinal fractures and the preservation or organic material.

TABLE 1 Microscopic changes in burned bone according to contemporary research

	0	100˚C	200˚C	300˚C	400˚C	500˚C	600˚C	700˚C	800˚C	900˚C+
Forbes (1941)	An increase in temperature results in a decrease in osteon size ➔									
Herrmann (1977)							Carbon colouration <700˚C	Organic matter cremated. Crystals fused >700—800˚C		
Bradtmiller and Buikstr (1984)							Microstructure preserved. Increase in osteon size 600˚C			
Nelson (1992)	An increase in temperature results in a decrease in osteon size and increase in Haversian canal diameter ➔									
Brain (1993)			Carbon in lacunae. Microstructure preserved 200˚C	Lamellar is carbon. Cracks spreading from Haversian canals 300˚C	Cracks continue to develop 400˚C	Carbon has oxidised, producing pale colour 500˚C	Microstructure visible. Cracks spread across surface area 600˚C	Matrix shrunk 700˚C	Microstructure disappeared. Fusion of crystals 800˚C	
Hummel and Schutkowski (1993)	An increase in temperature results in a decrease in osteon size ➔									
Cattaneo et al. (1999)								Osteons shrink <800˚C		Significant changes in microstructure <1000˚C
Hanson and Cain (2007)	All histological structures are visible Unheated—470˚C									
					Carbon accumulating. Cracking emanating from Haversian canals 380—482˚C					
							Accumulation of carbon in is minimal. No cracking			
							590 ˚C			
						Histological structure has disappeared. Carbon deposits extensive. Cracks spreading from Haversian canals 482—620˚C				
						Carbon deposits still occur. Cracks spreading outwards from the Haversian canals 462—705˚C				
Squires et al. (2011)				Preservation of microstructure, organic material with some crystal fusion. Dark in colour 300—600˚C				Less microstructure and >50% organic material. Hydroxyapatite fusion 60—900 ˚C		No microstructure. Complete hydroxyapatite fusion >900˚C
Absolonová, Veleminsky, Dobisikova, Beran, and Zocova (2013)							Microstructure similar to unburned bone 600˚C	Osteon and Haversian canals shrink 700˚C	Osteon and Haversian canals shrink further 800˚C	
Castillo et al. (2013)		Collagen deformation 100—300˚C			Vitreous crystalline formations. Crystalline polymers 400—600˚C			Rounded and cubical crystals. Loss of homogeneity 700—800 ˚C		Granular surface >900˚C
Wolf, Streit, Dokládal, and Schultz (2017)									Inorganic bone structures are in good condition 750—850˚C	

The categories described by Squires et al. (2011) and Castillo et al. (2013; Table 1) were established using qualitative methods. Qualitative analyses can be problematic when examining burned bone samples as they are based on the examiners experience and knowledge. Unfamiliarity with bone microstructure and the changes that can occur as a result of extreme heat can result in inaccurate interpretations of burning conditions. In addition, Squires et al. produced burned bone thin sections for microscopic analysis. However, the slide thickness ranged from 60 to 100 μ. This is because the archaeological bones were extremely fragile and often broke when cut with a microtome. Such variation in sample thickness has also been reported by similar studies conducting thin-section analysis (Holden, Phakey, & Clement, 1995), which could hinder the visibility of the sample's microstructure.

With this in mind, the authors aim to produce a new method for producing burned bone thin sections and intend to propose a new method for analysing thin sections using quantitative petrography; this approach aims to increase the reliability of assessing burning conditions and reduce interobserver bias.

2 | MATERIALS AND METHODS

2.1 | Thin-section production of modern animal bone

The authors created thin sections of the bone heated to known temperatures and durations to establish known burning categories. Eleven sections of pig femur measuring 2 cm long were burned in an industrial furnace at controlled temperatures ranging from 100°C to 1100°C (in 100°C increments) for 45 min. This resulted in one bone sample per temperature, which is a small sample but suitable for the current pilot study, which formed part of a PhD project. These samples were impregnated in a solution of Epoxy Resin RX77IC/NC and HY951 Hardener at a ratio of 10:1. The resin blocks were cut using a WOKO cutter and mounted onto glass slides using a mixture of RT151-BU-256 resin and RT151-BU-250G hardener at a ratio of 4:1. Once mounted, the blocks were left to set under a pressure plate overnight. Next, the excess resin block was cut from the glass slide using a LOGITRIM saw and polished to 40 μ using a LOGITECH.

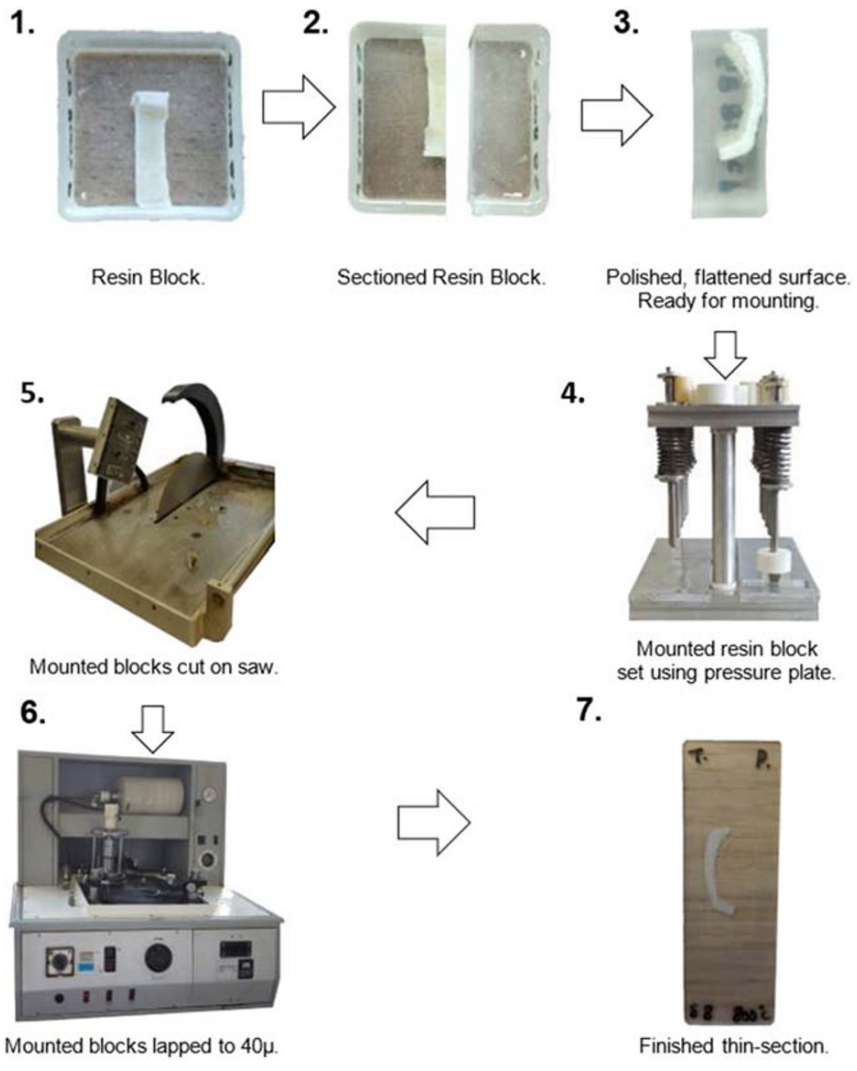


FIGURE 1 Flow diagram of burned bone thin-section production [Colour figure can be viewed at wileyonlinelibrary.com]

Figure 1 shows the stages of thin-section production in line with the methodology outlined above.

2.2 | Quantitative petrography

Quantitative petrography has traditionally been applied in geology to examine the composition of rocks and soils by counting the organic inclusions. More recently, it has been used in archaeological studies to count inclusions in pottery fragments and ceramic building material to establish how materials were sourced and made (Machine, 2017; Sutton, 2017). In this study, microscopic H-I alterations in burned bone were examined using the PETROG motorised stepping stage and 2018 PETROG software provided by Conwy Valley Systems Limited. The set-up involves attaching the PETROG motorised stepping stage (a metal frame in which the thin section is secured and moved along via a motor) to the platform of a Leica DM EP microscope with a Leica DF 295 camera. The 2018 PETROG software loaded on the associated computer is used to identify and count microscopic features in the thin sections using the software dictionary, developed from the criteria provided by Squires et al. (2011). The data can then be exported to a Microsoft Excel spreadsheet for quantification and SPSS for statistical analysis.

2.3 | Statistical analysis

The petrographic data from the 11 modern thin section was imported to IBM SPSS Statistics 24. A *k*-means cluster analysis was performed to statistically group the H-I alterations. This test was used as it is an unsupervised way of grouping unlabelled data and is more appropriate than a hierarchical cluster or a two-step cluster because it is better suited for large data sets (Kaur & Kaur, 2013).

2.4 | Interobserver study

An interobserver study was also carried out to examine the validity of this new method. Five participants were recruited: three anthropologists and two non-anthropologists. Each of the anthropologists had undertaken postgraduate studies in human bone, whereas the two non-anthropologists had studied archaeology at undergraduate level but had no previous anthropological experience. None of the participants had used quantitative petrography before. Each participant was required to analyse two thin sections of archaeological human bone. Archaeological samples were examined here because specialists utilising quantitative petrography would be using it primarily to examine archaeological burned bone. These samples come from the Anglo-Saxon cemetery of Elsham, North Lincolnshire (Burial EL75HL), and the Roman cemetery of Folly Lane, Hertfordshire (Burial 2). These thin sections were chosen for analysis as they displayed substantially different H-I microscopic changes. The examination of two thin sections is a small sample but suitable for the current pilot study, which formed part of a PhD project. The lead author was present during the study to answer any questions the participants may have; however, care was taken to ensure that any answer given did not influence the results of the study. Ethical approval to conduct the interobserver study was granted by the ethics committees of Staffordshire University and Reading University.

3 | RESULTS

3.1 | Categories of burning intensity using modern standards

The *k*-means cluster analysis identified four burning intensity groups based on the quantities of H-I alterations of the 11 porcine femora samples (Table 2). These results are consistent with other publications (Castillo et al., 2013; Squires et al., 2011), which indicates that the data are reliable.

Category I: Between 100–400°C, the microstructure of the bone is well preserved; Haversian systems are consistently circular, with no malformation, and smaller features including Volkmann's canals, osteons and canaliculi are relatively unaltered. Between 48.3% and 53.6% of the sample area consists of organic material, whereas the remainder is composed of well-defined microfeatures.

Category II: The depletion of organic material and the fusion of hydroxyapatite crystals become more frequent at temperatures between 500°C and 600°C. At this stage, microfeatures are still identifiable, but they are less well preserved and show signs of deterioration, whereby 14.7%–18.3% of the sample area displays poorly defined microstructures.

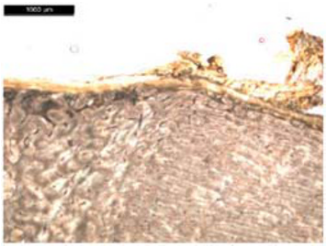
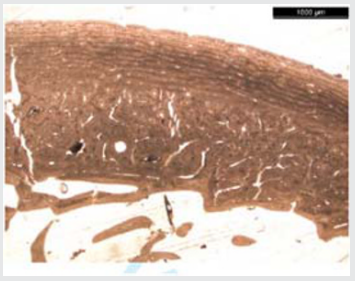
Category III: The degeneration of microscopic features and the increase in hydroxyapatite fusion become more apparent in temperatures ranging between 700°C and 900°C. All organic material within the sample area has decomposed, and 49%–59.6% of the microscopic composition is fused.

Category IV: For temperatures in excess of 1000°C, a minimum of 86.3% of the sample area displays complete hydroxyapatite fusion, evident from the lack of discernible osteons, Volkmann's canals and canaliculi. A few misshaped Haversian systems still remain, comprising up to 14% of the sample area.

3.2 | Interobserver study

Of the five examiners included in this study, four of the participants produced the same results, yielding an agreement level of 80% with

TABLE 2 Burning categories identified from *k*-means cluster test from the modern pig samples

Category	Temperature	Micrograph
I	100–400°C	
II	500–600°C	
III	700–900°C	
IV	1000–1100°C	

observations made by the authors (Table 3). Only one of the examiners (second anthropologist) obtained different results. The reason for this was simply due to the examiner repeatedly selecting the wrong option from the dictionary of H-I alterations.

4 | DISCUSSION

4.1 | Thin-section preparation

Creating thin sections of modern and archaeologically burned bone is challenging. In the past, researchers have struggled to produce consistent thin sections that have all the same thickness (Hanson & Cain, 2007; Simmons, Goodburn, & Singhrao, 2016; Squires et al., 2011). The method recommended in this paper encourages a standardised approach that will hopefully facilitate further comparative research.

As the bone is a porous material, the process of impregnating a fragment in resin often leads to air pockets that can obscure the appearance of the sample area. This study shows that evacuating the resin mixture before and after it is poured over the bone fragment helps to remove all the oxygen before the resin has set. This produces a consistent sample area that is not obscured by any air bubbles. To date, this approach has yet to be applied in other studies producing thin sections of burned bone (Cattaneo et al., 1999; Hanson & Cain, 2007; Simmons et al., 2016; Squires et al., 2011). However, it has been successfully applied to the analysis of other porous materials, including soils, metals and ceramics (Granger, 1967; Jongerius & Heintzberger, 1975).

Previous studies that have explored the histomorphology of burned bone employed a microtome (Castillo et al., 2013; Holden et al., 1995; Schotsmans, Fletcher, Denton, Janaway, & Wilson, 2014; Squires et al., 2011), which is a tool used to cut samples for thin-sectioning (e.g. Squires et al., 2011). This technique can

TABLE 3 Results from interobserver pilot study

Examiner	Sample	Original category (by the authors)	New category (by the participants)	Agreement (%)
First anthropologist	1	IV (1000–1100°C)	IV (1000–1100°C)	100
	2	II (500–600°C)	II (500–600°C)	
Second anthropologist	1	IV (1000–1100°C)	III (700–900°C)	50
	2	II (500–600°C)	II (500–600°C)	
Third anthropologist	1	IV (1000–1100°C)	IV (1000–1100°C)	100
	2	II (500–600°C)	II (500–600°C)	
First non-anthropologist	1	IV (1000–1100°C)	IV (1000–1100°C)	100
	2	II (500–600°C)	II (500–600°C)	
Second non-anthropologist	1	IV (1000–1100°C)	IV (1000–1100°C)	100
	2	II (500–600°C)	II (500–600°C)	

be problematic when used to section burned bone, often resulting in the slice breaking due to its friable nature, producing an uneven sample area that is hard to accurately examine under a microscope (Squires et al., 2011). An alternative to this approach involves grinding or polishing the resin block mounted (Cattaneo et al., 1999; Simmons et al., 2016), which can be a time-consuming process when conducted by hand (Simmons et al., 2016). To make the process more efficient, this study employed a LOGITECH, which is a lapping machine with automated plate flatness control that reduces a bone sample to a preprogrammed thickness. This guarantees consistent sample thickness and avoids breaking the sample. Other studies examining bone histology have also used this lapping technique to process large numbers of samples (Cattaneo et al., 1999; Holden et al., 1995).

The quantitative PETROG software and equipment used in this study were provided by Conwy Valley Systems Limited. The software's design is flexible and can be applied to other modern and ancient materials including ceramics, building materials, bones and soil samples. It is therefore an invaluable addition to any multidisciplinary department or laboratory. It is also compatible with Windows operating systems. The program exports the collected data into a spreadsheet from which statistical analysis can be carried out, for example, imported into SPSS. The automated stepping stage is also compatible with most microscopes and can be assembled easily. However, this set-up is another example of a specialist equipment that is not currently widely available.

Despite the benefits of this new approach, it is time consuming. On average, a batch of 12 samples takes 5 days to produce. This means that future studies using larger sample sizes will need to carefully consider the time needed for sample preparation prior to analysis. As a result, this could slowdown the research process, especially when compared with other methods that require less time-consuming sample preparation, such as SEM. In addition, the machines used in this procedure are specialist pieces of equipment that are not readily found and do require expert training.

Pigs are often used in forensic research as human proxies, as their body size, fat distribution and bone microstructure are, to some extent, similar to that of humans (Schotsmans et al., 2014); however, it is important to acknowledge that the results achieved may not be fully true of human bone (Thompson, 2002). The samples analysed in this study composed sections of a porcine femur, which were burned in an industrial furnace. Studies have found that the distribution of fatty tissue on a body can impact the resultant H-I changes (DeHaan, 2015). For example, DeHaan (2015) explains that fat is the best fuel in the body for burning, though the effects of heating on this material depends on its thickness and its thermal properties, as well as the duration of the fire. In the present study, the porcine samples used were defleshed (soft tissue removed) before firing, which would have had an impact on the resultant microscopic H-I alterations. In addition, we are aware of the limitations of differential bone microstructure of pigs and humans, but the results obtained in this study were consistent with other publications examining adult human bone (Squires et al., 2011), indicating that they are reliable. Future research would benefit from using complete animal carcasses and taking multiple samples from different parts of the body.

4.2 | Quantitative versus qualitative analysis

The results obtained from using quantitative petrography in the analysis of burned bone compliment those ascertained by previous histological research (Absolonová et al., 2013; Castillo et al., 2013; Squires et al., 2011) and those derived from the examination of macroscopic colour (Ullinger & Sheridan, 2015). This is encouraging and, when teamed with the data from the interobserver study, shows that this method is reliable. Quantifying the microscopic features in burned bone produces large numerical databases that can be easily used in statistical analysis, and the categorisation of data using a *k*-means cluster analysis helps to overcome issues with interobserver bias.

The new categorising system concerning microscopic changes in burned bone here has generated narrower temperature ranges than those established in contemporary research (Squires et al., 2011). This results in a more accurate interpretation of the material. The *k*-means cluster statistical analysis identified significant groups among the modern animal bone samples burned between 100°C and 1100°C that most studies had only previously been established using visual assessment (Castillo et al., 2013; Hanson & Cain, 2007; Squires et al., 2011).

On the basis of the standards devised from the porcine samples employed in this research, it was observed that minimal microscopic alteration takes place at temperatures between 100°C and 400°C, which is understandable considering that these temperatures are used in cooking and do not result in skeletonisation. At this stage, petrographic analysis found that between 48.3% and 53.6% of the sample area consisted of organic material, which includes carbon and collagen (Ellingham et al., 2015), whereas the remainder constitutes well-preserved microfeatures such as Volkmann's canals and canaliculi. This is to be expected and represents the dehydration stage of burned bones transformation whereby hydroxyl bonds start to break up, resulting in the loss of water (Ellingham et al., 2015). Squires et al. (2011) and Hanson and Cain (2007) also observed the preservation of microstructures at lower burning temperatures.

Between 500°C and 600°C, the sample area demonstrates depleted microscopic definition, whereby between 14.7% and 18.3% of the sample area constitutes poorly defined features, including Haversian systems and Volkmann's canals. This represents the decomposition and removal of carbon and collagen within bone's matrix (Hanson & Cain, 2007; Squires et al., 2011). Interestingly, Absolonová et al. (2013) found that burned bone's microstructure resembled that of an unburned bone at 600°C. This may be a result of the difference in burning duration; in the current study, bone sections were fired for 45 min, whereas Absolonová et al. (2013) placed their samples in a furnace for 30 min. As proposed by Squires et al. (2011), variation in firing duration is a significant factor in the preservation of bone's microstructure (Gonçalves, 2011).

By 700°C, all organic material is removed from the sample area due to pyrolisation. Haversian systems are the only discernible microfeature remaining, and their form has become misshapen due to the decomposition of bone's organic component and fusion of hydroxyapatite, which constitutes 49%–59.6% of the sample area. Similar depletion in bone's microstructure has also been reported by Brain (1993) and Cattaneo et al. (1999).

At 1000°C, 86.3% of the sample area is completely fused, representing the melting and coalescing of the crystal matrix (Ellingham et al., 2015). Brain (1993) and Castillo et al. (2013) report that complete fusion occurs at lower burning temperatures, namely, 800°C. However, the practice of counting individual microscopic features across the entirety of the sample area, rather than conducting a visual overview of the bone sections, has identified few degenerated microscopic features as this temperature and raising the level of crystal fusion to 1000°C.

It is important to consider that this is a pilot study with a comparatively small sample size; future research would greatly benefit from not only applying this quantitative approach on a considerably larger scale but also by making comparisons of both modern and archaeological samples. It is also necessary to point out that the categories of burning presented here do not take burning duration into consideration. All bone samples were fired for 45 min to resemble the experimental design of similar burned bone studies (Thompson, Islam, & Bonniere, 2013). On a microscopic level, Holden et al. (1995) has pointed out that short burning periods would leave the endosteum and inner cortical bone incompletely cremated, resulting in different structural alterations.

5 | CONCLUSION

This paper has presented a new method for preparing uniform thin sections of burned bone, which will hopefully improve comparability between the results of both qualitative and quantitative histomorphological studies. However, it is important to consider that it is a relative time-consuming process that requires specialist equipment and training. Future studies would need to take this into consideration when developing a research design. This article has also proposed a new quantitative method to determine burning intensity based on the quantification of H-I changes in burned bone. The use of the PETROG software proved to be reliable and quick when analysing samples. Additionally, the software is user-friendly, as demonstrated by the success rate of the interobserver study. It is possible that this particular method helps to ensure observer standardisation in analysis and minimises interobserver bias.

As this is a pilot study that formed part of a larger research project, the sample sizes are considerably small, which reduces the confidence of the data presented. It would therefore be insightful to apply quantitative petrography using a larger sample size and potentially in combination with other methods used to reconstruct burning conditions, such as Fourier Transform Infrared - Attenuated Total Reflectance spectroscopy (FTIR-ATR). Future research would also benefit from employing the measuring tool on the PETROG software to quantify the shrinkage of microstructure in burned bone; an assessment of such shrinkage could potentially be used for biological age and sex assessment in cremated remains.

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