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PAPER

ODONTOLOGY; CRIMINALISTICS

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Comparative Analysis of the Effects of Heat on the PCR-Amplification of Various Sized DNA Fragments Extracted from *Sus Scrofa* Molars*

ABSTRACT: This study examined the effects of heat on the amplification of DNA from the dental pulp of *Sus scrofa* molars and investigated the protection afforded to the pulp tissue by the dental enamel, alveolar process, and soft tissue of the head. Segments of defleshed maxilla and mandible encasing the first molar (n = 60) were subject to a range of temperatures for 15 min. Dental pulps were retrieved. Amplifications using three-primer and four-primer multiplexes showed no degradation of the largest fragment following exposure to 450°C. Amplifications in the three-primer multiplex (283 bp) were successful following exposure to 525°C in maxillary samples only. This study revealed the enamel density of maxillary molars to be greater than mandibular molars in *Sus scrofa*. Following incineration of intact heads for 15 min (n = 10) and 1 h (n = 4) at an average temperature of 625°C, amplifications of the largest fragment (450 bp) were successful from both maxillary and mandibular teeth.

KEYWORDS: forensic science, DNA analysis, polymerase chain reaction, burnt human remains, dentition, pulp tissue, *Sus scrofa*

From a forensic perspective, fire is arguably one of the most destructive forces in our society (1). A physical fire can result in soft tissue damage so extensive that conventional methods of identification are precluded. Because of the durability of the mineralized dental structures and the protection afforded by the soft and hard tissue mass of the head, teeth resist postmortem degradation and can withstand extreme environmental insult (2) and incineration (3,4) better than bone. Even in severe conflagrations, such as vehicle fires, explosions, and aircraft crashes, preservation of the dentition is common and often used as a primary or contributory method of identification (5,6).

In instances where antemortem records are not available for comparison, or where destruction of the dentition by fire leaves too little dental data for comparison, alternative methods of identification are required. A valuable source of DNA evidence is contained within the dentition (7), and this can be extracted and profiled to confirm identity by comparison to a known antemortem sample or to DNA profiles from relatives of the individual (8). With the development of the polymerase chain reaction (PCR) (9), it has become possible to analyze DNA that is severely degraded, of low copy number, and present only in minute quantities (10–12). PCR is a sensitive technique in which an exponential accumulation of a specific DNA fragment can be generated.

Due to its sensitivity, PCR has been widely used to profile DNA from a variety of forensic samples, including teeth (13,14). Several studies have looked at the effects of incineration on the extraction and amplification of DNA from the pulp of human teeth using PCR-based analysis (15–17). A major limitation with all such studies is that the sample utilized generally consists of isolated teeth. The insulating capacity of the bone and tissue mass of the head was not considered in such studies. As a consequence, results obtained from such works have no comparability to forensic situations where the teeth tend to remain *in situ*. While in many of these studies, PCR-based analyses were also performed on cremated remains from forensic caseworks, all too often the conditions of incineration are unknown. Consequently, the temperature and duration of incineration required for DNA to become too degraded for PCR-based analysis remain largely unknown.

The present study was designed so that the degree of DNA degradation occurring as a result of incineration could be further examined using *Sus scrofa* dentition as a human surrogate. The insulating capacity of the hard enamel casing, the alveolar process, and soft tissue mass of the head was of interest because the dentition of fire victims usually remains *in situ* within the head during incineration.

The aims of the current project were threefold: (i) to investigate the relationship between chamber furnace temperature and the corresponding temperature within the pulp cavity of embedded (still within the corresponding alveolar bone) *Sus scrofa* first molars so that the insulating capacity of enamel might be assessed, (ii) to examine the degree of degradation of nuclear DNA within the pulp cavity of embedded first molars at increasing temperatures and to determine the temperature at which DNA in the pulp tissue becomes

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too degraded for PCR analysis, and (iii) to assess the degree of protection afforded to *Sus scrofa* teeth by the soft and hard tissues of the head and to compare the success rates of target amplification by PCR to that obtained from defleshed jaw segments.

Materials and Methods

Sample Selection and Preparation

Permanent molars ($n = 88$) were utilized from 6-month-old, healthy, organically produced *Sus scrofa.* All four first molars (M1) were utilized from each *Sus scrofa*—left and right maxillary (upper) M1; left and right mandibular (lower) M1. The jaws were defleshed, and the segments of bone encasing the first and adjacent second molar and second premolar were cut from the left and right mandible and maxilla using a striker saw. Mandible and maxilla segments were clearly labelled. All samples were refrigerated at 4°C prior to heating. The heating of each sample took place within 3 days (maximum) of slaughter to ensure that putrefaction of the pulp did not affect results.

Experimental Conditions for Furnace Incineration

Jaw segments encasing the first molar were heated in a type 201 chamber furnace (Carbolite, Hope Valley, U.K.) at temperatures of 150, 225, 300, 375, 450, 525, and 600°C (five mandibular and five maxillary segments were heated per temperature [$n = 70$]). Ten segments (five mandibular and five maxillary) were left unheated as a control sample. The chamber furnace was preheated to the designated temperature prior to sample heating. Segments were placed separately in crucibles and heated for 15 min.

Incineration of Sus scrofa *Heads in the Field*

Four whole, fleshed *Sus scrofa* heads were obtained for incineration. A fire was started using charcoal, kindling, and thin blocks of wood within a metal container equipped with vents for air circulation. Heads were burned individually with the mouth facing into the hottest part of the fire. Three heads were burned for 15 min, and one head was burned for 1 h. Fire temperatures were recorded at regular intervals by placing a type K thermocouple probe (0–1370°C range; Omega Engineering Ltd., Manchester, U.K.) in close proximity to the molars. *Sus scrofa* heads were exposed to temperatures fluctuating between 450°C and 850°C. After cooling, segments of bone encompassing the first molar were removed.

Extraction of First Molars and Pulp Tissue

Control segments and heated segments were placed in a vice with plastic sheeting surrounding the sides and bottom of the segment. The teeth were left exposed and raised slightly from the vice so that the encasing bone was compressed rather than the tooth crown. Compressive strength applied to the segment was sufficient so that each tooth was slightly pushed up out of its socket. The crowns of the M1 were then gripped gently with pliers and extracted from the sockets. Because of the fragility of teeth heated at 450°C, an alternative method was used to extract the M1. The segments were very gently compressed in the vice to crack the surrounding bone, which was then picked away from the root structure using fine forceps. For samples heated at 150–450°C, the extracted M1 was securely wrapped in a clean piece of filter paper. The parcel was sealed in a sterile plastic bag and struck with a hammer to crack the tooth open and expose the pulp cavity and radicular canals. Broken fragments were retained in the plastic bag. All visible pulp tissues were removed using sterile fine forceps and placed in sterile 1.5 ml Eppendorf tubes.

At 525°C, the teeth were too fragile to be removed intact from the alveolar process. It was, however, possible to remove the crowns from the root sections by hand, thereby accessing the pulp chambers. The contents of each pulp chamber were lightly scraped into Eppendorf tubes using a sterile dental spatula. Pulp tissue within the root sections was not utilized as it was not possible to remove the fragile roots from the surrounding alveolar bone following exposure to this temperature. All pulp samples were stored at −20°C immediately after retrieval.

DNA Analysis

DNA extractions from pulp samples were performed using the QIAmp DNA Mini Kit (QIAGEN Ltd., Crawley, U.K.) following the tissue protocol. Extraction from fresh *Sus scrofa* muscle was used as a positive control. All samples were stored at −20°C following extraction. The quantity of DNA was measured using the Picogreen® double stranded DNA (dsDNA) quantification technique (Invitrogen Ltd., Renfrew, U.K.).

Several primers were chosen for use in this study (Table 1) (18,19). PCR was carried out initially using a three-primer multiplex (101, 200, and 283 bp) because the PCR was optimized most efficiently for this combination. PCR was repeated for all samples using a less sensitive four-primer multiplex, optimized specifically to intensify a 450 bp band, not present in the three-primer multiplex. PCR was carried out in PE 9700 PCR systems (Applied Biosystems, Foster City, CA) and samples were examined by electrophoresis of amplified DNA in an agarose gel matrix stained with ethidium bromide. Results were visualized using a UV lamp and photographed.

Pulp Cavity Temperature Experiment

Preparation of jaw segments for the pulp temperature experiment followed the same procedure as earlier. The occlusal surface of the M1 was penetrated down to the level of the pulp cavity using Midwest carbide dental burs (Dentsply Professional Division, Des Plaines, IL) and rough cut diamond burrs (Brasseler, Croydon, U.K.). Prior to drilling, dental X-rays were taken of several segments. The X-rays showed the distal area of the M1 providing the largest pulp cavity area. From the X-rays, the drilling distance required to penetrate the pulp cavity was determined. The dental

TABLE 1—*Primers used in the PCR amplification of the specific DNA fragment from the* Sus scrofa *dental pulp.*

Primer	GenBank Accession	Forward Primer (5′ → 3′)	Reverse Primer (5′ → 3′)	Expected Product Size (bp)	References
1	AY285525	CATGCTGCAGGTGTAACTGG	TCTCTGCTTTTGGTGTGTGC	101	(18)
2	AY285462	GTTTGCATGGACGTTTATTGG	GAGCAGATGTCGCTACTGACC	200	(18)
3	AY285456	TGGGGATTTTTATTTCATTGTC	GTTGCTGCCTTGGTTTTTTG	283	(18)
4	BV103614	CCCTGGACAGCCTCATAGAC	TTGACTGTCTTGGGCACG	450	(19)

burr was restricted by a rubber ring of required thickness positioned above the tip of the burr to ensure the cavity floor was not penetrated.

A type K 36-inch chromel versus alumel wire (Maplin Electronics Ltd., Bournemouth, U.K.) was inserted into the drill hole and held secure by a grip attached to a standing clamp. Care was taken to ensure the wire was not in contact with the cavity floor. Resin reinforced glass-ionomer luting cement (GC FugiCem, GC America Inc., Alsip, IL) was applied to secure the wire in place. While no bonding cement is specifically designed to withstand high temperatures, the FugiCem cement was chosen in particular because of its high percentage inorganic filler content and low shrinkage properties. Robinson et al. (20) heated a range of dental cements and showed that those having a high inorganic and low organic filler content could withstand the highest temperatures. Prior to drilling, the occlusal surface of the M1 was conditioned with an acid etch to improve the bonding capabilities of the cement. A bonding time of 1 h was allowed. The alumel wire was plugged into a type K thermocouple device (0–1370°C range) so that the pulp chamber temperature could be monitored. Four jaw segments (two mandibular and two maxillary) were heated for 15 min at each of the following temperatures: 150, 300, 450, 600, 750, and 900°C. Pulp cavity temperatures were recorded at 1 min intervals for the duration of heating.

Results

Effects of Heat on Teeth

Segments were visibly unaffected by incineration following exposure to temperatures of 150–300°C. Enamel became brittle, and segments were increasingly charred at temperatures of 375°C and above. The pulp first showed signs of desiccation at a temperature of 375°C and carbonization occurred following exposure to 525°C. Following incineration at this temperature, a notable weakness at the cement/enamel junction resulted in the crown easily breaking away from the rest of the tooth to expose the pulp cavity. Except for slight charring of the lingual enamel surface, teeth extracted from whole heads incinerated in the field were visually unaffected by the fire. The effects of each incineration temperature on teeth and pulp tissues are described in Table 2.

Maxillary and Mandibular Enamel Density

Dental X-rays of mandibular (left column) and maxillary (right column) segments are shown in Fig. 1. The brightness of the image observed is directly related to density—the whiter the image the greater the density. The enamel of maxillary molars appears to have greater density than that of mandibular molars.

DNA Recovery from Pulp Chamber

The mean quantity of DNA extracted from molars within each temperature set is displayed in Table 3. The associated standard deviation (SD) and percentage relative standard deviation (%RSD) are also shown. The RSD, which refers to the absolute value of the coefficient of variation, expresses the precision of an assay. The closer to zero the %RSD, the smaller the variation in the quantity of DNA retrieved from each tooth in the temperature set. In general, %RSD values were not particularly close to zero as a result of several factors: (i) the difficulty experienced in cracking the teeth uniformly, particularly in the lower temperature sample sets (150–300°C), and consistently retrieving all available pulp tissue from each tooth; (ii) variation in the amount of pulp present because of differences between the maxillary and mandibular pulp chamber areas and also natural variation in tooth sizes across the *Sus scrofa* sample; and (iii) differences in the degree of heating experienced within the pulps in any one temperature set because of differences in the density of maxillary and mandibular enamel.

Figure 2 shows the difference in mean DNA yield obtained from mandibular and maxillary pulps over the temperature range studied. Segment X-rays (Fig. 1) show the pulp chamber areas of mandibular first molars to be larger than maxillary first molars. In control samples and samples incinerated at 150°C, 225°C (segment molars), and 625°C (outdoor incineration of fleshed heads), DNA

TABLE 2—*Description of the effects of specific incineration temperatures on teeth and pulp tissue.*

Incineration Temperature	Physical Effects of Heat on Jaw Segments and Ease of Molar Extraction	Physical Effects of Heat on Pulp Tissue and Ease of Pulp Recovery
150°C 225°C 300°C	Little visual change from control segments. Much force required to extract M1 from alveolar process	Little visual change from control segments. Much force required to crack the hard enamel and expose the pulp cavity. Pulp is fleshy and moist in all samples
375°C	Segment shows signs of charring. Intermediate force required to extract M1	Enamel is slightly brittle—access to pulp chamber is simplified by obtaining a cleaner break. Pulp adjacent to chamber walls is desiccated and has to be peeled away
450°C	Brown-black charring over entire segment. Enamel becomes noticeably brittle and fragments frequently detach from underlying dentin, which is a burnt brown color. Care required to extract M1	Enamel is noticeably brittle. Pulp is desiccated with a papery texture, dark brown in color. Pulp has to be peeled away from cavity and radicular canal surfaces
525°C	Entire segment is charred black. Enamel displays a blue tinge and is extremely brittle and fragile. M1 could not be extracted from alveolar process in tact	Enamel is extremely brittle. Pulp is completely carbonized in most samples and has to be scraped off cavity walls. Radicular canals not accessible
600°C	Entire segment is blue/black in color. M1 crumbles on contact	Cannot differentiate between pulp, dentin and enamel. Pulp not retrieved
625°C*	Buccal side of teeth slightly charred, lingual side unaffected by fire	Pulp visibly unaffected by fire in all samples

*Field incineration of whole fleshed *Sus scrofa* heads.

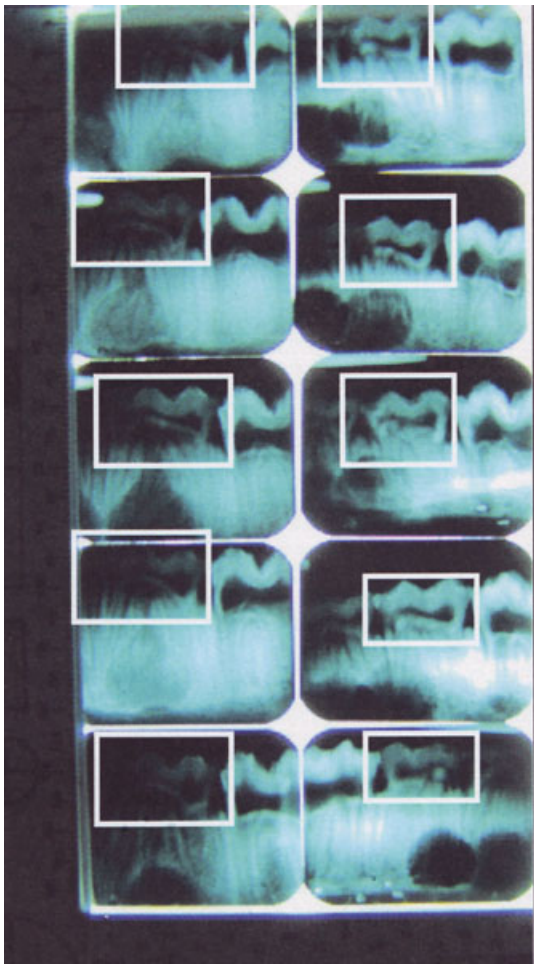

FIG. 1—*Maxillary (right) and mandibular (left) segment X-rays. First molars are highlighted. Maxillary enamel is brighter (whiter) and therefore has greater density than mandibular enamel. NB. mm scale used.*

yields from mandibular pulp were greater than from maxillary pulps. This trend was reversed in segment molars incinerated at temperatures of 375°C and above, where maxillary pulps yielded more DNA than mandibular pulps. This might be attributed to the mandibular pulps experiencing a greater degree of heating, and in turn pulp desiccation, because of mandibular enamel being less dense. This effect is most pronounced in the 450°C and 525°C temperature sets. At 525°C, maxillary pulp yielded 45 times more DNA than mandibular pulp. A technical difficulty arose with samples heated at 300°C, and as a result data for this set were lost.

In samples heated at 375°C, total DNA exceeded all other samples apart from the control and field incineration samples. Following exposure to this temperature, the teeth became slightly brittle, allowing the tooth to be cracked open with greater ease than that experienced in samples heated at lower temperatures. This allowed more complete access to the pulp cavity and radicular canals resulting in better retrieval of pulp tissue. At temperatures above 375°C, there was an inverted correlation between overall DNA yields and increasing temperatures. This can be attributed to the increase in pulp desiccation at higher temperatures and the general difficulty experienced in retrieving pulp tissue because of the fragile state of the teeth.

Total DNA recovery from field incineration samples burned for 1 h exceeded all other temperature sets, including control samples and field incineration samples burned for 15 min. This reflects that, as with the 375°C sample set, the enamel became slightly brittle following burning for 1 h, which allowed greater ease of access to the pulp chamber and radicular canals. In addition to the ease of pulp recovery, the particularly low %RSD obtained for this sample set (2.2%) may be attributed to the lack of size variation between the four molars analyzed because they originated from a single animal.

PCR Amplification Using the Three- and Four-Primer Multiplex

Each sample exposed to incineration temperatures of 150, 225, 300, 375, 450, and field incineration at 625°C allowed the generation of a PCR product of 450 bp (the largest fragment analyzed) using the four-primer multiplex. No PCR products were amplified from samples incinerated at 525°C using the four-primer multiplex. However, all three product sizes were successfully amplified for 4 maxilla samples using the three-primer multiplex (Fig. 3). This demonstrates that the DNA was not degraded following exposure to this temperature. Amplification failure in the mandibular samples might be attributed to insufficient DNA extraction. The mean amount of DNA extracted from mandibular pulp (6.5 ng) was significantly lower than that extracted from maxillary pulp (290 ng). It is probable that the greater DNA yields in maxillary samples were the result of maxillary enamel having greater density and thus providing greater protection to the pulpal tissue from heat. The total amplification failure experienced in these samples when using the four-primer multiplex might be attributed to insufficient

TABLE 3—*Conditions of incineration and DNA quantity retrieved from* Sus scrofa *molars.*

Sample References	Furnace Temperatures (/C)	Duration (mins)	Weight Loss (%)	Mean DNA Quantity		
				Total (μg)	SD (μg)	RSD (%)
1–10	Control	0	–	19.52	3.84	19.7
11–20	150	15	4.66	15.15	2.17	14.4
21–30	225	15	13.52	12.84	1.87	14.6
31–40	300*	15	19.60	–	–	–
41–50	375	15	27.24	16.03	2.83	17.7
51–60	450	15	30.54	9.99	7.95	79.5
61–70	525	15	37.30	0.147	0.296	201.0
	Average Fire Temperatures (/C)					
71–80	625	15	–	16.76	4.04	24.1
81–84	625	60	–	20.10	0.43	2.2

Table 3 displays average values for conditions of incineration, percentage weight loss, and mean DNA quantity recovered from samples.
SD, standard deviation; RSD, relative standard deviation; sample ref 71–84 = outdoor incineration.
*No results for DNA quantity because of technical difficulties.

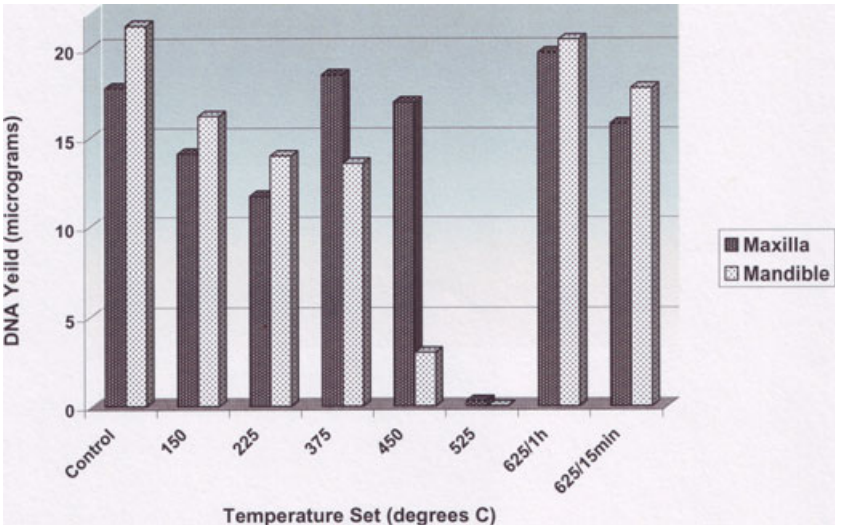

FIG. 2—*Bar chart comparing DNA yields obtained from maxillary and mandibular pulp. Where not stated incineration duration is 15 min. Control samples were not heated.*

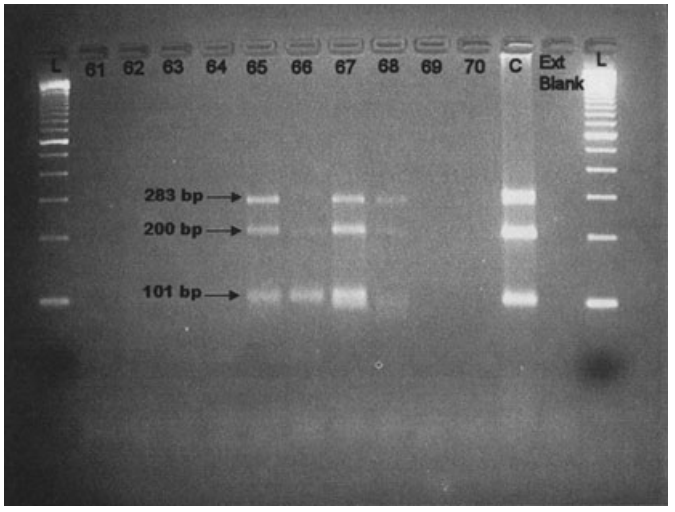

FIG. 3—*PCR products generated from molars (lane and sample ref. 61–70) heated at 525°C using three-primer multiplex. C, control sample (*Sus scrofa *muscle); L, 100 bp mol. wt. ladder; Ext Blank, extraction blank (control: H_2O). Amount of isolated DNA (ng) added in the PCR reactions of samples: Ref 61(6), 62(9), 63(23), 64(0), 65(347), 66(93), 67(934), 68(42), 69(4), and 70(15).*

optimization of the PCR for this primer set. Unfortunately, time restrictions prevented further optimization of this system.

Relationship Between Furnace and Pulp Cavity Temperature

The relationship between furnace and pulp cavity temperature is shown in Fig. 4. At each furnace temperature, maxillary molars experienced internal heating at a slower rate compared to the mandibular molars. At furnace temperatures of 150–450°C, pulp temperatures were lower in maxillary samples during the first 3–4 min. Both maxillary and mandibular pulp chamber temperatures tended to increase at the same rate following this period (Fig. 4). At furnace temperatures of 600 and 750°C, there were marked differences between mandibular and maxillary pulp chamber temperatures for periods of 12 and 9 min, respectively (Fig. 4). Maxilla samples experienced significantly lower pulp cavity temperatures during these heating periods. It is likely that the differential heating recorded during this experiment was a result of maxillary enamel having greater density than mandibular enamel.

At furnace temperatures of 300 and 450°C, the pulp temperatures in both maxilla and mandible samples was maintained at around 100°C (boiling point of water) for *c.* 4–6 min. This might indicate the utilization of available heat for water vaporization during this period. At 600°C, the pattern was more pronounced in the maxillary samples.

At furnace temperatures >450°C, the insulating capacity of the enamel appeared to be rapidly reduced with pulp chamber temperatures almost reaching the temperature of the furnace at the end of the 15-min period. At furnace temperatures of 450°C and below, the pulp chamber temperatures were significantly lower than the furnace temperature at the end of the heating period.

Discussion

In the present study, manual retrieval of dental pulp became difficult following exposure of segments to 450°C and increasingly so at 525°C. At 525°C, the method of pulp retrieval was largely ineffective because of the extreme fragility of the dentition and carbonization of the pulp. Consequently, with the exception of field incineration samples (i.e., teeth in whole heads), the potential to analyze DNA from teeth subjected directly to temperatures >525°C was precluded.

Although the present study did not succeed in determining the degree of degradation that occurs with exposure to increasing temperature, it has shown that following incineration at 450°C for a 15-min period, it is still possible to amplify a 450 bp DNA fragment from the dental pulp of maxillary and mandibular molars embedded in defleshed jaw segments by PCR (Fig. 3). Following exposure to 525°C for the same duration, the largest PCR product amplified was 283 bp, and this was only possible from the pulp of maxillary molars. Significantly, less DNA was extracted from mandibular pulp compared to maxillary pulp. This might be attributed to maxillary enamel having greater density than that of mandibular enamel and thus affording greater protection to the pulp tissue from heat and subsequent carbonization. The amplification failure experienced in the 525°C temperature set when using the four-primer multiplex is not indicative of DNA degradation. It is known from the results of the three-primer multiplex that DNA was not degraded following

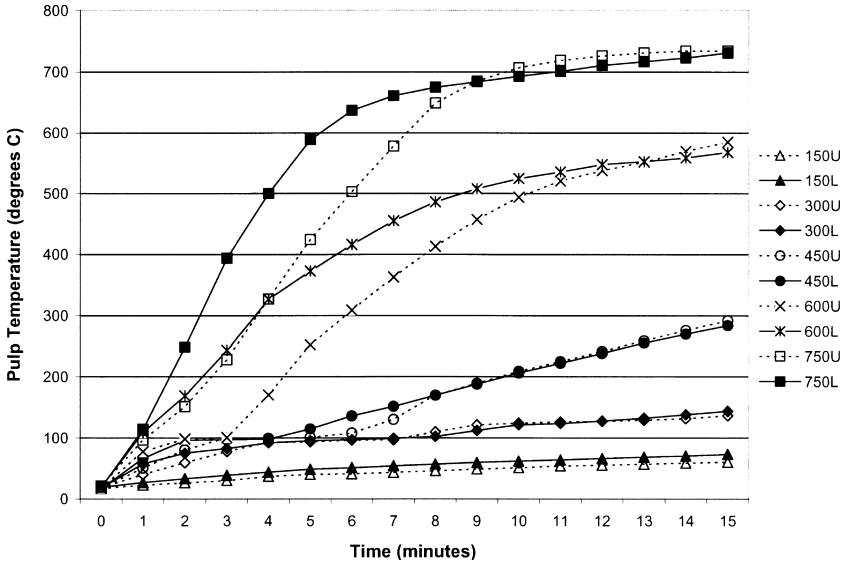

FIG. 4—*Line graph displaying pulp cavity temperatures recorded at 1 min intervals for each furnace temperature. Zero minutes represents the control temperature (starting temperature). Legend: U = upper (maxilla); L = lower (mandible).*

exposure to this temperature. With further optimization, it is likely that DNA would have been amplified using the four-primer system.

The sensitivity of the detection could have been improved by using fluorescently labelled primers in the PCR and visualizing the labelled fragments on a genetic analyzer. Unfortunately, both financial and time constraints prevented the development of a suitable system for use in the present study. Further improvement in sensitivity could have been obtained with real-time PCR (qPCR) using fluorescent reporter probes, e.g., TaqMan® probes (Applied Biosystems), to increase specificity. qPCR simultaneously amplifies and quantifies targeted DNA regions and has been shown to be significantly more sensitive than the general DNA assay used here and is the standard method for analyzing amplified human DNA in forensic DNA laboratories (21). Unfortunately, the authors did not have access to qPCR at the time the study was conducted. In addition, had a more precise technique been used to extract pulp tissue from the teeth, such as using a dental drill to access all areas of the pulp cavity and radicular canals, more pulp may have been recovered, and the chance of amplifying the collected DNA might have been improved.

The DNA amplified in the 450°C set using both primer systems (450 bp) is larger than any allele that could be generated using the AmpF*l*STR SGM plus PCR amplification kit (Applied Biosystems), indicating that the equivalent DNA recovered would be suitable for full DNA profile production. The DNA amplified from the 525°C set using the three-primer multiplex (283 bp) is approximately the same size as the largest possible allele in the AmpF*l*STR MiniFiler PCR amplification kit (Applied Biosystems) and thus should also be sufficient for full profile production using this kit for human equivalents.

In the present study, whole *Sus scrofa* heads burned in the field (average temperature of 625°C) yielded substantial amounts of DNA, compared to segments heated in the chamber furnace at 450°C and 525°C (Table 3), with no associated difficulty in pulp retrieval. The largest fragment analyzed (450 bp) was amplified from all samples burned for 15 min and 1 h. Generally, in the many studies where isolated teeth have been used to examine the effects of incineration on DNA in the dental pulp, the smallest amplicon analyzed could not be consistently amplified following incineration. In separate studies, Alvarez Garcia et al. (17) and Urbani et al. (16) could not consistently amplify the X–Y amelogenin gene (106 and 112 bp) following incineration of isolated teeth at 200°C for 10 and 15 min (Fig. 5). Murakami et al. (22) incinerated isolated teeth at 250°C for 30 min and could not amplify the same gene from any sample (Fig. 5). In each of the studies mentioned earlier, PCR analyses were also performed on dental pulp from cremated remains and although the conditions of incineration were not known, the state of the remains indicated exposure to high temperatures and for a considerable duration. In each case, the teeth were in relatively good condition, and the extracted DNA was successfully amplified. This provides evidence as to the significant amount of protection afforded the teeth by the soft and hard tissue mass of the head and the alveolar bone.

The value of comparisons between data resulting from a controlled burn of sustained temperature versus field incineration in which the temperature fluctuates dramatically (as does the period of burn at each) is an important consideration for understanding

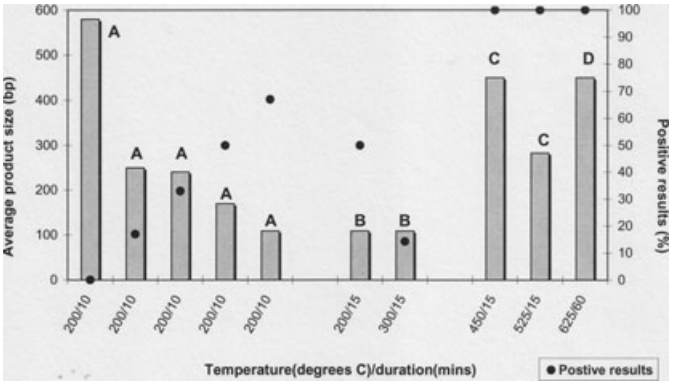

FIG. 5—*Comparison of amplification results obtained following incineration of isolated teeth and teeth embedded in jaw bone. (A) Represents the results of Alvarez Garcia et al. (17) obtained from isolated human teeth; (B) represents the results of Urbani et al. (16) obtained from isolated human teeth; (C) represents the results of the present study from* Sus scrofa *teeth embedded in jaw bone (furnace incineration); and (D) represents results obtained from* Sus scrofa *teeth* in situ *in whole heads (field incineration).*

these results and also for future research. This may have an unquantifiable bearing on the interpretation of results in this study. It should be stressed, however, that the temperature of the field furnace rarely dropped below 450°C, and the pulp tissue was nevertheless visually unaffected in all samples. This gives some indication of the protection afforded by the tissue mass of the head, which is of considerable relevance to most forensic cases and itself points to important lines of future research. That said, for the future, teeth within jaw segments and teeth within intact heads should ideally be tested under identical thermal conditions (if that is possible) so that direct and more meaningful comparisons can be made.

In real fire situations, direct exposure of the dentition to the high temperatures of the fire is often prevented by the protective soft and hard tissues of the head (23). Consequently, carbonized pulp tissue is rarely found, especially in the well protected molars. Thus, the method of cracking the molar open and retrieving pulp tissue would be far more applicable to forensic cases than it has been for the jaw segments incinerated in the present study. In comparison with other DNA containing sites within the tooth, the pulp has been recorded as yielding the strongest amplification signals using PCR (24) and more intact DNA can be extracted from the pulp alone compared to whole teeth (7). Bearing this in mind, attempts should always be made to extract the dental pulp from the teeth of fire victims for DNA analysis. However, in particularly intense fires of long duration human teeth can exhibit the characteristics of extreme fragility and complete pulp carbonization as observed in the present study. As a result, distinguishing pulp from the surrounding tooth structure may not be possible. In such cases, crushing the whole tooth may be the best approach to maximize DNA yield (25).

The present study along with casework examples demonstrates the significant variation in results obtained from isolated teeth compared to those obtained from teeth embedded in jawbone and surrounded by soft tissue. The potential difference in thermal effects experienced by teeth subjected to a controlled burn to those in realistic fire situations, where the temperatures and the duration of those fluctuate, further highlights the unsuitability of experiments conducted on isolated and *ex situ* teeth. The use of isolated teeth in temperature-related experiments is, therefore, not meaningful because the relationship between temperatures used experimentally and those experienced by teeth *in situ* in real fire situations cannot be ascertained. The data generated from studies utilizing isolated human teeth cannot realistically contribute to human identification in fire-related forensic casework, or to the determination of fire temperatures based on the condition of those teeth.

The visible effects of incineration on *Sus scrofa* teeth observed during this study were found to be consistent with general trends described for human teeth (26–28). In addition, the size and morphology of *Sus scrofa* teeth closely resemble human teeth (29). Close similarities between humans and 6-month-old *Sus scrofa* jaw tissue and tongue thickness have also been recorded previously (23). Duffy et al. (23) also found that the nuclear morphology of pulp from *Sus scrofa* and human teeth showed the same stability following incineration at 100°C for 1 h. The combined evidence suggests that there is a good basis for comparison in terms of thermal effects on the pulp tissue. However, an inconsistency between the human and *Sus scrofa* dentition was observed during this study. Maxillary molars were observed to have greater enamel density compared to mandibular molars. Such a variation has not been observed in the human dentition at any stage of development. It is reasonable to assume that either the maxillary or the mandibular dentition of *Sus scrofa* would have enamel density more closely matching that of humans. The set of *Sus scrofa* dentition determined to be the most representative of humans could then be used analogously for future incineration studies.

By using fleshed *Sus scrofa* heads experimentally and by utilizing the dentition most comparable to humans for data collection, DNA degradation after incineration can be investigated in realistic forensic settings. Furthermore, acquiring organically reared, healthy *Sus scrofa* ethically, and in large sample numbers is no challenge and statistically viable results can therefore be obtained. Data obtained from such studies would certainly be more realistic than that obtained using isolated human teeth. Analysis of the degree of degradation of DNA extracted from human teeth in forensic casework, where the average temperature and duration of the fire are known, can be compared to data obtained from experimental research conducted on *Sus scrofa*. By using real-time PCR specific for *Sus scrofa* and analyzing amplified DNA using fluorescent primers to enhance sensitivity, the degradation of recovered DNA can be determined more effectively, and the results obtained can be more accurately extrapolated to human DNA analyses from heat-exposed samples. With the availability of such data from *Sus scrofa* studies, it may be possible to extrapolate temperatures and durations required for total loss of DNA in human teeth.

Conclusions

This study demonstrated that DNA from the dental pulp remains a reasonable sample for PCR identification following incineration of embedded, defleshed jaw segments at 525°C for 15 min and whole heads at 625°C, even after 1 h. It also highlighted the inconsistency of results from incineration of isolated teeth. In such studies, most authors could not consistently amplify the smallest amplicon investigated following incineration, even at 200°C for 10 min. It is evident from this study that the alveolar process and soft tissue mass of the head provide a substantial amount of insulation to the pulp chamber during heating.

Observations made during these experiments indicated that trends previously observed in human teeth exposed to increasing temperatures are consistent with teeth similarly exposed from *Sus scrofa*. This combined with close similarities between the two species in soft tissue thickness promotes the experimental use of whole *Sus scrofa* heads as a sample dentition representative to that of humans. Research on DNA degradation after incineration using *Sus scrofa* can provide statistically viable data and comparable to the incineration of humans. Such research will be of particular value to forensic casework.

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References

1. Mayne Correia PM. Fire modification of bone: a review of the literature. In: Haglund WD, Sorg MH, editors. Forensic taphonomy: the postmortem fate of human remains. Boca Raton, FL: CRC Press, 1997;275–86.
2. Schwartz TR, Schwartz EA, Mieszerski L, McNally L, Kobilinsky L. Characterization of deoxyribonucleic acid (DNA) obtained from teeth

subjected to various environmental conditions. J Forensic Sci 1991;36:979–90.
3. Spitz WU. Thermal injuries. In: Spitz WU, editor. Medicolegal investigation of death. 3rd edn. Springfield, IL: Charles C Thomas, 1993;3.
4. Fisher BAJ. Techniques of crime scene investigation. 7th edn. Boca Raton, FL: CRC Press, 2004.
5. Nambiar P, Jalil N, Singh B. The dental identification of victims of an aircraft accident in Malaysia. Int Dent J 1997;47:9–15.
6. Dumancic J, Kaic Z, Njemirovskij V, Brkic H, Zecevic D. Dental identification after two mass disasters in Croatia. Croatian Medical Journal 2001;42:657–62.
7. Yamada Y, Yamamoto K, Yoshi T, Ishiyama I. Analysis of DNA from teeth and application to forensic dental medicine. Japanese Journal of Legal Medicine 1989;43:420–3.
8. Olaisen B, Stenersen M, Mevåg B. Identification by DNA analysis of the victims of the August 1996 Spitsbergen civil aircraft disaster. Nat Genet 1997;15:402–5.
9. Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA, et al. Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle-cell anaemia. Science 1985;230:1350–4.
10. Paabo S, Gifford JA, Wilson AC. Mitochondrial DNA sequences from a 7000-year old brain. Nucleic Acids Res 1988;16:9775–87.
11. Saiki RK, Walsh PS, Levenson CH, Erlich HA. Genetic analysis of amplified DNA with immobilized sequence-specific oligonucleotide probes. Proc Natl Acad Sci U S A 1989;86(16):6230–4.
12. Saiki RK. Amplification of genomic DNA. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: a guide to methods and applications. San Diego, CA: Academic Press, 1990;13–17.
13. Hanaoka Y, Minaguchi K. Sex determination from blood and teeth by PCR amplification of the Alphoid satellite family. J Forensic Sci 1996;41:855–8.
14. Lessig R, Edelmann J. Individualisation of dental tissue: an aid for odontological identification? J Forensic Odontostomatol 1995;13:1–3.
15. Williams D, Franzen T, Lissett V, Adams C, Lewis M, Tysoe C, et al. Sex determination by PCR analysis of DNA extracted from incinerated, deciduous teeth. Sci Justice 2004;44:89–94.
16. Urbani C, Dubreuil-Lastrucci R, Kramer B. The effect of temperature on sex determination using DNA-PCR analysis of dental pulp. J Forensic Odontostomatol 1999;17:35–9.
17. Alvarez-Garcia A, Munoz I, Pestoni C, Lareu MV, Rodriguez-Calvo MS, Carracedo A. Effect of environmental factors on PCR-DNA analysis from dental pulp. Int J Legal Med 1996;109:125–9.
18. Fahrenkrug S, Wagner M, Morrison L, Alexander LJ. Map assignments of 373 previously unreported porcine microsatellites. Anim Genet 2005;36:76–86.
19. Freking BA, Nonneman DP, Rohrer GA, Fahrenkrug SC, Smith TPL, Keele JW Development of a comprehensive SNP-based comparative linkage map for pigs. (unpublished). Clay Centre, NE, USA, 2004. Available at: http://www.ncbi.nlm.nih.gov (accessed November 5, 2008).
20. Robinson FG, Rueggeberg FA, Lockwood PE. Thermal stability of direct dental esthetic restorative materials at elevated temperatures. J Forensic Sci 1998;43:1163–7.
21. VanGuilder HD, Vrana KE, Freeman WM. Twenty-five years of quantitative PCR for gene expression analysis. BioTechniques 2008;44:619–26.
22. Murakami H, Yamamoto Y, Yoshitome K, Ono T, Okamoto O, Shigeta Y, et al. Forensic study of sex determination using PCR on teeth samples. Acta Med Okayama 2000;54:21–32.
23. Duffy JB, Waterfield JD, Skinner MF. Isolation of tooth pulp cells for sex chromatin studies in experimental dehydrated and cremated remains. Forensic Sci Int 1991;49:127–41.
24. Malaver PC, Yunis JJ. Different dental tissues as a source of DNA for human identification in forensic cases. Croatian Medical Journal 2003;44:306–9.
25. Smith BC, Fisher DL, Weedn VW, Warnock GR, Holland MM. A systematic approach to the sampling of dental DNA. J Forensic Sci 1993;38:1194–209.
26. Gustafson G. Resistance of teeth and dental restorations. In: Gustafson G, editor. Forensic odontology. London: Staple Press, 1966;178–91.
27. Harvey W. Identification after fire, drowning, air crashes and other disasters. In: Harvey W, editor. Dental identification and forensic odontology. London: Henry Kimpton Publishers, 1976;67–87.
28. Muller M, Berytrand MF, Quatrehomme G, Bolla M, Rocca JP. Macroscopic and microscopic aspects of incinerated teeth. J Forensic Odontostomatol 1998;16:1–7.
29. Robinson C, Kirkham J, Weatherell JA, Richards A, Josephen K, Fejerskov O. Mineral and protein concentrations in enamel of the developing permanent porcine dentition. Caries Res 1988;22:321–6.

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