Sandra L. Myers,[1] D.M.D.; John M. Williams,[2] D.D.S.; and James S. Hodges,[3] Ph.D.

Effects of Extreme Heat on Teeth with Implications for Histologic Processing*

REFERENCE: Myers SL, Williams JM, Hodges JS. Effects of extreme heat on teeth with implications for histologic processing. J Forensic Sci 1999;44(4):805–809.

ABSTRACT: This pilot study examined teeth subjected to extreme heat under laboratory conditions, and the subsequent effect of decalcification and histologic processing. Physical and microscopic findings were evaluated in relation to temperature and duration of thermal insult. Microscopic examination following decalcification and histologic processing revealed changes including severe tissue fragmentation, vapor bubbles within dentinal tubules, altered histologic staining, charring and tissue shrinkage. Dentin appeared to be the most reliable microscopic identifier of incinerated dental tissues. Temperatures above 600°C strongly predicted tooth disintegration following decalcification. This finding has implications in incineration cases where histologic evidence must be maintained and examined intact.

KEYWORDS: forensic science, incineration, dentition, histologic processing, histology

A precise understanding of physical and histological changes in teeth subjected to high temperatures is of great importance in forensic medicine (1). The type and severity of structural damage provide valuable clues in fire and criminal investigations, especially when only dental evidence remains. For example, extracted teeth when subjected directly to the fire of a Bunsen burner will often crack like glass due to moisture content. Teeth subjected to more gradual heating to the same temperatures will survive despite charring and becoming very brittle (1,2). Preserving fragile incinerated teeth for physical examination and/or histologic preparation is extremely important and relies on a thorough understanding of the structural changes in dental tissues subjected to heat (3–5). The main goals of this pilot project were to investigate: a) the structural damage in freshly extracted teeth subjected to heating at different temperatures and for different lengths of time in the laboratory, and b) the effect of decalcification and histologic processing on these heat-treated teeth. The results of this pilot project are not meant to provide a direct description of what happens to teeth in an intact body in a real fire. The results will, however, provide heretofore unavailable but valuable data on the results of heating teeth to high temperatures.

[1] Assistant professor, Division of Oral Pathology, School of Dentistry, University of Minnesota, Minneapolis, Minnesota.

[2] Deputy Medical Examiner, Hennepin County Medical Examiner's Office, Minneapolis, MN.

[3] Senior research associate, Division of Biostatistics, School of Public Health, and Director, Biostatistics Core, Minnesota Oral Health Clinical Research Center, School of Dentistry, University of Minnesota, Minneapolis, Minnesota.

* Presented in part at the 51st Annual Meeting of the American Academy of Forensic Sciences, February 1999, Orlando FL.

Received 24 Sept. 1998; accepted 20 Oct. 1998.

Methodology

Incineration and Histological Methods

This pilot project used 66 freshly extracted permanent teeth obtained as discarded waste from patients presenting to a general dental office for treatment. There were 5 canines, 19 incisors, 15 premolars, 26 molars, and one tooth, type not specified. Both carious and restored mandibular and maxillary teeth were used in the study. Teeth restored with full crowns and/or a history of endodontic treatment were excluded. After extraction, the individual teeth were debrided with a solution of 3% hydrogen peroxide and rinsed thoroughly with tap water. Within 30 min the teeth were placed in a preheated Ney Mark Four furnace and subjected to heating at constant selected temperatures (ranging from 300°C to 1400°C) for varying lengths of time (ranging from 30 to 90 min). On removal from the oven, the teeth exhibited varying degrees of charring, fragility, and tendency to crumble. They were allowed to cool and then placed in closed storage containers at room temperature. After one week, the teeth were fixed in formalin and subjected to a typical decalcification protocol in 1 M hydrochloric acid during which 42 of the 66 teeth (64%) completely disintegrated. Six of the disintegrated teeth were selected randomly and the residue was filtered and collected. Twenty-four teeth survived decalcification but became extremely soft and gelatinous in consistency. The surviving teeth and disintegrated tooth residue were submitted for routine processing and embedding in paraffin. Finally, all specimens were sectioned at 4 microns, mounted on glass slides, and stained with hematoxylin and eosin.

Statistical Methods

Two methods were used to characterize which teeth disintegrated in decalcification: logistic regression and classification trees (as implemented in the S-Plus computing package, version 3.3, Release 1) (6). The logistic regressions used as their outcome the binary variable disintegrated/didn't disintegrate, and used as explanatory variables temperature and time (continuous variables) and tooth type (a categorical variable). Explanatory variables were tested for significance using the likelihood ratio test (7). The tree analysis used the same outcome and explanatory variables. Its recursive algorithm chose at each stage the split (or threshold) that induced the maximum reduction in deviance (i.e., $-2 \times$ log-likelihood). Splits were retained as long as the resulting deviance reduction exceeded 3.84, the 95% point of the χ^2 density on 1 degree of freedom.

Results

Microscopic Findings

After sectioning, only two of the 24 surviving specimens (both molars) grossly retained the morphological appearance of a tooth on the glass slides. Enamel, normally completely removed by decalcification, was not present for histologic examination. Microscopic evaluation of all specimens revealed fragmented tooth sections with identifiable dentin and, in 16 of 24 specimens, pulpal remnants and cementum with or without attached periodontal tissues (Table 1). Tissue fragments identified as pulp were located within pulp chambers (Fig. 1). Periodontal ligament tissues were identified adjacent to cementum-covered portions of root dentin. Inflammatory and connective tissue cell nuclei, along with small vascular channels, were recognizable within some of the pulpal remnants (Fig. 2). Both cellular and acellular cementum were identified along with several tiny attached and unattached cementicles.

Thermal effects observed microscopically within the tissue sections included shrinkage, increased tissue fragmentation, charring, and alterations in hematoxylin and eosin staining. Fragmentation of the dental tissues, particularly dentin (which makes up the bulk of a tooth), was a prominent feature with the greatest destruction in areas where severe to total charring had occurred. The thermal insult caused variations in tissue staining, often producing dramatic and

TABLE 1—*Dental tissues identified in: (A) incinerated teeth surviving decalcification and processing, and (B) residue of totally disintegrated teeth.*

(A)

Specimen	Pulp	Dentin	Cementum	PDL*	Temp/Time/Tooth#
1(R16)	•	•	•	•	300/30 (02)
2(R28)	•	•	•	•	300/90 (17)
2(R29)	•	•	•	•	400/30 (02)
1(R17)	•	•	•	•	400/30 (17)
2(R30)	•	•	•	•	400/60 (03)
1(R18)	•	•	•	•	500/30 (16)
1(R19)	•	•	•	•	500/30 (20)
2(R31)		•	•		550/30 (04)
2(R34)		•			550/30 (10)
2(R32)		•			550/60 (05)
2(R35)		•	•	•	550/60 (08)
2(R33)		•			550/90 (06)
3(R7)	•	•	•	•	600/30 (01)
3(R8)		•	•		600/30 (04)
3(R9)		•	•	•	600/30 (07)
3(R10)	•	•	•	•	600/30 (14)
1(R20)	•	•	•	•	600/30 (16)
2(R37)		•	•		600/60 (06)
2(R38)		•			600/60 (07)
2(R39)		•			600/60 (09)
2(R40)		•			600/60 (25)
4(R25)		•	•		650/30 (08)
4(R19)		•			700/30 (15)
2(R41)		•			700/30 (29)

(B)

Specimen	Pulp	Dentin	Cementum	PDL*	Temp/Time/Tooth#
4(R30)		•			650/30 (02)
4(R28)		•			650/30 (13)
4(R18)		•			700/30 (14)
4(R17)		•			700/30 (29)
4(R41)		•			750/30 (02)
4(R39)		•			750/30 (25)

* Periodontal ligament.

FIG. 1—*Photomicrograph of pulp-dentin interface within pulp chamber. (Hematoxylin and Eosin; original magnification ×870).*

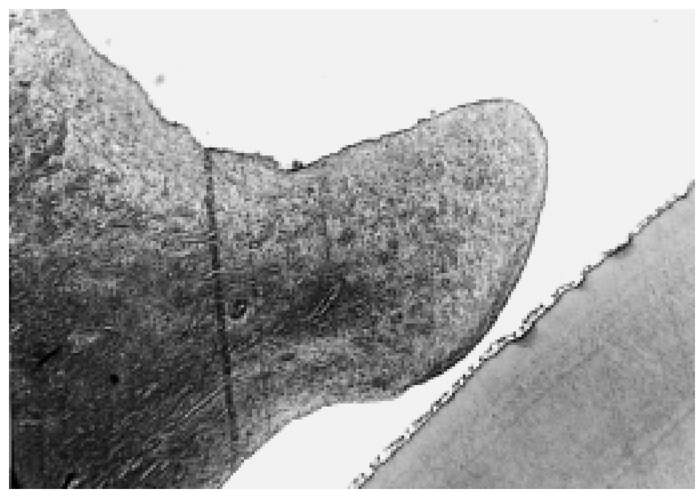

FIG. 2—*Photomicrograph of pulp tissue with identifiable small vascular channels and cell nuclei. (Hematoxylin and Eosin; original magnification ×87).*

varied differences in coloration. Relatively normal hematoxylin and eosin staining was observed in areas exhibiting the initial effects of heating such as vapor bubbles within the dentinal tubules. Along the external surface of some sections of dentin, a discrete bright orange-colored band was observed, possibly representing thermal changes preceding charring. This orange coloration, in more severely affected regions, gradually progressed to orange-brown, then to orange-gray and finally to totally black charred areas (Fig. 3).

The tubular nature of dentin was identified in all 24 histologic sections of the surviving teeth as well as in all six histologic preparations of filtered residue from disintegrated teeth (Table 1). Dentinal tubules were identified cut either along their long axis or in cross-section. Widening of the dentinal tubules was a feature in regions that exhibited initial thermal changes where the expansion may have been promoted by intratubular water vapor (Figs. 4 and 5). A distinctive woven "wicker-basket" configuration, with separations between the individual tubules, characterized regions with

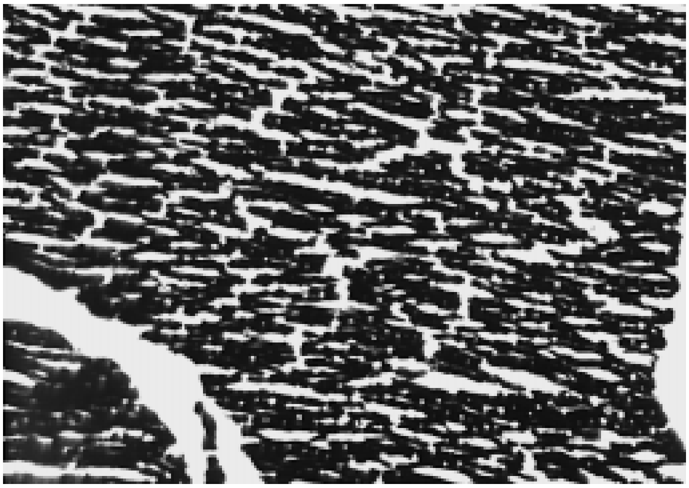

FIG. 3—*Photomicrograph of charred dentin. (Hematoxylin and Eosin; original magnification ×870).*

severe thermal damage (Fig. 6). This appearance is likely due to a combination of loss of inorganic matrix from decalcification, shrinkage due to drying and finally incineration and consequent loss of the intertubular organic matrix. Exogenous metallic pigment consistent with amalgam was observed on one section within the attached periodontal tissues and adjacent to the dentin surface on several other sections that did not exhibit charring. Grains of the metallic material were observed to extend down within individual tubules at some distance from the surface, providing evidence of prior amalgam restoration at these sites.

Disintegration Findings

Table 2 gives the numbers of teeth examined at each temperature and time. Because of the exploratory nature of this pilot study, a standard experimental design was not used. Instead, experimental conditions were selected in search of gross changes in the outcome.

Disintegration of heated teeth during decalcification was strongly related to the temperature at which they were heated

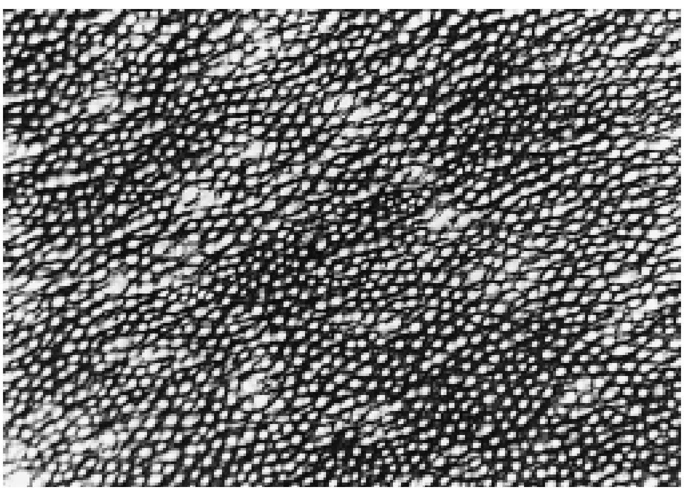

FIG. 4—*Photomicrograph showing widening of the dentinal tubules. (Hematoxylin and Eosin; original magnification ×555).*

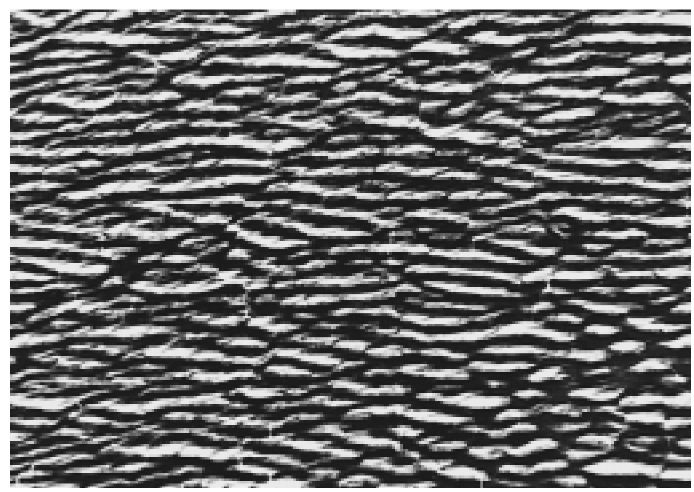

FIG. 6—*Photomicrograph showing "wicker-basket" configuration of incinerated dentinal tubules. (Hematoxylin and Eosin; original magnification ×870)*

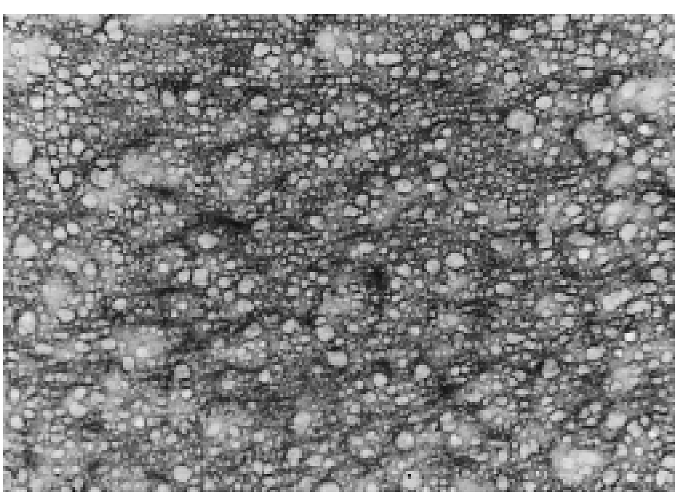

FIG. 5—*Photomicrograph showing intratubular water vapor bubbles. (Hematoxylin and Eosin; original magnification ×870).*

TABLE 2—*Numbers of teeth at each temperature and time; percent of teeth disintegrating at each temperature.*

Temperature (°C)	Time (min) 30	45	60	90	Total n	% Disintegrating
300	1	0	0	1	2	0
400	2	0	1	0	3	0
500	2	0	0	0	2	0
550	2	0	2	1	5	0
600	5	4	5	0	14	36
650	11	0	0	0	11	91
700	17	0	0	0	17	88
750	7	0	0	0	7	100
800	1	0	0	0	1	100
900	1	0	0	0	1	100
1000	1	0	0	0	1	100
1200	0	1	0	0	1	100
1400	1	0	0	0	1	100
Total	51	5	8	2	66	64

TABLE 3—*Logistic regression results.*

Variables	Compared to	χ^2	df	p
temp	null model	45.4	1	$<$0.001
time	null model	10.2	1	0.001
tooth type	null model	0.13	3	0.99
temp + time	temp only	0.018	1	0.89
temp + time	time only	35.2	1	$<$0.001
temp + tooth type	temp only	1.11	3	0.77

(Table 2). Table 3 summarizes the logistic regression results. In univariate logistic regressions, both temperature and time were related to the frequency of disintegration ($p < 0.001$ and $p = 0.001$, respectively), while tooth type was not ($p = 0.99$). In multivariate logistic regressions, the combination of temperature and time gave a significant gain in predictive power compared to time only ($p < 0.001$) but not compared to temperature only ($p = 0.89$). These results suggest that while temperature is truly predictive of disintegration, time only appears to be predictive because it is correlated with temperature in this experimental design. Specifically, the longer times were tested only at the lower temperatures (Table 2). Consequently, in the univariate logistic regressions, increased temperature was associated with an increased chance of disintegration, while increased time was associated with a decreased chance of disintegration. Tooth type added no predictive power to temperature in a multivariate logistic regression (Table 3; $p = 0.77$).

The tree analysis produced only three splits. First, teeth were split according to whether temperature was less than or greater than 625°C. Those with temperatures greater than 625°C were not split further; 92% (36/39) of these teeth disintegrated. Teeth with temperatures less than 625°C were split further into those with a temperature of 600°C and those with lower temperatures. The latter were not split further; 100% (12/12) did not disintegrate. Fourteen teeth had a temperature of 600°C; they were split into those having a time of 30 min (5 teeth), none of which disintegrated, and those having times of 45 or 60 min, of which 56% (5/9) disintegrated. The latter pattern is not as promising as it may seem, because all four teeth heated at 600°C for 45 min disintegrated, while only one of five teeth heated at 600°C for 60 min disintegrated. This anomaly is not explained by tooth types.

Discussion

The study of incinerated teeth, including their histology, is an important part of forensic science. Forensic investigators have mainly used the unique nature of teeth to identify victims. However, morphologic and microscopic tissue alterations caused by incineration may provide useful information about the temperature and duration of exposure to fire. Controlled laboratory incineration of teeth and their histologic evaluation have the potential to create valuable data for these tissues.

Distinctive features of teeth and their prior restoration are preserved even after fragmentation and/or incineration. Scanning electron microscopy (SEM) of incinerated dental hard tissues shows distinct surface changes, grooves, and striations from the use of a dental drill (8). Linear and cross-sectional views of dentinal tubules have been identified by SEM in almost totally destroyed human dental remains, confirming that the residual fragments were teeth (9). Globule or pearl formation of the dentin has been seen in some teeth heated to around 1000°C, and variations in the appearance of dentinal tubules in incinerated teeth, with distinct intertubular separation, have been observed (1,10). Microscopic examination of incinerated teeth is complicated by the profound effects of dessication and histologic processing on the organic and inorganic components of dental hard tissues.

Enamel, which is approximately 96% mineral, is highly susceptible to the decalcification required for routine histologic sectioning and is therefore not readily studied in this manner (11). Dentin, approximately 70% mineral, 20% organic and 10% water, is susceptible to decalcification, but less so than enamel (11). This provides opportunity for studying dentin structure in routine histologic sections. Approximately 56% of the mineral content of dentin is located within an organic matrix composed of type I collagen (11). This mineral content may help stabilize the collagen in dentin against thermal denaturation and shrinkage, as previously reported for bone collagens (12). A framework of organic material, primarily collagen, is left behind after decalcification and maintains the form of a tooth.

The organic component of the dental hard tissues, primarily collagen within the dentin, is eventually totally destroyed by incineration at high temperatures. If decalcification follows the thermal insult, as in this study, total disintegration results from breakdown or loss of the organic component in addition to loss of the mineral component. In the present work, all incinerated teeth were fixed in formalin and decalcified with hydrochloric acid. Teeth surviving decalcification were semitranslucent and, for the most part, soft, gelatinous, and difficult to section. Those that did not survive decalcification appeared as charred fragments and residue at the bottom of the decalcification containers. Yumikura and Hirano fixed incinerated teeth in formalin, decalcified them with nitric acid, and then impregnated them with gelatin before histologic sectioning (13). This method of impregnating incinerated teeth, to stabilize structure during sectioning, aided in maintaining proper histologic orientation of the tissues.

Several factors may have influenced the outcome of this study. For example, since all the teeth were discarded as waste, data such as patient age and eruption status of the tooth were not available. In individuals below the age of 20, normal permanent teeth have high volumes of well-vascularized pulp tissue containing many odontoblasts. This, in addition to the presence of odontoblastic processes within dentinal tubules, means that young teeth have high water content. Older teeth, in contrast, have lower water content because lifelong secondary dentin deposition produces progressively smaller pulp chambers and less well-vascularized pulp tissue. Increased numbers of empty dentinal tubules (so-called "dead tracts" without odontoblastic processes), and reduced size of the dentinal tubules, due to deposition of intratubular dentin, further decrease water content in older teeth. These age-related changes result in more brittle teeth and less permeable dentin (11). Dentinal deposition, pulp, and odontoblastic changes are closely related to the presence or absence of caries and dental restorations.

Restored teeth were included in the study if they did not have full cast crowns or exhibit endodontic treatment. Measurements of the teeth and estimates of the volume of restoration were not recorded. The presence, absence, extent and type of existing restorations and the presence, absence and extent of caries were not described for the teeth. Only a limited number of teeth were incinerated and processed in this pilot study, with irregular allocation of teeth to the temperature/time combinations.

This pilot project, despite the small number of teeth used, presents several interesting findings for forensic investigators. For instance, the temperatures to which the teeth were heated appeared to be strongly related to disintegration during decalcification. This finding has implications in incineration cases where histologic evidence must be maintained and examined intact. If dental remains have been subjected to temperatures over 600°C, the teeth, in all probability, will disintegrate during decalcification, and other methods of histologic evaluation, such as ground sections or SEM, should be used.

Another finding was that dentin, with its characteristic tubules, was the most reliable identifier of dental origin following thermal insult, decalcification and histologic processing. This was also true for the six teeth that underwent complete disintegration after decalcification and before histologic processing. This finding has implications in incineration cases requiring histologic examination of incinerated fragments for evidence of dental origin. In cases where such fragments have been decalcified and have disintegrated, the residual debris should be filtered, processed, and examined microscopically because evidence of dentinal tubules may still be present. Further laboratory investigations into structural changes in incinerated teeth may provide additional clues related to the temperature, intensity, or duration of thermal insult.

Additional laboratory studies of tooth incineration are planned to relate structural damage to time and temperature and to such factors as tooth age, type and restorative history. It would be useful to have a way to stabilize incinerated dental remains for decalcification and histologic processing. In the future, laboratory methods can be applied to investigating more realistic situations including teeth in intact incinerated bodies. Routine histologic evaluation of incinerated dental remains can provide additional investigative avenues both in the areas of fire investigation and victim identification for forensic investigators.

Acknowledgments

The authors would like to thank Mary Warrings for histologic preparation of the tissue sections, Carol Rose for help in preparing the manuscript, and Dr. Robert Gorlin for critical reading of the manuscript. Dr. Hodges was supported by NIH/NIDR grant P30-DE09737.

References

1. Schirnding H. The teeth and their significance in forensic medicine, with special regard to the identification of corpses. Dental Cosmos 1934;76:853–9.
2. Zillner E. Beitrag zur Lehre von der Verbrennung. Nach Befunden an Leichen beim Ringtheaterbrande Verunglückter. Vjschr. gerichtl. Med. 1882;37:65–72, 237–46.
3. Griffiths CJ, Bellamy GD. Protection and radiography of heat affected teeth. Forensic Sci Int 1993;60(1–2):57–60.
4. Gustafson G. Forensic Odontology. 1966, New York:American Elsevier Publishing Company, Inc.
5. Gejvall NG. Bestämning av brända ben från forntida gravar (Determination of burned bones from prehistoric graves). Fornvännen 1947;42:39–46.
6. S-PLUS Guide to Statistical and Mathematical Analysis, Version 3.3. 1995, Statistical Sciences, a Division of MathSoft, Inc.: Seattle: Chapter 10.
7. Rao CR. Linear statistical inference and its applications. 2nd ed. 1973, New York: Wiley, Section 6e.3.
8. Fairgrieve SI. SEM analysis of incinerated teeth as an aid to positive identification. J Forensic Sci 1994;39(2):557–65.
9. Carr RF, Barsley RE, Davenport WDJ. Postmortem examination of incinerated teeth with the scanning electron microscope. J Forensic Sci 1986;31(1):307–11.
10. Harsanyi L. Scanning electron microscopic investigation of thermal damage of the teeth. Acta Morpholog Academ Sci Hungaricae 1975; 23(4):271–81.
11. Ten Cate AR. Oral histology: development, structure, and function. 4th ed. 1994, St. Louis: Mosby-Year Book, Inc.
12. Bonar LC, Glimcher MJ. Thermal denaturation of mineralized and demineralized bone collagens. J Ultrastruct Res 1970;32:454–61.
13. Yumikura S, Hirano K. Über eine neue Nachweismethode für verbrännte oder verkohlene Deninschicht. Kokub. Zass 1936;10:36–42.

Additional information and reprint requests:
Sandra L. Myers, D.M.D.
Department of Oral Science
University of Minnesota
16-122 Moos Tower
515 Delaware Street S.E.
Minneapolis, MN 55455