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SECTION IDENTIFICATION

Behavior In Vitro Of The Dentin-Enamel Junction In Human Premolars Submitted To High Temperatures: Prediction Of The Maximum Temperature Based On Logistic Regression Analysis

Carlos Mejía[1], Adriana Herrera[1], Ana-Isabel Sánchez[2], Sandra Moreno[3], Freddy Moreno[3]

[1]School of Dentistry, Universidad del Valle, Cali, Colombia
[2]Faculty of Health Sciences, Pontificia Universidad Javeriana, Cali Colombia
[3]Basic Sciences of Health, Faculty of Health Sciences, Pontificia Universidad Javeriana, Cali Colombia

Corresponding author:* *fmorenog@javerianacali.edu.co

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ABSTRACT

Objective:* *The aim of the study was to provide scientific evidence that would permit DEJ separation to be used as a parameter to estimate the temperature to which burnt, carbonized or incinerated cadavers or human remains had been subjected. Materials and methods: A descriptive pseudo-experimental study was carried out in vitro using cone beam tomography to determine the physical behavior of the dentine-enamel junction in 60 human premolars submitted to high temperatures (200°C, 400°C, 600°C, 800°C and 1000°C). Results: Spearman's concordance and correlation index was used to determine the relationship between longitudinal separation of the dentine-enamel junction (mm) and temperature (°C) and a simple linear regression model developed to show that once micro- and macrostructural changes are initiated in the enamel and dentine. Conclusions: The dentine-enamel junction begins to separate from the cervical towards the occlusal as temperature increases.

KEYWORDS: forensic science, forensic odontology, dental tissues, dentine-enamel junction, high temperatures, medico-legal documentation

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INTRODUCTION

In the past five years increasing numbers of studies have described changes in dental tissues and dental materials to show repetitive patterns and allow correlation between in vitro and in vivo observations. The objective of these studies has been to generate useful markers for forensic odontological identification and documentation of medico-legal autopsies of individuals who have died as a result of the high temperatures generated in, for example, catastrophic fires, car and airplane crashes, terrorist attacks, bombings, post-mortem cremation to impede identification of the individual and ante-mortem cremation as a method of torture.

Teeth are able to resist temperatures of up to 1200°C without important loss to the dental macrostructure[1]; complete cremation of a cadaver between 700 – 800°C for 1 hour or more is reported in the literature without loss to the dental macrostructure of the teeth[2]. However there have been few investigations into the changes that occur in the dental tissues when teeth are submitted to high temperatures. The earliest studies focused principally on the macroscopic description of changes in color, fissures and cracks, fragmentation and bursting of the mineralized dental tissues[3-6]. They did not identify microscopic markers with sufficient scientific reliability to allow these results to be applied to the documentation of medico-legal autopsies during the identification of cadavers or burnt, carbonized and incinerated human remains.

However, in some of these studies it was reported that as temperatures increased enamel separated from dentine at the level of the dentine-enamel junction (DEJ)[5,7]. This finding has been associated with combustion of the organic matrix (a non-fibrillar component consisting of glycoproteins, glucosaminoglycans and proteoglycans, together with a fibrillar-collagen component) and physical-chemical changes in the inorganic component (crystals of calcium hydroxyapatite) of the enamel and dentine, producing reduction in the tissue volume and loss of continuity solution in the DEJ.

The purpose of the present study was to use cone beam tomography to determine the in vitro behaviour of DEJ in human premolars submitted to high temperatures so that a predictive marker of the maximum temperature to which the tooth had been subjected could be established. This marker could be used for forensic odontological identification in medico-legal necropsies

MATERIALS AND METHODS

This was a cross-sectional, pseudo-experimental and descriptive study in vitro that used cone beam tomography to describe the behavior of the DEJ in human premolars, to determine whether a causal relationship exists between the phenomenon of enamel-dentine separation and high temperatures. The null hypothesis was that human premolars, on being subjected in vitro to high temperatures, did not present significant changes in the DEJ.

SAMPLE

Once authorization was obtained from the Human Ethics Committee of the Universidad del Valle according to the Declaration of Helsinki, 60 premolar teeth were collected. The teeth were obtained from patients attending the oral and maxillofacial surgery clinics of the Universidad del Valle for orthodontic extractions. All of the teeth were caries free, fracture free and had not been subject to previous dental treatment

The teeth were washed with non-sterile water to eliminate the remains of blood and tissues, and then immersed in 5% chloramine T (100 g sodium tosylchloramide diluted in 2L distilled water) for fixing. The teeth remained in chloramine T for 1 week and were subsequently immersed in saline solution at room temperature to maintain conditions of 100% humidity according to the norms of ISO/DIS 11405:2003[8].

Finally the teeth were classified and distributed randomly into different groups that would be subjected to a range of high temperatures. As a control group 10 teeth were not submitted to high temperatures to establish criteria of normality (Table 1).

Table 1. Classification and distribution of the sample

Groups	**Control**	**Intervention**				
	Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**
Temperature	**0°C**	**200°C**	**400°C**	**600°C**	**800°C**	**1000°C**
Number of teeth	**10**	**10**	**10**	**10**	**10**	**10**

APPLICATION OF HIGH TEMPERATURES

Teeth of the intervention group were placed individually in trays coated with Cera-fina® Whipmix® and introduced in groups of 10 into an earthen oven (Thomas® Small Benchtop 1256®) previouslv calibrated to five different ranges of temperature (200ºC, 400ºC, 600ºC, 800ºC, 1000ºC) with a rate of increase of 10ºC per min from an initial temperature of 28ºC until each of the test temperatures was reached. For example, 10 teeth corresponding to the 200ºC group were exposed to a temperature range of 28 - 200ºC, the oven was allowed to cool to room temperature and the teeth removed. Ten teeth of the 400ºC group were then subjected to a temperature range of 28 - 400ºC, the oven allowed to cool as before and the teeth removed. The same procedure was used in succession for the 600ºC, 800ºC and 1000ºC groups. This protocol was standardized by the Oral and Maxillofacial Surgery Study Group of the School of Dentistry at the Universidad del Valle[7].

The in vitro model considered was carried out in an oven rather than in a direct flame against the background that in the various literature reports the maximum temperature reached was 1000°C over a period of 25-30 min and then subsequently falling to 500°C. At this stage all the oxygen had been consumed by combustion and the organic materials reduced to carbon (carbonization) or until the compounds of calcium, phosphates, silica or other oligoelements were sinterized (incineration)[9]. This in situ "muffler effect" is comparable to the changes undergone by the perioral tissues and facial musculature, as well as the bone, dental and periodontal tissues[3].

MEASUREMENTS

After being submitted to high temperatures the teeth were embedded in acrylic autopolymerized resin (New Stetic®) to facilitate their manipulation in the tomograph i-CAT® 3-D Cone Beam Dental Imaging System. The tomographs were taken longitudinally (in the buccal-lingual plane), with a voxel size of 0.2, exposure time of 14.7 s, tomographic section thickness of 1 mm, distance between sections of 1 mm, real-size images of 1:1 and 0% transverse distortion.

A preliminary study was carried out with five premolars which were each submitted to a specific temperature (200°C, 400°C, 600°C, 800°C and 1000°C respectively) with one premolar as a control. This was done in order to calibrate the observation equipment and standardize it for the observer according to the behavior of the dental tissues (enamel and dentine) and

DEJ at each specific temperature. The expert and observer used i-CAT® 3-D Dental Imaging System software to measure the length of the DEJ separation, represented by a radiolucent space between the enamel and dentine, from the most apical enamel of the cervical third of the tooth up to the longitudinal projection in the occlusal direction. In the same way the level of occlusal separation was correlated with the coronal thirds (cervical, medial and occlusal) of each sample (Figure 1). These measurements were used to estimate the degree of concordance by means of the intra-class correlation coefficient using the Epidat® 4.0 software and assuming a confidence level of 95.0%. The results show a correlation of 0.9455 for the inter-observer criterion (observer vs. expert) and 0.9658 for the intra-observer criterion (observer vs. observer). The values 0 = no correlation, 1 = direct correlation and -1 = inverse correlation were used as references.

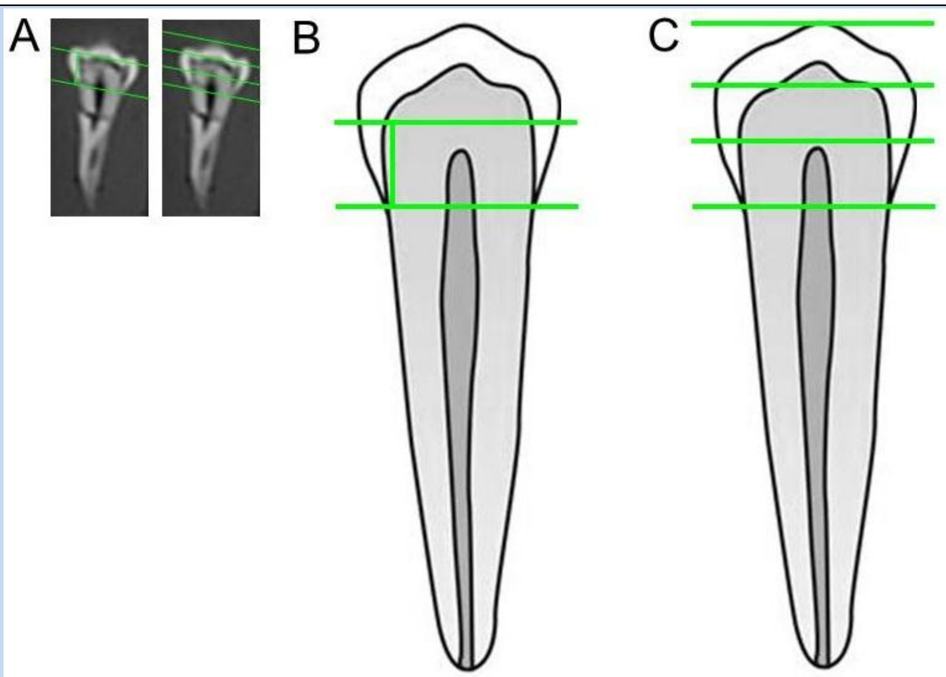

Fig. 1: Measurement of the extension of the radio lucid band that is compatible with the length of the separation of the DEJ from the cervical region to the occlusal region, and its correlation with coronal dental thirds. A. Cone Beam Tomography of a tooth subjected to high temperatures; B. A premolar tooth diagram in which the measurement taken of DEJ from the cervical region to the occlusal region is observed; C. A premolar tooth diagram in which the coronal dental thirds are observed.

STATISTICAL ANALYSIS

The measurements for DEJ separation in the cervico-occlusal direction were processed with the software IBM SPSS Statistics® 21 by means of descriptive (measurements of central tendency) and non-parametric statistics. The a priori plan of analysis involved systematization of the variables (Table 2) and analysis of satisfaction of the assumed statistics to determine whether the variables were dependent or independent. The non-parametric Kolmogorov-Smirnov test was used to evaluate normality of the sample distribution, with $P > 0.05$ being taken as normal. Levene's test was also used to contrast the homogeneity of variances, the

null hypothesis being P >0.05 when variances are equal.
Taking the temperature as an independent variable and the extent of the right and left sides of the DEJ separation and the coronal thirds as dependent variables, the Kolmogorov-Smirnov test confirmed the null hypothesis that there is normality in the extent of the right side of the DEJ separation (P = 0.886) and that of the left side of the DEJ separation (P = 0.442). Levene's test indicated that the alternative hypothesis could be accepted because there was inequality of variances in the extent of the right (P = 0.015) and left (P = 0.002) sides of the DEJ separation.

Spearman's correlation coefficient determines the level of association or dependence between an ordinal and numerical variable (in this case temperature and extent of the right and left sides of the DEJ separation), or between two ordinal variables (temperature vs. right and left coronal thirds). Interpretation of Spearman's correlation coefficient indicated that the values here varied between -1 and +1 (the more the coefficients approached a value of a +1, the greater the degree of correlation between the associated variables; 0 signified that no correlation existed). The null hypothesis corresponded to P > 0.05 when there was no correlation.

Finally a simple linear regression model was developed in which the extents of the right and left sides of the DEJ separation (numerical dependent variables) were analyzed as a function of the temperature (independent ordinal variable) to which the teeth were subjected. The null hypothesis corresponded to P > 0.05 when the model was not applicable.

RESULTS

When a tooth is exposed to high temperatures it may undergo structural changes that depend the maximum temperature reached, so that it may remain intact at 200°C, burnt at 400°C (change of color and formation of fissures and cracks), carbonized at 600°C (reduced to carbon by incomplete combustion), incinerated between 800°C and 1000°C (reduced to ashes), and finally explodes at 1200°C (radicular and coronal burst)[4-7].

With respect to the junction between the enamel and the dentine, at 200°C no macro-structural changes were observed that affected the DEJ when teeth were compared before and after being submitted to high temperatures, apart from a loss of shine (opacity of the enamel) associated with dehydration (Figure 2A). At 400°C the enamel turned brown and opaque (revealing the coronal dentine that presented signs of combustion of the extracellular organic matrix); while in the cervical region loss of continuity of the enamel was observed due to longitudinal and transverse fractures, as well as its separation from the dentine, indicating loss of DEJ continuity (Figure 2B). At 600°C the enamel became chalk-white due to combustion of the inorganic matrix; the coronal dentine that was visible through the fragmented enamel appeared grayish due to the change of phase from carbonized to incinerated while in the cervical region fragmentation of the enamel could be observed due to deepening of surface cracks and fissures, suggesting loss of DEJ continuity (Figure 2C). At 800°C the incinerated enamel assumed a grayish color since the coronal dentine became transparent in certain regions even when carbonized, while in the cervical region greater separation of the enamel from the dentine was observed due to loss of DEJ continuity (Figure 2D). Finally, at 1000°C the crowns of the teeth turned white due to incineration of the enamel and dentine. DEJ separation was much more obvious in the cervical region due to bursting and separation of the enamel, as well as the reduced volume of the root compared to that of the crown (Figure 2E).

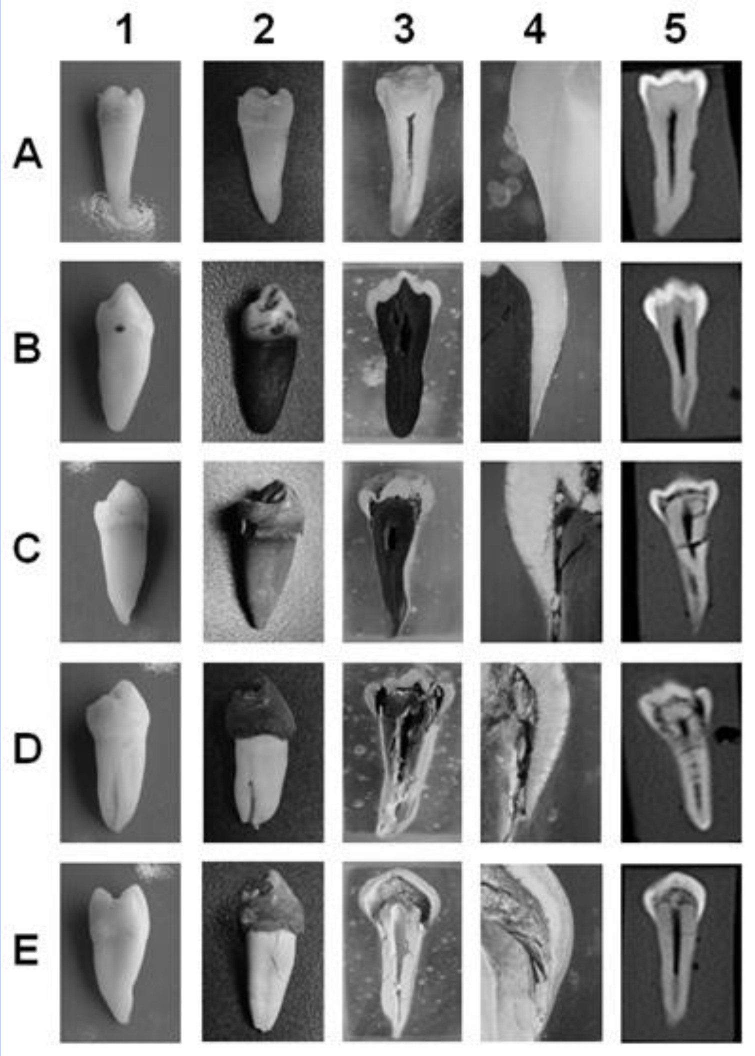

Fig. 2: Premolars subjected to high temperatures. A. 200°C; B. 400°C; C. 600°C; D. 800°C; and E. 1000°C. 1. Photograph of a tooth before being subjected to high temperatures; 2. Photograph of a tooth after being subjected to high temperatures; 3. Sagittal section of a tooth placed on acrylic resin and after being subjected to high temperatures; 4. Details of the separation of the DEJ in a tooth submitted to high temperatures and placed on acrylic resin; and 5. Cone Beam Tomography of a tooth subjected to high temperatures; a radio lucid band is seen to illustrate the separation between the enamel and dentine at the DEJ.

Based on these results Spearman's coefficient of correlation and concordance supported the alternative hypothesis, since there were dependent correlations between temperature and the extent of the right and left sides of the DEJ separation (P = 0.000). A dependent correlation was observed between the extent of right side of the DEJ separation with the coronal right (P = 0.000) and left (P = 0.002) thirds and the extent of the left side of the DEJ separation with the coronal right (P = 0.001) and left (P =0.002) thirds. Dependent correlations were also observed between temperature and the right (P = 0.001) and left (P = 0.002) coronal thirds (Table 3).

The simple linear regression model (adjusted using the R2 determination coefficient) showed that separation of the DEJ on the right is explained by a 40% change in temperature and that of the left side by a 64% change, allowing us to accept the alternative hypothesis (P = 0.000). In the same way it was determined that for each 200°C rise in temperature, DEJ separation widened from 0.72 - 0.76 mm (Table 4). Thus the simple linear regression model developed in this study is valid and useful, the null hypothesis being rejected in all cases.

Table 2. Systematization of variables

Variables	Definition	Type	Measurement Scale	Indicator
Temperature.	Independent variable	Ordinal	Qualitative	Temperature in °C at which separation of the DEJ is observed.
Separation of the right side DEJ.	Dependent variable	Numeric	Quantitative	Separation of the DEJ in mm from the most apical side of the enamel.
Separation of the left side DEJ.	Dependent variable	Numeric	Quantitative	Separation of the DEJ in mm from the most apical side of the enamel.
Right coronal third.	Dependent variable	Ordinal	Quantitative	Longitudinal region of the crown that involves separation of the DEJ.
Left coronal third.	Dependent variable	Ordinal	Qualitative	Longitudinal region of the crown that involves separation of the DEJ.

Table 3. Spearman´s correlation coefficient

Variables	Temperature	Separation of the right side DEJ	Separation of the left side DEJ	Right coronal third	Left coronal third
Temperature	1.000				
Separation of the right side DEJ	0.069 *P* = 0.000	1.000			
Separation of the left side DEJ	0,832 *P* = 0.000	0.610 *P* = 0/000	1.000		
Right coronal third	0.642 *P* = 0.000	0.874 *P* = 0.000	0.584 *P* = 0.000	1.000	
Left coronal third	0.687 *P* = 0.000	0.564 *P* = 0.000	0.803 *P* = 0.000	0.556 *P* = 0.000	1.000

DISCUSSION

Moreno et al[10] argued that separation of the DEJ began at 200°C, becoming more evident at 400°C with bursting of the cervical enamel. At 600°C and 800°C they described the complete separation between enamel and dentine at the level of the cervical and median thirds. Finally between 1000°C and 1200°C the enamel fragmented and separated totally from the dentine like a cap. The authors concluded that this fracturing phenomenon at the DEJ level could be associated with alteration of the extracellular matrix of both mineralized tissues. Enamel has a high inorganic content (96 - 99%) composed of a large quantity of calcium phosphate in the form of hydroxyapatite crystals, so that on being subjected to high temperatures it rapidly loses its scarce inorganic content, producing a strong contraction that induces changes in the organization of these crystals. Dentine has a high inorganic content and contains 12% water. It is protected by enamel in the crowns of the teeth, permitting a certain margin of thermal contraction compared to enamel, causing the latter to fracture at the DEJ level and separate at temperatures of over 400°C.

Although other authors have described this phenomenon none have explained it micro-structurally (4). Ferreira et al (11) pointed out that significant differences exist in the separation of the enamel and dentine at the level of the DEJ. Aramburo et al[12] and Vasquez et al[13] observed that this pattern of fracture between the enamel and dentine at the DEJ level is gradual as temperature increases.

In the present study, the spontaneous separation of the enamel and dentine by the action of temperature behaved as reported in the literature. At 200°C, although no tomographic changes were observed, there was combustion of the organic matrix and cohesive failures of the dentine in proximity to the DEJ. The process of carbonization of the mineralized tissues was initiated between 400°C and 600°C; the enamel rapidly lost its scarce inorganic content and hydroxyapatite crystals gradually increased in size and fused with each other (synterization). The dentine lost volume through dehydration and combustion of the inorganic component. This difference in the dimensional contraction of the dentine is the reason why the enamel separates at the level of the DEJ, initially in the cervical third of the tooth and then gradually up to the medial third of the tooth. This can be observed in tomographs as a radiolucent band. Finally between 800°C and 1000°C, the enamel is totally compacted and the dentine gradually begins the process of incineration of its inorganic component and synterization of the scarce inorganic component. Contraction of the dentine is much more evident and the enamel separates completely allowing a much wider radiolucent band to be observed in the tomographs.

Using cone beam tomography it was possible to determine that a gradual separation of the DEJ moving in a cervical/ occlusal direction was initiated between 200°C and 400°C and became more obvious as the temperature increased until total separation was reached at 1000°C. This constant repetitive phenomenon allowed correlation of the separation of the DEJ in the different coronal thirds as a function of temperature, thus making it possible to predict the behavior of DEJ separation as temperature increased. Thus, Spearman's correlation coefficient showed a relationship between temperature, extent of the separation of the DEJ on the right and left sides and the coronal right and left thirds. In this way the simple linear regression model predicts that on increasing the temperature DEJ separation advances from the cervical third progressively up to the occlusal third at approximately a rate of 0.72 - 0.76 mm per 200°C.

CONCLUSIONS

This in vitro study demonstrates that significant changes occur in the DEJ of human premolars when they are submitted to high temperatures in vitro, and that longitudinal separation of the DEJ constitutes a useful parameter to estimate the temperature to which a tooth has been subjected.

This research may eventually contribute to the documentation of medico-legal autopsies in cases of identification where forensic odontology is used and where decedents have been subject to burning, carbonization or incineration. The collection and analysis of a tomography, could demonstrate the behavior of the DEJ and measure the separation of the enamel and dentine represented by the radiolucent space. The features of this radiolucent space could contribute to the estimation of the temperature to which the tooth had been subjected; below 200°C the DEJ shows no changes, between 400°C and 600°C it is separated in the cervical third of the tooth, and between 800°C and 1000°C it is separated in the occlusal third of the tooth together with other changes such as the complete separation of the enamel in the form of a cap.

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