Histo-morphologic and gravimetric changes of teeth exposed to high temperatures - An in-vitro study

Nisha Sam[1], Sivakumar Trivandrum Thanappan[2], Anna. P. Joseph[2], Varun Baby Amma Raghavan Pillai[2], Vinod Mony[2]

[1] Central Research Laboratory Vinayaka Mission's Sankarachariyar Dental College Salem Tamilnadu (VMRF-DU). [2]Department of Oral and Maxillofacial Pathology, PMS College of Dental Science and Research, Thiruvananthapuram, Kerala, India.

Corresponding author: nisharanjit1320@gmail.com

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ABSTRACT

Background: Fire intelligence is the multidisciplinary basis of reconnaissance, which includes determining the origin, cause, and identification of fire victims. Fire is a destructive force capable of inflicting significant damage. Destruction of soft tissue in fire disasters makes victim identification nearly impossible. Teeth are hard and resilient and withstand such conditions. Analyzing the precise morphological, stereomicroscopic, histological, and gravimetric findings can extract valuable information from dental evidence in forensic investigations.

Materials and Methods: Thirty-six mandibular premolar teeth extracted for therapeutic purposes were exposed to high-temperature gradients. Macroscopic, stereomicroscopic, histological, and dry weight analyses were performed at each temperature gradient.

Results: The colour of teeth changed from yellowish orange to metallic black bronze to chalky white. Stereomicroscopy showed intact teeth at 100°C, gradual micro-cracks at 500°C, and a fully fractured crown at 900°C. Decalcified sections revealed dilatation of dentinal tubular pattern at 300°C. Dentinal tubules showed appearance of vapour bubbles at 400°C, resulting in loss of typical architecture. In the ground sections, alterations in scalloping nature of dentino-enamel junction, coalescing radicular dentinal tubules, and sand cracking appearance of teeth were noted at 100°C, 300°C, and 900°C, respectively. Significant reductions in the weight of the teeth samples were observed with higher temperatures.

Conclusion: From the morphological, histological, and gravimetric changes in a tooth caused by fire, it might be possible to determine the temperature and duration of fire exposure, and the cause of the fire.

INTRODUCTION

Unidentified human remains can be accurately identified using dental identification and is commonly accepted as evidence in Court. Dental evidence typically survives much better than soft tissue evidence such as facial characteristics or fingerprints. Human teeth are the hardest substance in the body because teeth are calcified and are resistant to environmental conditions that destroy soft tissue evidence. As a result, the teeth cannot be damaged by water immersion or decay and only minimally degrade over time. The association of human casualties with fire accidents is a common scenario in forensic investigations. Various fire sources, such as fuel explosions, bomb explosions, airplane accidents, etc., expose the human body to high temperatures, resulting in soft tissue mutilation, making human identification difficult.[1] Teeth can also be destroyed by heat when temperatures exceed 1000 °C, and the teeth are not protected by the soft tissue of the cheeks and lips. The most important task a pathologist can perform during an autopsy is determining the cause of death. In terms of legality, law enforcement agencies must prove beyond a reasonable doubt that the deceased died from causes other than natural causes. Teeth exposed to heat change their structure and morphology. The aim of the present study is to examine macroscopic, stereomicroscopic, histological, and gravimetric changes in teeth exposed to heat at different temperatures ranging from 100 °C to 900 °C.

MATERIAL AND METHODS

The present observational study was conducted after obtaining clearance from the institutional ethical committee (IEC No:-PMS/IEC/2016/10). The samples included in the study were thirty-six mandibular premolar teeth that were extracted for orthodontic purposes from patients with an age range of 15 to 25 years. The study sample teeth were divided into four groups of nine each. The samples in each group were exposed to controlled temperatures ranging from 100 °C to 900 °C in a burn-out furnace (Unident), and then analysed. Group 1 was analysed visually for changes in colour and morphologic changes in crown and root. Group 2 was analysed stereomicroscopically for macroscopic changes using a Magnus MLX stereomicroscope (2X magnification), and digital photographs were taken using a Nikon SLR5500. Ground sections were prepared from Group 3 using Arkansas stone (thin sections of 2 mm were prepared) and fixed on microscope slides. Subsequently, the images were captured with a photomicrograph. In group 4, the samples were first decalcified with OSTEOMOLL (decalcifier solution, Merck), sections of 3 μm thickness were prepared using a Leica semi-automatic microtome, and stained with hematoxylin and eosin. The decalcified sections were analysed using a Labomed SP-Achro microscope and photomicrographs. For gravimetric analysis before and after the exposure to temperature, the teeth were weighed using a precision electronic balance (model KD-HN).

RESULTS

Morphological analysis showed the following changes in colour and macroscopic structure at different temperatures. (Figure:1 showed the colour and morphological changes of crown and root). At 100°C, a band appeared on the crown. At 200°C, the colour of the crown changed to a slightly grey and white appearance. At 300 °C, the colour of the crown and root changed to a brownish-orange shade, and cracks were visible on the tooth. At 400°C, the crown had turned into a metallic blackish bronze colour, while the root had turned charcoal black. The crown and root appeared to be intact, with a vertical crack on the crown surface. At 500 °C, the colour of the crown changed to shiny grey black with blackish areas. The crown part showed chipping of the enamel shell, pitting, pits, and grooves. A portion of the dentine was lost. The colour of the root and crown changed to grey at 600 °C. The entire crown fractured, and surface roughness was visible on the apical 1/3rd of the tooth. The crown and root had a grey-blue outer surface at 700 °C, while the enamel's inner surface was grey, and the dentine was black. The coronal 1/3rd of the root was fractured. At 800 °C, the outer surface of the crown appeared to be greyish blue in colour, and the inner surface was bluish grey along with deep white cracks in the crown area. The crown and root fractured and appeared very brittle. At 900°C, the crown and root colour changed to chalky white. The crown fractured into fragments, and the apical 1/3rd of the root appeared thin. (Table:1)

Stereomicroscopic analysis of teeth exposed to different temperatures showed structural changes. (Figure:2 showed stereomicroscopic appearance of teeth exposed to 100°C to 900°C). At 100°C, the enamel showed a mottled appearance and roughness at the tip of the root.

At 200°C, the micro-cracks were visible at the crown and root, and the cervical portion showed roughness. At 300 °C, the crown displayed a brownish band of discoloration and enamel loss in the cervical margin, as well as surface irregularities. The root showed micro-cracks from the cervical margin to the root tip and a scorched appearance at the root tip. At 400°C, the crown portion was split longitudinally and showed a crusted appearance. The cervical margin of the tooth showed a gun powder-like appearance. The root exhibited micro-fractures and a crack line from the cervical margin to the root tip. The upper 1/3rd part of the root showed a "matted appearance." At 500°C, the crown showed the enamel split from the cervical margin, peeling of the cementum layer, and along with the dentine structure, the crown was lost. The enamel portions of the crown came out like a cap at 600°C, and the following changes were visible: the micro-cracks became more prominent, the surface layer showed pits, and the cemental layer was completely lost. The crown portion split apart from the root, and the dentino-enamel junction was marked with a line at 700 °C. At 800°C, the tooth was fractured into multiple fragments. At 900°C, the tooth showed a chalky white appearance.(Table 2)

Figure 1. Colour and morphological changes of crown and root

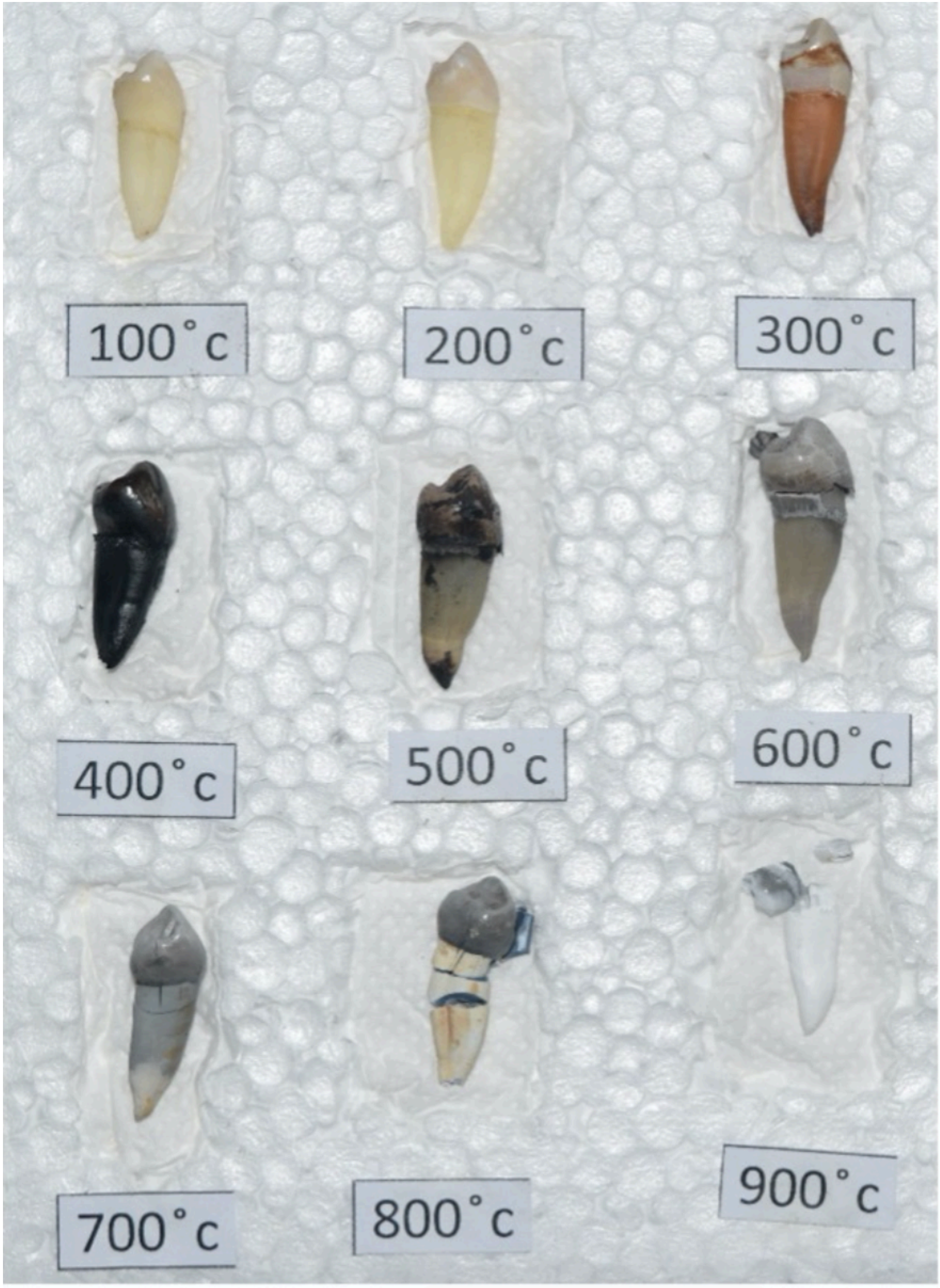

Table 1. Colour and morphological change in crown and root

Temperature	Colour changes of crown and root		Morphological changes of crown and root
	Colour of Crown	Colour of Root	
100°C	Band like appearance	No colour change	No structural variations
200°C	Slight greyish white	No colour change	Crown - cracks appeared.
300°C	Band like brownish orange	Brownish orange	Crown - cracks
400°C	Metallic blackish bronze	Charcoal black	Crown-- intact, Vertical crack. Root-intact grainy on apical 1/3rd and cervical line
500°C	Glistening greyish black with blackish areas	Greyish black with apical 1/3rd blackish area.	Crown--enamel shell cracking off, pitting defects, pit and grooves. A portion of dentine was lost.
600°C	Grey in colour	Dark greyish in colour	Entire crown fracture, surface roughness on apical 1/3rd
700°C	Outer surface greyish blue in colour. Inner surface – enamel greyish dentine black.	Greyish blue in colour, apical 1/3rd yellowish white. Root tip of teeth ivory in colour	Fractured at coronal 1/3rd of the root.
800°C	The outer surface is greyish blue in colour. Inner surface bluish greyish white.	Outer yellowish white, Inner surface – bluish	Deep cracks on the crown portion. Tooth fractured with crown and root – fragile
900°C	Outer and inner surfaces chalky white in colour.	Outer and inner surfaces chalky white.	Crown fractured into fragments with roughness, thin apical 1/3rd of the root.

Figure 2. Stereomicroscopic appearance of teeth exposed to 100°C to 900°C

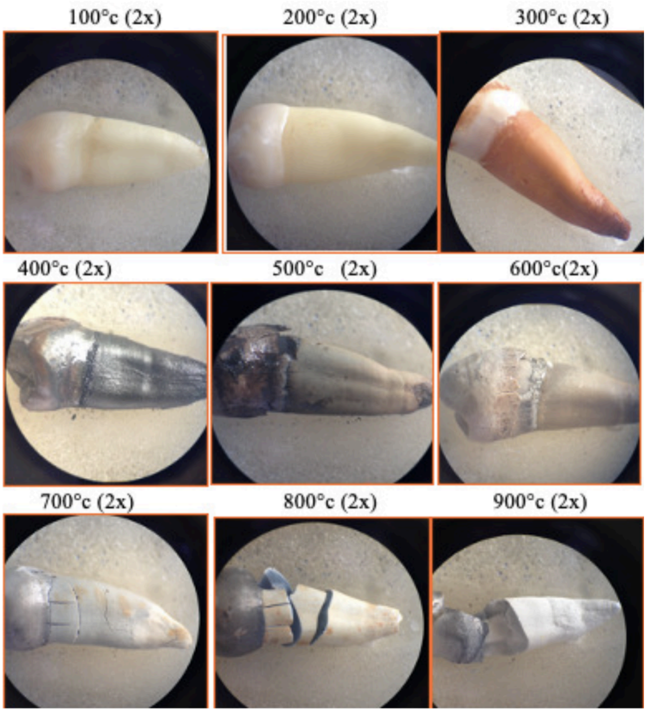

Table 2. Stereo microscopic analysis

Temperature	**Stereo microscopic analysis of crown and root**	
	Crown	**Root**
100°C	The mottled appearance of enamel	Roughness on the tip of the root. Cervical band/ discoloration.
200°C	Micro-cracks on crown and root	Roughness in the cervical area
300°C	Brownish band of discoloration of loss of enamel in the cervical margin of surface irregularities.	Micro-cracks from cervical margin to root tip and scorched appearance root tip
400°C	Splits longitudinally, crusted appearance. The cervical margin shows a gun powder appearance.	Micro-fractures, crack line from cervical margin to root tip, upper 1/3rd part showed matted appearance. Lined appearance in the middle area. Rough apex.
500°C	Enamel splits from the cervical margin, eggshell cracking, micro-cracks increase.	Peeling of root surface layer -the cementum, layer of dentine structure lost. Tip of root charred. Structural loss.
600°C	Enamel comes out like a cap; micro-cracks are prominent, pits on the surface, more crack lines appear; band-like crack on the cervical area	loss of cementum.
700°C	Portion splits apart, enamel appears grey, and dentino-enamel junction can be demarcated with a line	Fracture lines
800°C	Fragile	Vertically and horizontally. Multiple fractures.
900°C	Completely shattered, chalky white appearance.	Irregular surface, fracture of crown and root.

Ground sections of teeth were analysed up to 800°c. (Figure.3 showed histological analysis (ground section) of teeth exposed to 100°C to 900°C). At 100°C, the tooth section showed alterations in the scalloping nature of the dentino-enamel junction. At 200°C, the radicular dentinal tubular pattern was distorted. The radicular dentinal tubules coalesced, and the apical portions of the dentinal tubule structure deformed at 300°C. The enamel and dentine surface of the tooth section had a fur-like appearance at 400°C. The tooth section appeared amorphous and blackish at 500°C, with irregular margins. It had a sand cracking appearance at 600°C. At 700°C, the section showed an amorphous appearance. The tooth section appeared greyish black amorphous with irregular margins at 800°C. (Table 3)

Decalcified sections of teeth were evaluated up to 400°C. (Figure.4 showed histological analysis (decalcified section) of teeth exposed to 100°C to 900°C). At 100°C, the sections showed normal architecture. At 200°C, the apical 1/3rd of the radicular dentine showed changes in dentinal tubular pattern. Dentinal tubules dilated when heated to 300 °C. At 400°C, the loss of normal architecture and dentinal tubules resulted in the appearance of vapour bubbles.(Table 4)

From gravimetric analysis (Figure. 5 showed gravimetric analysis of teeth samples 100°C to 900°C), it was identified that at each temperature before and after exposure, a consistent reduction in the weight of the teeth was observed at above 300°C, with a steep decrease from 400°C to 900°C (Table 5).

Figure 3. Histological analysis(Ground Section)of teeth exposed to 100°C to 900°C

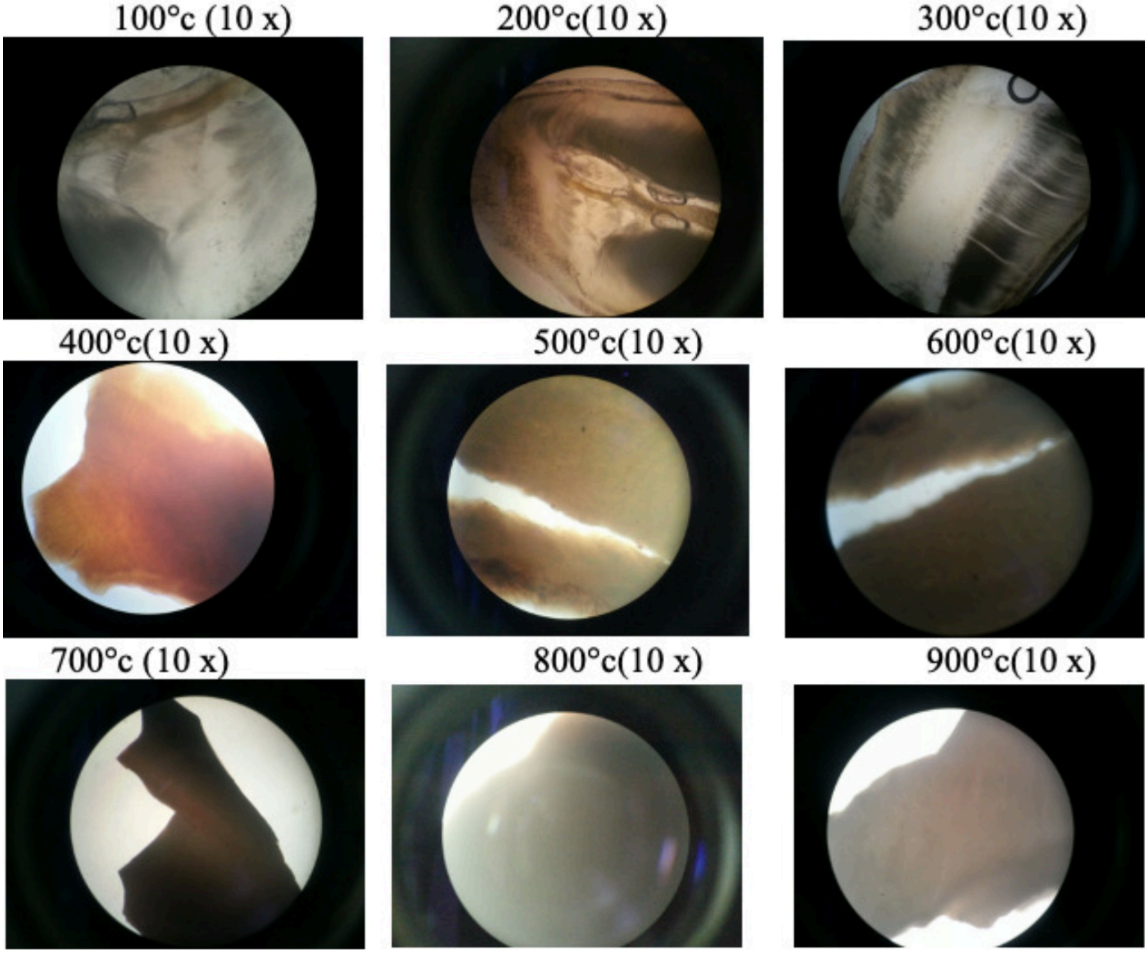

Table 3. Histological analysis(ground section)

Temperature	**Histological analysis (Ground Section)**
100°C	Alterations in the scalloping nature of dentino-enamel junction
200°C	Radicular dentinal tubules structure and pattern distorted
300°c	Radicular dentinal tubules were coalesced. Apical portions of dentinal tubule structure deformed
400°c	Appears dark brownish in colour, enamel, and dentine surface fur-like appearance
500°c	Amorphous blackish with irregular margins
600°c	Sand cracking appearance
700°c	Amorphous
800°c	Greyish black amorphous irregular margins

Figure 4. Histological analysis (Decalcified Section) of teeth exposed to 100˚C to 900˚C

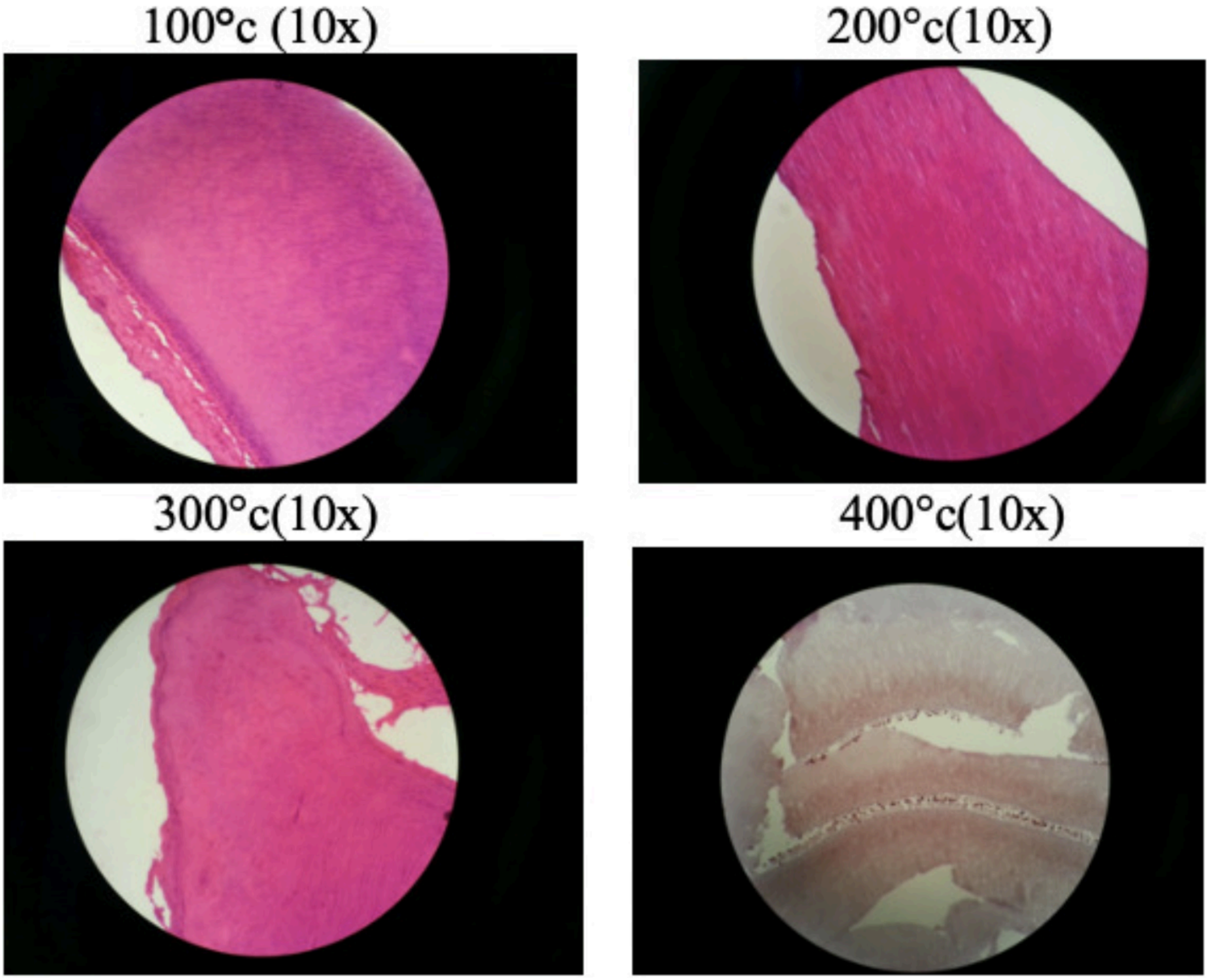

Table 4. Histological analysis (decalcified section)

Temperature	Histological analysis (Decalcified section)
100˚C	Normal architecture
200˚C	Apical 3^{rd} of radicular dentine shows changes in dentinal tubular pattern
300˚c	Dilated dentinal tubules
400˚c	Loss of normal architecture, dentinal tubules show vapour bubble appearance.
500˚c and above	Teeth disintegrated completely during decalcification

Figure 5. Gravimetric analysis of teeth samples 100°C to 900°C

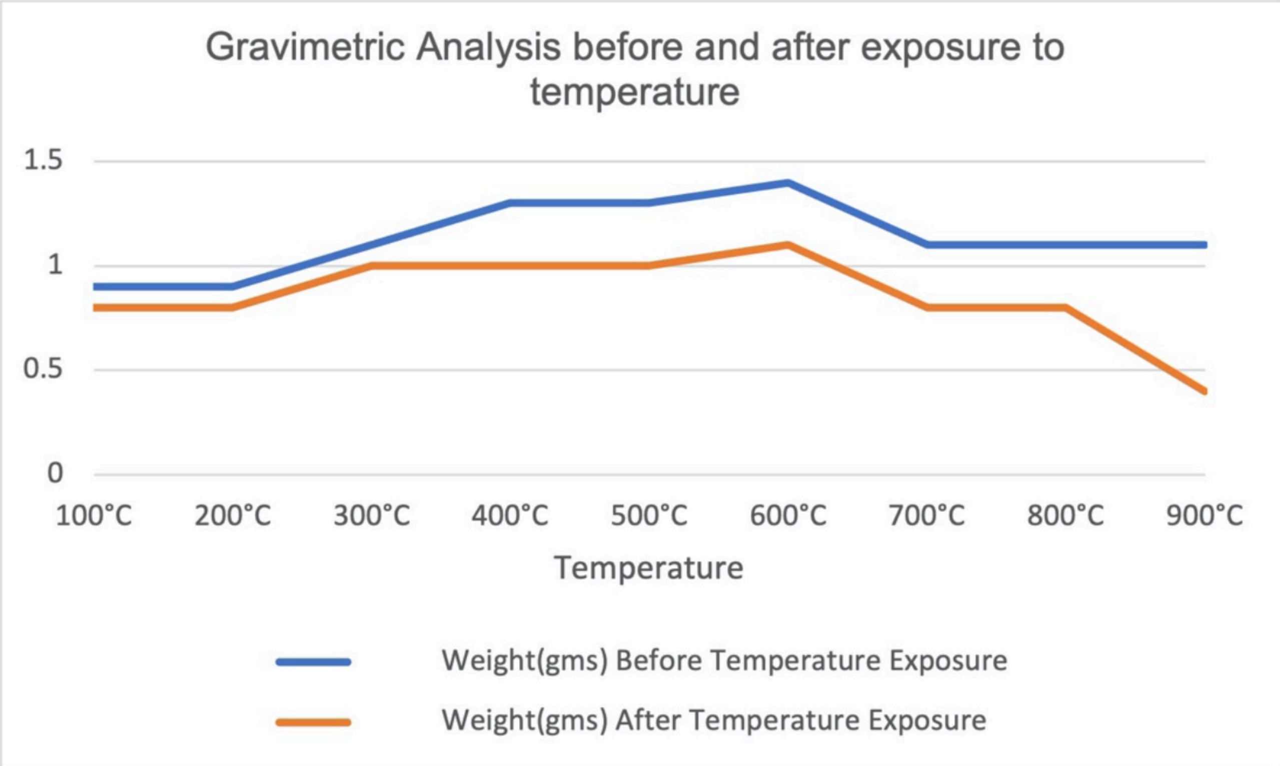

Table 5. Gravimetric analysis of teeth samples

Temperature	Weight (gms)	
	Before Temperature Exposure	After Temperature Exposure
100°C	0.9	0.8
200°C	0.9	0.8
300°C	1.1	1.0
400°C	1.3	1.0
500°C	1.3	1.0
600°C	1.4	1.1
700°C	1.1	0.8
800°C	1.1	0.8
900°C	1.1	0.4

DISCUSSION

Teeth are the hardest structure in the human body and can provide valuable dental evidence in the forensic investigation of fire accidents. Studies by Anderson on odontological identification in fire victims showed that 50 % of the victims had no injury, while 25% of cases showed injuries to the anterior teeth only. The present study revealed that the tooth withstands temperatures of up to 900°C and can provide evidence for forensic investigation.[2,3]

The temperature might vary for different fire accidents; some of the sources of fire accidents such as domestic fire reach temperatures of 649°C, burning of kerosene: 65°C to 220°C, burning of gas cylinders: 100°C to 200°C, car accidents: 220°C to 990°C, incinerator: 850°C to 1093°C, combustion of gasoline: 800°C to 1100°C, cremation: 871°C to 1009.3 °C and airplanes: 1000 °C to 3000 °C. However, chemical fires can exceed several thousand degrees. [4]

In the present study, the morphological, histological, and gravimetric changes of teeth exposed to high temperatures were evaluated. Colour change was the most distinguishing morphological feature for each temperature range from 100 °C to 900 °C. Specific colour changes from yellowish orange to charcoal black, passing through greyish blue and chalky white, were observed in the crown and root. Similar changes were described by Merlati G, Priyanka, Patidar KA, and Moreno et al. [5-8]

From the stereomicroscopic evaluation of the study, it was observed that the teeth were affected from 300°C with the progressive formation of micro-cracks, enamel splits, peeling of dentine and cementum, and fracture of crown and root at 900°C. The findings of the present study were comparable to the study conducted by Dhobley et al.[9]

Most of the currently available evidence is based on the morphological and stereomicroscopic analysis of charred tooth residues. There is an apparent lack of data on the ground sections and decalcified sections on teeth. In the present study, the authors evaluated the ground and decalcified sections. It was also observed that the ground sectioning of teeth was possible up to 800°C, beyond which the teeth became powdered. It showed an altered histological pattern of the dentinal tubules with amorphous changes at elevated temperatures. The teeth were decalcified up to 400°C; above that, the teeth completely disintegrated in the decalcifying solution. The dentinal tubules showed the appearance of vapour bubbles in the decalcified section, similar to that reported by Prakash et al.[10] No previous studies were conducted on gravimetric changes in burnt teeth. The present study determined that the weight of teeth exposed to temperatures over 300°C steadily decreased, with a steep decline between 400°C and 900°C. Small tooth fragments can be identified from the remains of the fire, and the exposure temperature can be reliably estimated. It was found that the teeth shattered into large particles at lower temperatures and at higher temperatures, the teeth shattered into numerous smaller particles. A similar observation was reported by Karkhanis.[11]

CONCLUSIONS

In the present study, the authors demonstrated structural changes at different temperatures in human teeth and thus provided valuable information about the thermal stress in teeth. The distinctive features of teeth exposed to different temperatures indicated the source of the fire and served as significant scientific evidence in forensic analysis.

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