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Histomorphologic and Gravimetric Changes of Teeth Exposed to High Temperature - *In vitro* Study

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Abstract

Background: In forensic dentistry, odontologists have been particularly interested in the investigation of burnt human remains. The purpose of our research is to provide morphological, stereomicroscopic, histological, and gravimetric findings from the investigation of the effects of thermal processes on teeth.Teeth, being the hardest substance in the body, give valuable information for forensic analysis. **Objective:** Histomorphology and gravimetric changes in teeth exposed to different ranges of high temperatures. **Methodology**: Thirty-six mandibular premolar teeth extracted for therapeutic purpose were taken and exposed to the varying higher temperatures. Macroscopic, stereomicroscopic, histological analyses along with the dry weight estimation were recorded at each temperature gradient. **Results:** From a lower temperature to a higher temperature, the specific colour change of the tooth was yellowish-orange, metallic black bronze and chalky white. Stereo microscopically, we observed intact teeth at 100°C; the gradual formation of micro cracks, crown-enamel separations from the cervical margin, eggshell cracking at 500°C; and a completely shattered crown at 900°C. Decalcified sections show dilation in the dentinal tubular pattern at 300°C. Loss of typical architecture was noted at 400°C, with dentinal tubules exhibiting the vapor bubble appearance. In-ground section alterations on the scalloping nature of dentino enamel junction, coalescing radicular dentinal tubules and sand cracking appearance of the tooth 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:** Incineration-induced morphologic, histologic, and gravimetric alterations may provide useful information regarding the temperature and duration of fire exposure. It might also assist in understanding the conditions of the fire.

Keywords: Fire, Odontology, Teeth, Temperature

Introduction

Unidentified human remains can be accurately identified using dental identification. In addition, it is commonly accepted in evidence in the trial. Dental evidence typically survives much better than soft tissue evidence such as facial characteristics or fingerprints. The human tooth is the hardest substance in the body, more rigid than bone. Since they are calcified, they are resistant to environmental conditions that destroy soft tissue evidence. As a result, teeth cannot be damaged by water immersion, putrefaction, or degradation. Human disaster associated with fire accidents is a common scenario encountered in forensic investigation. Varying sources of fire such as fuel explosion, bomb blast, plane accidents, etc., expose the human body to a high temperature causing mutilation

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of the soft tissues, thereby making human identification difficult[1]. Teeth can, however, be destroyed by heat in rare cases when temperatures exceed 1000°C and the teeth are not protected by the soft tissues of the cheeks and lips. The most crucial task a pathologist may accomplish during an autopsy is determining the cause of death. In terms of legality, law enforcement must demonstrate beyond a reasonable doubt that the deceased died of causes other than natural causes.

Aim of the Study

The study aims to examine macroscopic, stereo microscopical, histological, and gravimetric changes in teeth exposed to heat at different temperatures ranging from 100°C to 900°C.

Materials and Methodology

The study was conducted on thirty-six mandibular premolar teeth extracted for orthodontic purposes, with an age range of 15 to 25 years. Four groups of nine premolar teeth were studied (Group 1 - morphological analysis, Group 2 - stereomicroscopy, Group 3 - ground section, and Group 4 - decalcified section). The samples were exposed to controlled temperatures ranging from 100°C to 900°C in a burnout furnace. Stereomicroscopy, ground sections, and decalcification were performed on the samples. Magnus MLX stereomicroscope was used to examine the samples. Images were captured. Using a Leica semiautomatic microtome, sections 3µm thick were prepared of each sample after decalcification with osteomol (10% HCL). Hematoxylin-eosin was used to stain the sections. Using a Labomed SP-Achro microscope and a photomicrograph, the sections were analyzed. The samples were used for the ground section, sectioned by using Arkansas stone, thin sections of 2 µm were prepared and fixed on microscope slides. Images were captured with a photomicrograph.

Results

At 100°C, crown showed the mottled appearance of enamel, cervical band/discoloration of the crown, an alteration in the scalloping nature of dentino-enamel junction and roughness on the tip of the root. At 200°C, the crown appeared slight greyish white patchy. There were micro-cracks on crown and root, roughness in the cervical area, radicular dentinal tubules structure and pattern distorted. The apical 3rd of radicular dentin showed changes in dentinal tubular pattern. At 300°C, the crown showed band like brownish-orange, and the root appears brownish orange. The crown showed loss of enamel in the cervical margin and surface irregularities. Root began to deform, producing micro-cracks from cervical margin to root tip and scorched appearance. Radicular dentinal tubules were coalescing, dentinal tubule structure deformed at the apical region and dilated. Loss of normal architecture and dentinal tubules showed the vapor bubble appearance in decalcified section. At 400°C, crown began to appear metallic blackish bronze, intact and vertical crack. The root appeared charcoal black, intact and grainy on apical 1/3rd and cervical line. Enamel and dentinal surface showed fur-like appearance. Teeth disintegrated completely during decalcification. At 500°C, the crown was seen as glistening greyish black with patchy blackish areas, crown-enamel shell cracking off, pitting defects, pit and grooves. The root has appeared greyish black with an apical 1/3rd patchy blackish area and a portion of dentin was lost. Peeling of root surface layers and layer of dentin structure lost and amorphous blackish with irregular margins. At 600°C, Gray in color, entire crown fracture and surface roughness on apical 1/3rd. Enamel could come out like a cap; micro-cracks were prominent, more crack lines appeared, bands like crack on the cervical area were seen and cementum was lost. Sand cracking appearance was noticeable in-ground section. At 700°C, outer surface appeared grayish-blue in color. Inner surface enamel was greyish and dentin was black apical 1/3rd yellowish white. (Root tip of tooth ivory) Fractured at coronal 1/3rd of the root. At 800°C outer surface of the crown was greyish blue in color and the inner surface was bluish greyish white. The root appeared outer surface yellowish-white, inner surface-bluish, deep cracks on crown portion. Tooth fractured with fragile crown and root-greyish black amorphous irregular margins. At 900°C, outer and inner surface chalky white crown was fractured into fragments with patchy roughness, thin apical 1/3rd of the root. Root showed irregular surface. (Table 1, 2, 3, 4, 5) (Figure 1, 2, 3, 4) On gravimetric analysis, there was a consistent reduction in the weight of teeth above 300°C, with a steep decline from 400 to 900°C.

Figure 1. Morphological Variations of teeth.

Table 1. Morphological Variations of teeth

Temperature	Color Changes		Morphological variations
	Color of Crown	Color of Root	
Normal	No color change	No color change	No Anatomical/Structural variation
100°C	Band like appearance	No color change	No structural variations
200°C	Slight greyish white patchy	No color 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 & cervical line
500°C	Glistening greyish black with patchy blackish areas	Greyish black with apical 1/3rd patchy blackish area.	Crown--Enamel shell cracking off, pitting defects, pit and grooves. A portion of dentin was lost.
600°C	Gray in color	Dark grayish in color	Entire crown fracture, surface roughness on apical 1/3rd
700°C	Outer surface grayish blue in color. Inner surface – Enamel greyish Dentin black.	Grayish blue in color, apical 1/3rd yellowish white. (Root tip of tooth ivory)	Fractured at coronal 1/3rd of root.
800°C	Outer surface greyish blue in color. Inner surface bluish greyish white.	Outer yellowish white, Inner surface – Bluish	Deep cracks on crown portion. Tooth fractured with crown and root – Fragile
900°C	Outer and inner surface chalky white in color.	Outer & Inner surface chalky white in color.	Crown fractured into fragments with patchy roughness, thin apical 1/3rd of root.

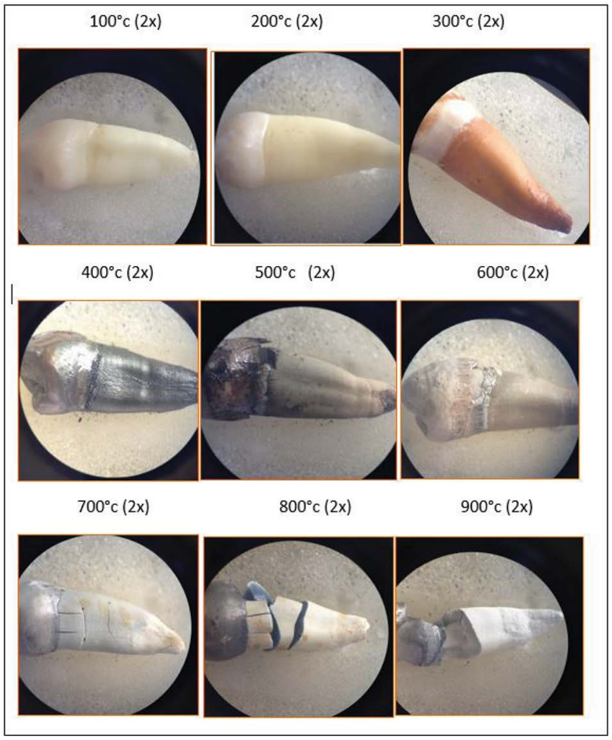

Figure 2. Stereomicropic analysis.

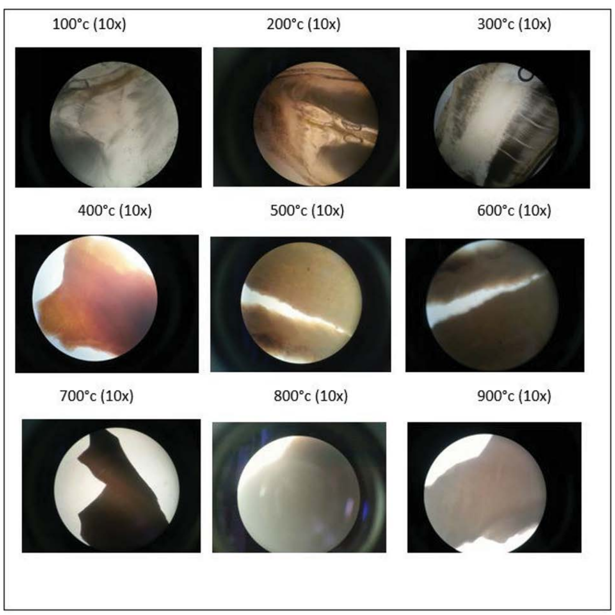

Figure 3. Histological Analysis (ground section).

Discussion

In this study, the morphologic, stereo microscopical, histologic, and gravimetric changes of teeth exposed to high temperatures were assessed, keeping in mind that teeth are still intact following a fire accident to serve as a tool of identification. Further, for different fire accidents the temperatures may vary. For example, some of the sources for fire accidents such as house fire reach temperatures of 649°C, combustion of kerosene 65°C to 220°C, combustion of gas cylinder 100°C to 200°C, car crash 220°C to 990°C, incinerator 850°C to1093°C, combustion of petrol 800°C to1100°C, cremation 871°C to 1009.3°C and aircraft 1000°C to 3000°C but chemical fires can exceed several thousand degrees[2]. Based on Andersen Classification the fire injuries to teeth and jaws are classified into six grades: no injury, injury to an anterior tooth, Unilateral injury to anterior and posterior teeth, Bilateral injury to anterior and posterior teeth, fragments of jaw bones including teeth or roots, and no dental remains[11,12].

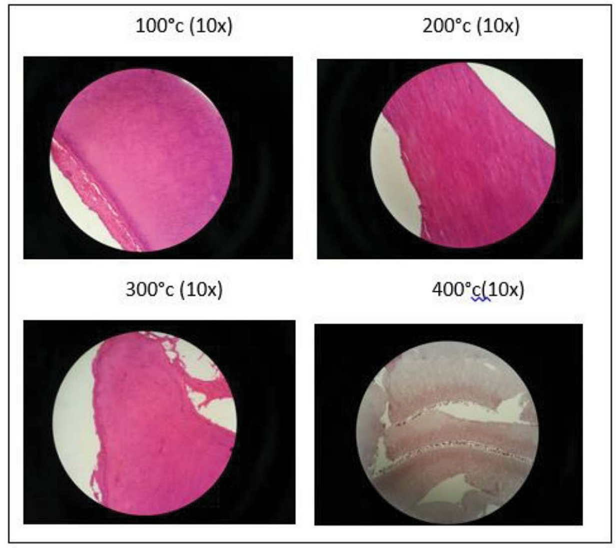

Figure 4. Histological Analysis (Decalcified section).

The morphological appearance is not same for teeth at different temperatures. Loss of translucency of enamel has been attributed to loss of water. Our study observed and recorded various macroscopic, stereomicroscopic, histologic characteristics and weight differences in teeth when exposed to different temperatures. On macroscopic examination, specific color changes were noted with increasing temperature varying from yellowish orange to charcoal black.

The most obvious observation and characteristic for each range of temperature was color change. The study's macroscopic heat-induced color changes resemble prior observations of unrestored human teeth[13,14]. The stereomicroscope revealed an intact tooth at 100°C and a mottled appearance at 200°C. The color changes following

Table 2. Stereomicroscopic analysis

Temperature	Stereo Microscopic Analysis
100°C	Mottled appearance of Enamel, Roughness on the tip of the root. Cervical band/ discoloration.
200°C	Micro cracks on crown and root. Roughness in cervical area.
300°C	Crown--Brownish band of discoloration of, Loss of enamel in cervical margin of, Surface irregularities. Root-- Micro cracks from cervical margin to root tip & Scorched appearance root tip
400°C	Crown-- splits longitudinally, crusted appearance. Cervical margin shows gun powder appearance. Root--Micro fractures, the Crack line from cervical margin to root tip, upper 1/3rd part shows 'Matted appearance', Lined appearance in the middle area. Rough apex.
500°C	Crown--Enamel splits from cervical margin, Egg shell cracking, Micro cracks on increased. Root --peeling of root surface layers, Layer of dentin structure lost. Tip of root charred. Structural loss.
600°C	Crown-- Enamel comes out like a cap, Micro cracks are prominent, pit on the surface, more crack line appears, Band like crack on the cervical area Root -- loss of cementum.
700°C	Crown-- portion splits apart, Enamel appears grey and DEJ can be demarcated with line. Root--Fracture lines
800°C	Fragile Root-- vertically & horizontally. Multiple fractures.
900°C	Crown-- completely shattered, chalky white appearance. Root -- Irregular surface, Fracture of crown and root.

thermal exposure are similar for a given temperature. Similar results have been reported in previous studies as well[5–7]. Invisible carbonization was attributed to the loss of glossiness on the surface of crown and root after 400°C by Rotzscher *et al.*,[8]. A pinkish discoloration is present on the root surface after exposed to 1000°C. Previous researchers reported similar observations, although the reason for discoloration was unknown[10]. Structural alterations such as micro cracks, fragmentation, and loss of tooth integrity were noted with temperatures ranging from 300°C to 900°C. According to Hughes and White, teeth are dehydrated, causing dentin materials to become brittle and dentin-enamel junctions to weaken. Intertubular tensile stress is responsible for the origin of cracks near dental pulp cavities, which can cause cracks to modify through structurally modified enamel and dentin[11,15]. In human enamel, water exists as adsorbed water, which is lost continuously and reversibly from 20°C to 200°C, and lattice water, with irreversible loss at 250°C to 300°C. Translucency continued to deteriorate with an increase in temperature. Delattre claims that the teeth of a burnt victim remain intact, have superficial discoloration, become charred, burned, and burst apart[3,4].

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 are coalescing. Apical portions of the dentinal tubule structure deformed
400°c	Appears dark brownish in color, Enamel and dentinal surface fur like appearance
500°c	Amorphous blackish with irregular margins
600°c	Sand cracking appearance
700°c	Amorphous appearance
800°c	Greyish black amorphous irregular margins

Table 4. Histological analysis (Decalified section)

Temperature	Histological Analysis (Decalcified section)
100°C	Normal architecture
200°C	Apical 3rd of radicular dentin shows changes in dentinal tubular pattern
300°c	Dilated dentinal tubules
400°c	Loss of normal architecture, dentinal tubules shows the vapor bubble appearance.
500°c and above	Teeth disintegrated completely during decalcification

Table 5. Weight of teeth before and after temperature exposure

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

According to Rafael Fernandez's research, when bone is exposed to temperatures of 100-200°C, longitudinal fractures occur in cortical and trabecular bone, while crystallization occurs from 200°C higher. At 300°C and 400°C, organic material disappears, fractures become more evident, and connective bone tissues distort. Similarly, at this temperature, crystalline formation expands in size. In the collagen and extracellular matrix, crystalline linear macromolecular polymers develop between 500°C and 600°C. The number of crystalline formation increases between 600°C and 800°C. The bone structure also changes from a laminar pattern with a homogeneous structure to a more crystalline structure. The structure becomes entirely crystalline at 900°C, however, it remains amorphous and granular in shape[13].

Ground and decalcified sections showed an altered histological pattern of dentinal tubules with amorphous changes at increased temperatures. Decalcification fluid disintegrates the tissue with higher temperatures, as collagen frameworks are destroyed, and collagen structures are worn out. There was a consistent reduction in weight of teeth above 300°c, with a steep decline from 400 to 900°c.

Conclusion

In our study, we were able to identify structural changes at varying degrees of temperature on human teeth, thereby providing valuable information about the thermal exposure when dental evidence remains. The distinctive characteristics of teeth exposed to different temperatures provide a clue to the source of the fire and serve as significant scientific evidence in forensic analysis.

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