Analysis of dental hard tissues exposed to high temperatures for forensic applications: An in vitro study

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Abstract

Aim: The aim of this study was to observe and record the macroscopic, radiographic, and microscopic findings obtained after subjecting the teeth to high temperatures. Materials and Methods: An in vitro study was conducted to observe macroscopic, radiographic, and microscopic changes in dental hard tissues in 60 unrestored non carious extracted human teeth. The teeth were grouped based on age: Below 30 years, 30–40 years, and above 40 years. The teeth from each age group were further divided into five subgroups, and each subgroup was subjected to a particular temperature: 200°C, 400°C, 600°C, 800°C, and 1000°C. Results: Various degrees of changes in relation to temperature were observed macroscopically, radiographically, and microscopically. The histological examination was limited for teeth exposed to 200°C. Conclusion: This investigation was carried out to study the gross changes, radiographic changes and histological changes in dental hard tissues exposed to high temperatures, which is an important part of forensic science. The aforementioned alterations caused by heat may provide useful information about temperature ranges and duration of exposure to high temperatures.

Key words: Color changes in teeth, forensic odontology, high temperatures, teeth incineration

Introduction

Forensic odontology plays a significant role in identification of individuals in case of crime investigations or during autopsy. Forensic odontology becomes even more important when bodies are decomposed, skeletonized, or mutilated. This is because dental hard tissues have some morphological characteristics, and perhaps restorations, anomalies, or pathologies, that make them as specific as a fingerprint. One of the most important tasks of the forensic odontologist is the identification of deceased individuals. In case of a fire disaster there are two main questions that arise: The cause of fire and the identity of the victim(s). As heat destroys or changes the recognizable features of the original appearance of the body (mostly soft tissue), research into the cause of death and identification of the victim...
become more challenging. Teeth are the hardest structures of the human body, thus they are highly resistant to cadaveric decomposition and long burial periods, and teeth can also survive the action of temperatures as high as 1100°C; for these reasons the dental hard tissue examination can help solve some mysteries.\textsuperscript{[3]}

Knowledge of the physical and histological changes taking place in the dental hard and soft tissues due to extreme heat is necessary for concrete interpretations.\textsuperscript{[4]} There have been only a few studies where such changes were observed. For instance, Merlati \textit{et al.} characterized the changes caused by heat on intact teeth and in those restored with amalgam, resin, and metallic crowns using stereomicroscopy and a scanning electron microscope. They reported that there is a consistent correlation between exposure temperature and the associated changes with it, and it is possible to create a reliable baseline data. This data could be used in real life scenarios to estimate the temperature to which the dental hard tissues could have been exposed.\textsuperscript{[4]}

The aim of this research was to observe and record the macroscopic, radiographic, and microscopic findings obtained after subjecting the teeth to high temperatures in the laboratory.

\textbf{Materials and Methods}

A total of 60 freshly extracted, unrestored permanent teeth fixed in 10\% formalin\textsuperscript{[5]} were collected and categorized according to the age of the patient. Group A comprised teeth from patients aged under 30 years, Group B comprised teeth from patients aged 30–40 years, and Group C comprised teeth from patients aged above 40 years. Each group included five subgroups, with each subgroup consisting of 1 incisor, 1 canine, 1 premolar, and 1 molar. Grossly decayed teeth that had very little hard tissue left were excluded.

The teeth used for the study were rinsed thoroughly in tap water and cleaned with a toothbrush in order to remove any soft tissue remains. Deposits of calculus were removed using a manual scaler.

A pre–heat-exposure intraoral periapical radiograph (IOPA) was taken for all the teeth, and the radiographs were kept in small plastic wrappers with proper labeling to prevent any confusion.

Each subgroup was subjected to a particular temperature—200°C, 400°C, 600°C, 800°C, and 1000°C—in the electric muffle furnace [Figure 1]. The increase in temperature was gradual, starting with 18°C, with an increment of 25°C/min till the desired temperature was obtained. The desired temperature was maintained for 15 min, followed by gradual cooling to room temperature.

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{figure1.png}
\caption{Electric furnace used to subject the teeth to desired temperatures}
\end{figure}

A postexposure radiograph was then taken for all the teeth to compare the degree and severity of radiographic changes, if any. An investigator from the Department of Oral Medicine and Radiology blinded to the group was requested to compare the findings. The exposed teeth were then carefully sent to the Department of Oral and Maxillofacial Pathology, where macroscopic changes such as changes in color of the tooth and surface changes were observed and recorded. Another investigator from the Department of Oral and Maxillofacial Pathology, blinded to the groups, was asked to note the color changes and surface changes of the exposed teeth of each group. The exposed teeth were decalcified using 10\% nitric acid. The teeth were examined and changes were recorded all through the decalcification. The decalcified specimens were processed, sectioned, and stained with routine hematoxylin and eosin (H and E) stains for histological examination. Photomicrographs were then taken as a record of histological examination.

\textbf{Results}

\textbf{Gross changes observed}

At 200°C, the color of the crown remained white with a smooth surface. The surface of the root showed a few irregular cracks when seen under a magnifying glass.

At 400°C, gapping along the cementoenamel junction (CEJ) was observed, the color of the crown changed to brownish black, and the root turned black. The teeth at this
temperature were more brittle. The surface showed multiple irregular cracks.

At 600°C, the surface became irregular, with hard tissue flaking off. Enamel separated from dentin. The crown and the root showed black to grayish discoloration.

At 800°C, the tooth appeared totally burnt. The surface of the crown and the root became white. The color of the exposed dentin was found to be blue toward the pulpal region, gradually fading to white along the periphery.

At 1000°C, the crown crumbled to pieces. The color of exposed dentin changed from blue to gray, with white peripheral areas. Vertical fracture lines were seen originating from the center and radiating toward the periphery in the molars. The color of the root remained chalky white, with multiple irregular cracks [Figure 2].

Radiographic changes observed
It was found that at 200°C, the teeth were intact and no differences were observed by radiographic examination. When the temperature was increased to 400°C, gapping was seen along the dentinoenamel junction (DEJ) as well as the CEJ. At 600°C, complete separation of hard tissues was observed at the DEJ and CEJ. At 800°C, in addition to separation of tissues at the DEJ and CEJ, many fracture lines were noticed throughout the crown and the root. At 1000°C, complete disintegration of the crown occurred. Only the root remained [Figure 3].

Histological changes observed
It was observed that all teeth irrespective of the age group, when subjected to a temperature of 400°C and above, disintegrated completely during the decalcification procedure. Therefore, processing and sectioning of these teeth could not be performed. Stained sections could be obtained only for teeth subjected to 200°C. On the histological examination of H and E-stained sections, two distinct patterns, namely, vapor bubble pattern and wicker basket pattern were observed.

There was no difference in the radiographic, morphologic, and histologic appearance of the teeth according to the age of tooth [Figure 4].

It was also observed that a group of teeth exposed to direct heat for comparison had more cracks in the crown at 400°C, and the enamel was completely destroyed at 600°C. The radiographic changes showed more structural damage in teeth exposed to direct heat. No difference was observed histologically.

Discussion
The few studies conducted on this topic agree that more studies need to be done for conclusive evidence. The present study was conducted to observe and record the findings obtained after subjecting the teeth to high temperatures with slight variations in the exposure variables.

According to the results obtained in the present study, we found that teeth irrespective of any age group, which remained exposed to gradual increments of temperature, experienced less structural damage than teeth exposed to
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In the present study, the crowns of teeth exhibited a change in color ranging from whitish (200°C) to blackish discoloration (400°C) to grayish white (600°C), when exposed to high temperatures. This is in accordance with Merlati \textit{et al.} in 2002.\[4\] On the contrary, a study conducted by Moreno \textit{et al.} in 2009 reported a change in color to light brown at 200°C.\[6\] In this study we observed that teeth were not strongly affected by the temperature exposure till 200°C. However, above 200°C, the teeth were affected by a progressive formation of fissures. Many disintegrated crowns were recorded. But the radiographic properties of the root could be discerned till 1000°C. The findings mentioned above were in agreement with Savio \textit{et al.} in 2006.\[7\]

The histological evaluation was limited only to 200°C in this study, as the teeth exposed to higher temperatures were completely dissolved during demineralization. This finding contradicts the findings in a research conducted by Chauhan in 2010, where histological evaluation was possible up to 400°C.\[3\]

Irrespective of the age, it was not possible to differentiate dentin from cementum during histological evaluation. It also became progressively difficult to handle the tooth specimens due to their increasing fragility at higher temperature.

We found that the conventional histological method becomes complicated as teeth become increasingly fragile with increase in temperature, as described by Myers SL \textit{et al.}\[8\] and Chauhan.\[3\] This drawback was overcome by Fereira \textit{et al.} when they were able to preserve the specimen teeth by immersing the incinerated teeth in monomer and then embedding them in poly methyl methacrylate. The sections were cut, scanned, and then converted to CMYK format, following which the calorimetric pattern of the teeth was described.\[1\]

In the present study we could not propose any baseline data due to the reduced size of the sample, and also because the teeth were extracted for reasons that were not directly related to the objectives of this research. However, the findings can effectively contribute to the scarce resources on this particular topic. The examination of the teeth in the present study also provides valuable information about the temperatures a fire victim might be exposed to.

It was interesting to note that the experimental conditions have some limitations as the present study did not accurately represent the real-life scenario of a fire, explosion, etc. This study also did not take into account other possible variables present in reality, such as protection from direct exposure provided by structures such as hard and soft tissues surrounding the teeth or a dental appliance present in the mouth.

In the present study, once the predetermined temperatures were reached, the teeth were removed from the furnace and allowed to cool at room temperature. The controlled increment of temperature and careful handling of the specimens allowed us to observe the morphology of the tissues, a view also highlighted by Fereira \textit{et al.} in 2008.\[1\] We agree with Moreno \textit{et al.}, who observed that in reality many other factors may complicate the effect of fire, such as the time of exposure to fire, the type of fire, the source of fire, the speed of increase in temperature, and the material used to extinguish the fire.\[6\]

Further experiments are required that take into consideration the factors discussed above. Studies that focus on experimental setups which are closer to real life scenarios is the need of the hour.

\textbf{Conclusion}

Forensic odontology is of great importance in medicolegal identification procedures. Dental hard tissues (enamel and dentin) show a series of specific changes when subjected to heat directly and gradually. Their macroscopic and histological features reveal information on the temperature ranges to which they might have been subjected. From the observations discussed above, it can be concluded that dental evidences may provide clues to solve the mystery in fire investigations as dental structures are the last to be destroyed under extreme conditions, whether temperature, acid, or putrefaction.
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Conflicts of interest
There are no conflicts of interest.

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