MACROSCOPIC AND MICROSCOPIC CHANGES IN INCINERATED DECIDUOUS TEETH

S Karkhanis, J Ball, D Franklin

Centre for Forensic Science, The University of Western Australia, Crawley, Australia

ABSTRACT

Teeth are amongst the most resilient elements of the human skeleton and are thus often utilised in routine forensic investigation involving the identification of unknown remains. Teeth exposed to thermal stress have the potential to not only aid in identification, but also in understanding the circumstances surrounding the fire. Children are twice as likely to become victims of house fire because of an inability to safely evacuate from areas of danger. The literature demonstrates, however, that research on the effects of incineration on teeth is mostly restricted to the permanent dentition. The apparent lack of knowledge on the effects of incineration on deciduous teeth thus necessitates further research in this area. To this end, this study aimed to relate colour changes that occur post heating with fragility to aid in proper handling of samples in a forensic scenario and to determine the possibility of identifying incineration temperature based on tooth condition.

A total of 90 deciduous teeth, extracted as a part of routine clinical treatment, were exposed to temperatures ranging from 100°C to 1100°C for 30 minutes using a laboratory Gallenkamp oven. Unheated deciduous teeth were used as controls for the project. Post-incineration the teeth were analysed under a stereomicroscope and SEM to assess the morphological changes. A colorimetric assessment was also undertaken to evaluate colour changes due to thermal stress. It was possible to identify incineration temperature based on tooth condition when the colour changes, stereomicroscopic findings and SEM images were utilised collectively. It was concluded that thermally induced changes in primary teeth occur at lower temperatures in comparison to the permanent teeth. It was also established that post-incineration deciduous teeth are fragile and show a tendency to fragment after minimal exposure to thermal stress.
(J Forensic Odontostomatol 2009;27:2

Keywords: Forensic investigation, deciduous dentition, incineration, identification.

INTRODUCTION

Teeth, as compared with most elements of the human skeleton, have an inherent resistance to damage. Under certain circumstances, however, the form and texture of teeth can be altered and occasionally total disintegration may occur. Fire is a leading cause of mortality and morbidity in adults and children. Investigators have long recognised the forensic potential of evaluating charred dental remains, as they yield important data regarding not only individual identity, but also circumstances surrounding the fire. In a report by the National Fire Protection Association (USA), it was suggested that children are twice as likely as adults to become victims of a house fire.[1] Children are more susceptible to the fatal effects of fires because of their inability to safely evacuate themselves. A child's risk is further increased due to their smaller airways which is associated with a greater incidence of mucous obstruction.[2]

All of the research analysing burnt dental remains currently available in the literature has been conducted on adult mono- and multiradicular teeth. The apparent lack of published data on the effect of incineration on the deciduous teeth thus necessitates research in this area. The present study, therefore, aims to investigate the type and extent of macroscopic and microscopic changes that can occur in deciduous teeth when exposed to high temperatures. The study also aims to relate post-incineration colour changes with the condition (in particular the fragility) of the teeth, to assist in the proper forensic treatment of samples. The present study also considers if there is any relationship between incineration temperature and tooth condition.

MATERIALS AND METHODS

A total of 90 deciduous teeth were studied; 45 molars and 45 anteriors. One deciduous molar and one anterior tooth were used as the control samples and were thus not exposed to the experimental temperatures. All of the teeth analysed were acquired as part of routine clinical therapeutic procedures conducted in dental hospitals in Central India. The ethics approval for the study was granted by the Human Research Ethics Committee of The University of Western Australia (RA/4/1/1931). As the extraction of clinically sound teeth in children is not justifiable in routine clinical practice, the deciduous molars used in the study were carious to some degree and the anterior teeth showed marked root resorption.

Following extraction each tooth was rinsed with saline water to remove blood deposits and salivary coating. The teeth were then stored in 10% formalin for 15 days, after which they were removed from the solution and sent to the first author. Formalin (10% concentration) was used as the storage medium for the teeth because it is known to have a minimal effect on dentin permeability, in comparison with other storage media such as 70% ethanol and distilled water with thymol.[3] Furthermore, 10% formalin has been demonstrated to be effective in disinfecting and sterilising extracted teeth without altering their hardness.[4,5]

Incineration

Incineration was performed using a Gallenkamp oven* at experimental temperatures starting at 100˚C and incrementally increasing by 100˚C up to a maximum of 1100˚C. The teeth were placed in a porcelain stub with the buccal surfaces facing the oven chamber and exposed to a sudden thermal shock by inserting them into the oven individually when the experimental temperature was reached. Once placed in the oven chamber, the teeth were incinerated for a period of 30 minutes; the teeth were then removed and allowed to cool to room temperature.

*Manufactured by Weiss-Gallenkamp (United Kingdom)

** Manufactured by Leica Microsystems Pty Ltd, North Ryde, Australia

Stereomicroscopic and Colorimetric Analysis

Each incinerated tooth was first examined under a stereomicroscope; a Leica MZ6** with a Leica L2 cold light source to assess the extent of heat induced alterations in the crown and root of the teeth. The colour of the enamel, dentin and cementum were then quantified using a Munsell soil colour chart (year 2000 revised washable edition).

Scanning Electron Microscopic Analysis

The SEM analysis was performed using the ESEM mode, which eliminated the high vacuum in the microscope chamber, replacing it with a high pressure gaseous atmosphere. Although the enamel is the least hydrated of all tissues, a tooth placed in the SEM will become dry and cracks will develop. These changes would adversely affect the final results, therefore, the environmental mode was used. Furthermore, to make samples conductive, conventional SEM analysis requires specimens to be coated with a thin layer of a suitable material, such as gold, gold-palladium alloy, platinum, silver or aluminium.[6] This would have involved further handling of the fragile teeth, leading to artefacts and further disintegration. Accordingly, to minimise potential damage from handling the incinerated teeth, double sided carbon tape was used to secure the teeth on a glass slide, which was then placed on the microscope stage.

During the imaging procedure, standardised values for the different working parameters such as spot size, working distance and kV were not used, as each image had to be optimised individually depending upon the magnification. The SEM analysis was performed using a Phillips XL 30 SEM from The Centre for Characterization, Microscopy and Microanalysis, The University of Western Australia.

RESULTS

All incinerated teeth analysed showed surface changes on the crown that progressed from the cervical end towards the incisal edge. At 100˚C the cervical enamel appeared to have a 'melted' irregular surface and was pale yellow in colour. The crown retained its surface lustre and perpendicular fissures that formed a chequered pattern were evident. Disintegration of teeth due to these deep fissures was present even at the initial starting temperature of 100˚C. The teeth incinerated at 200˚C were characterised by surface bubbling on the root, which was a shiny black colour. In addition to these heat induced changes, the teeth exposed to 300˚C showed a light gray colour, knob or globule like formations on the predentinal surface (Fig.1) and an amorphous deposit on it. The enamel and dentin began to separate at 400˚C and the very pale brown enamel shell had disintegrated into numerous fragments.

At 500˚C the light gray coloured enamel lost its surface lustre that was apparent at all previous incineration temperatures. Another important morphological change at this temperature was a decrease in dentinal tubular diameter near the dentinoenamel junction (Fig.2). The teeth were extremely fragile after exposure to 600˚C and the cementum colour had changed to bluish black. The prismatic structure of the enamel was still preserved at 700˚C (Fig.3), but the dentinal tubular diameter continued to decrease, with the tubules being completely obliterated in some areas (Fig.4). Despite the fissuring at 800˚C the surface characteristics (Tomes' process pits) of the bluish gray enamel were still identified on the fragments. Due to the melting of the inorganic crystals, the predentinal surface had changed in appearance from globular to a spicular configuration (Fig.5). The cementum had changed colour to a very dark bluish gray.

The surface of the dentinal tubules at 900˚C presented characteristic 'star shaped' structures (Fig.6). These crystalline structures appeared to be emerging from the peritubular dentin matrix and were observed in all of the teeth exposed to 900°C. The cementum surface in the teeth exposed to 900°C had no characteristic structure and was thus unrecognizable (Fig.7). The external surface appeared to have melted and was covering a granular layer. In the teeth exposed to 1000˚C the dentinal tubules remained identifiable, but the lumen was obliterated due to the granular changes in the intertubular dentin (Fig.8).

The crown and root of the molars exposed to 1100°C were neutral-white in colour, with a pink tinge in the cervical one-third. The predentinal surface was a light greenish-grey colour. The Tomes' process pits observed on the enamel surface were not identifiable in the teeth incinerated at 1100°C; at this temperature the enamel surface had a granular appearance and the crystallites appeared to be melting. The characteristic topography of the crystallites in the enamel structure was observed in some areas, thus the enamel remained recognisable, even after exposure to the extreme and sudden thermal trauma (Fig.9). The tubular structure of the dentin remained intact after the incineration process and they had narrowed due to extreme granulation of the intertubular matrix (Fig.10). The cementum surface remained unidentifiable post-incineration. The surface had melted and some granulation was apparent. Some areas on the root had developed vesicles due to the complete melting of the surface, which exposed underlying dentin (Fig.11). The results from the stereomicroscopic, colorimetric and SEM analysis are described briefly in Table 1.

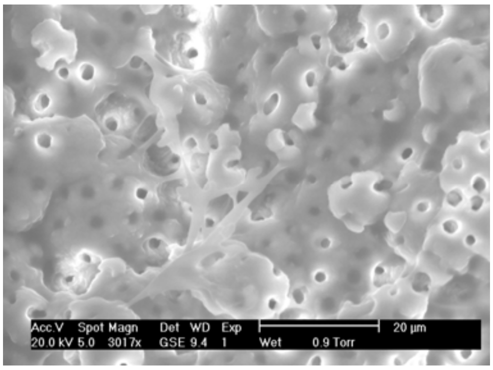


Fig.1: *SEM analysis of a deciduous mandibular second molar exposed to 300°C for 30 minutes. The predentinal layer showed knob like structures and the tubular diameter remained unaffected.*

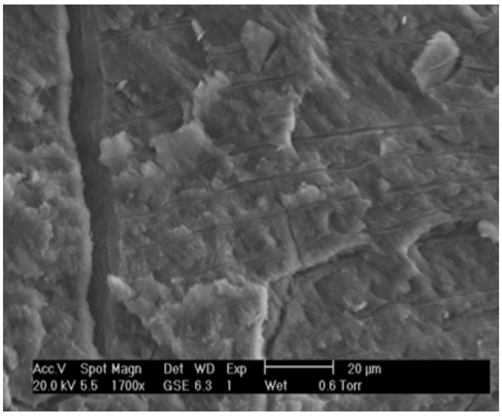


Fig.2: *SEM image of dentin after exposure to 500°C for 30 minutes. The tubular diameter near the dentino-enamel junction had decreased.*

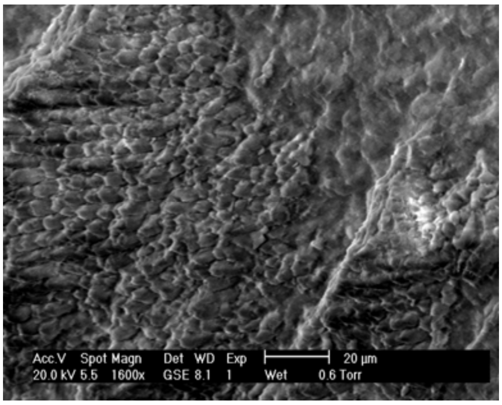


Fig.3: *SEM image shows the enamel subsurface structure. The morphology and the arrangement of the prismatic structure remained identifiable in the teeth exposed to 700°C for 30 minutes.*

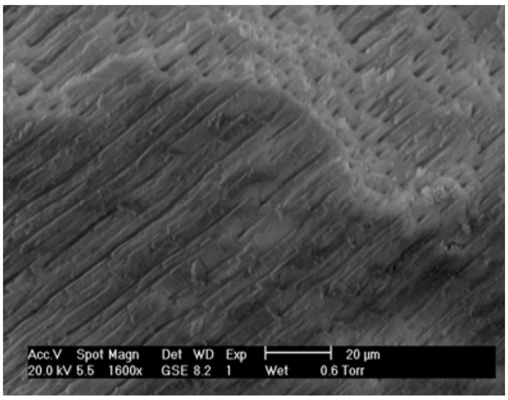


Fig.4: *The dentinal tubules at 700° appeared to be obliterated but the tubular morphology was well identified due to the hypermineralised peritubular dentin.*

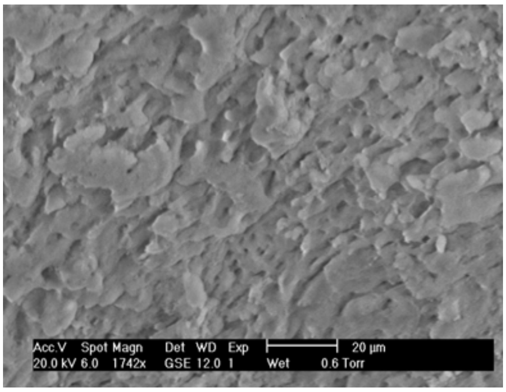


Fig.5: *SEM image of the predentinal surface exposed to 800°C showed a spicular appearance due to the melting of the inorganic salts.*

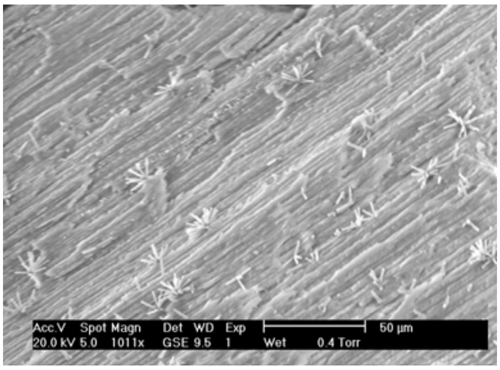


Fig.6: *SEM image of dentin after incineration at 900°C for 30 minutes. A characteristic feature observed was the presence of star shaped crystals, apparently originating from the peritubular dentin.*

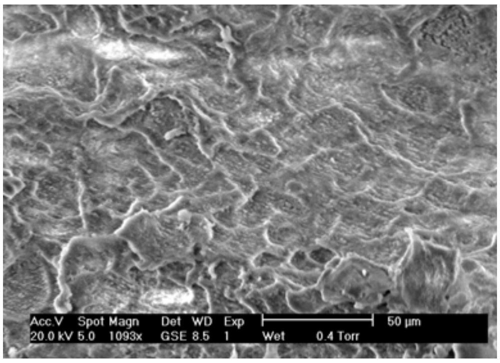


Fig.7: *SEM image of cementum after exposure to 900°C for 30 minutes. The surface had melted forming a continuous layer. This external surface was covering a granular tissue underneath it.*

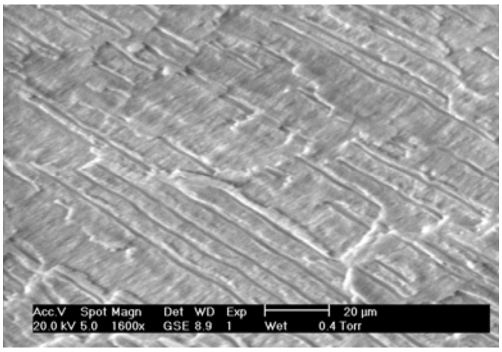


Fig.8: *SEM image of dentin incinerated at 1000°C for 30 minutes. Dentinal tubules remained identifiable but the lumen was obliterated due to the granular changes in the intertubular dentin.*

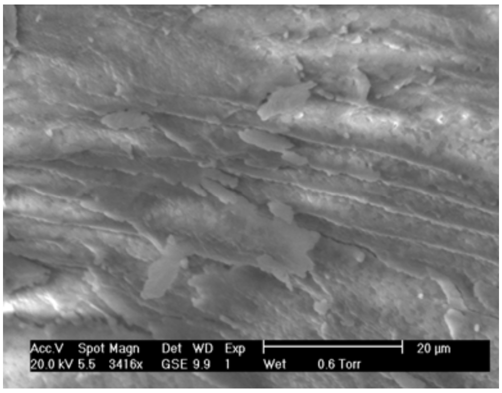


Fig.9: *SEM image of a tooth incinerated at 1100°C for 30 minutes. The arrangement of the organisational units was still preserved post-incineration.*

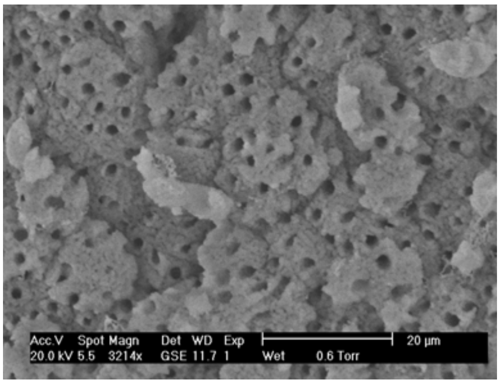


Fig.10: *SEM image of the predentinal zone of a tooth incinerated at 1100°C for 30 minutes showing the granulation of the intertubular dentin.*

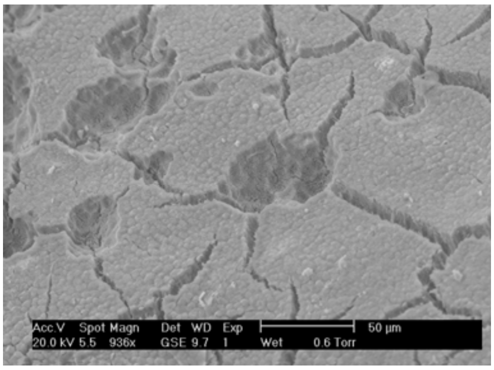


Fig.11: *SEM image of a tooth root exposed to 1100°C for 30 minutes. The cementum surface appeared to have melted into a granular tissue.*

DISCUSSION

The teeth, although resistant to most physical trauma, can become brittle and fragile when subjected to increased temperature. Disintegrated teeth are difficult to reconstruct for the purpose of post-mortem radiography and dental charting.[7] Understanding thermally induced changes in teeth can, therefore, assist forensic investigators to appropriately handle fragile dental tissues and then study the thermal record of the teeth. The purpose of the present study was to analyse thermally induced macro- and microscopic changes in deciduous teeth deemed to be important because children are more susceptible than adults to deaths in fires.

The teeth are protected from physical and thermal insults by the peri-oral musculature, lips and tongue. When exposed to fire, the soft tissues dehydrate and retract, thereby exposing the anterior teeth. As a result, the anterior teeth exhibit more thermal damage than the posterior teeth, which are protected by the tongue and buccal musculature. In a burns victim, therefore, the thermal damage observed in anterior teeth exceeds the damage in posterior teeth. The consequence of this differential damage is that estimation of temperature becomes complex due to the absence of uniformity in the structural changes in the hard tissues.[8]

It was observed in the review of current literature that there is an apparent lack of published data on effects of incineration on the deciduous teeth. This meant that a comparative analysis of the present results was limited. In this study the teeth were introduced in the oven at the experimental temperatures to simulate a thermal shock that would be induced by a fire; after 30 minutes exposure they were removed. This simulated exposure to a single thermal shock.

It was found that the colour of the teeth was the most important indicator of their relative fragility. This confirms previous research which suggests that blackened teeth are less fragile in comparison with remains that are grey or white in colour.[8] The results obtained from this study, however, also indicated that deciduous teeth disintegrated into smaller fragments (even at 100°C) when the colour change post-incineration was minimal. This has significant ramification for the forensic practitioner as due caution must be taken in handling remains which appear morphologically 'normal' (See below). Fragmentation of the teeth was also observed at 200°C and 300°C when the colour of the teeth post-incineration was shiny black. At temperatures of 600°C and above all the teeth were extremely fragile and could be fractured under digital pressure. The teeth disintegrated into larger fragments at lower experimental temperatures and into smaller and finer fragments as the experimental temperatures increased. At lower temperatures the teeth fragmented primarily due to fissures parallel to the long axis of the teeth.

On the basis of our experiments, we recommend that in an *in-vivo* forensic investigation, stabilisation of all deciduous teeth exposed to heat be performed, irrespective of colour change. This will minimise the possibility of subsequent fragmentation during examination and handling. This stabilisation could be achieved by impregnating with cyanoacrylate cement and with polyvinyl acetate (PVA). It has been suggested that clear acrylic spray paint is the preferred impregnation material that could be used to stabilise fragile dental remains post-incineration.[9] This would aid in conducting a conclusive radiographic and morphometric analysis of the teeth, and thereby retaining critical information for identification purposes.

At 100°C crazing of the enamel surface and colour changes were apparent. Fissuring of the enamel progressed from the cemento-enamel junction towards the incisal edge, which is expected because the enamel is thinnest cervically. When temperature increased, the colour of the teeth changed, and very dark brown patches were observed at the cervical margin at 200°C. The superficial enamel surface appeared to have melted as a result of the thermal shock it had been exposed to. This observation differed from those made in the permanent teeth by Harsanyi whereby colour changes were only observed up to 200°C.[10] The overall differences in colour and microstructure observed in the deciduous teeth, as compared to the permanent teeth, may be related to the fact that the deciduous enamel is more porous and has a larger area of organic/inorganic surface interface compared to the permanent teeth.[11] It is possible that the colour changes may be the result of the ultrastructural changes, such as the formation of intra- and inter-crystalline voids and the change in morphology and phase of enamel crystals. Furthermore, an increase in the positive birefringence of the enamel due to increased temperature is known to be associated with amplified opacity, thereby altering the colour of the hard tissue.[12]

The 200°C-300°C phase in the heating of deciduous teeth was characterised by bubbling on the root surface, which was more pronounced in the cervical one-third of the root. The root surface was shiny and silvery black from the remnants of organic distillation. This bubbly phase was described by Muller *et al*. at 300°C-400°C in the permanent teeth.[13] These changes were also observed at a lower temperature in the deciduous teeth, most likely due to the hypomineralisation of enamel and dentin, in comparison with the permanent teeth.[14] In the deciduous teeth incinerated at 300°C, the pulpal aspect of dentin was characterised by an amorphous deposit. A similar finding was reported by Wilson and Massey (1987) in the permanent teeth at 400°C.[15]

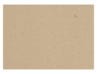
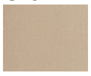
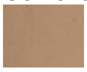
Enamel and dentin shrink with an increase in incineration temperature. As the water content of enamel is less than dentin, shrinkage is greater in the dentin and the resultant force leads to tissue separation progressing from the cervical margin; this is also likely related to enamel being thinnest cervically.[16] Also, the enamel did not separate from the dentin at the dentinoenamel junction, but during separation the enamel drew with it some portion of the coronal dentin, which was observed adhering to the under-surface of the enamel fragments. Separation of the enamel and dentin in the deciduous teeth was observed as early as at 400°C and by 500°C the enamel cap had completely undergone separation and had disintegrated into smaller fragments. This separation was observed at a lower temperature in the deciduous teeth, in comparison to the permanent teeth, where the enamel-dentin separation occurs at 450°C.[13] The reason for the early separation of enamel and dentin in primary teeth could be attributed to the higher organic content of the deciduous teeth.[16]

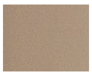
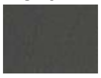
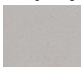
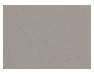
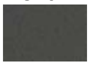
In the present study it was shown that at lower experimental temperatures the teeth fragmented into large particles, and as the temperature increased there was associated disintegration into numerous smaller fragments. The diameter of the dentinal tubules started to decrease at 500˚C; this morphological change was observed in the permanent teeth by Harsanyi and Muller *et al*, in both studies this did not occur until a temperature of at least 700°C.[10,13] The reduction in the tubular diameter of dentin gradually increased with the increase in the experimental temperatures. The tubules appeared to be obliterated due to the granulation of the inter-tubular dentin as early as at 700°C. A differential rate of obliteration of the tubules was observed at the experimental temperatures, which were more pronounced near the dentinoenamel junction. The differential rate of obliteration of the tubules could be attributed to their tapering structure. The dentinal tubules are approximately 2.5µm in diameter near the pulp and 900µm near the dentinoenamel junction.[16] Obliteration was also observed more in the dentin underneath the incisal edge and cusp tips. This is likely due to the dentinal tubules being more closely arranged in these areas. The tubular morphology was preserved because of the higher mineral content of the peritubular dentin.[16]

A unique feature observed in the dentin at 900°C was the presence of star shaped crystalline projections that appeared to arise from the peritubular dentin. The reason for this characteristic feature is currently uncertain. The cementum surface had melted into a continuous layer which covered a finely granular sub-surface and had lost its characteristic morphology. As the temperatures increased the enamel, dentin and cementum changed to a neutral white colour. At 1100°C the teeth had turned completely white with a pink tinge on the root and crown surface. The pulpal surface of the dentin at this stage was a light greenish gray colour in some areas, and pinkish in the coronal fragments, the reason for which could not be identified. A similar observation has been reported by Muller *et al*., where the root of permanent teeth appeared to be slightly pinkish at 1000°C.[13] The reason for this discolouration has not been ascertained in previous literature. The tubular morphology of dentin and the prismatic nature of enamel were identifiable at 1100°C.

The present study was a preliminary analysis of the changes induced by heat in deciduous teeth. Further extensive research is required to afford a more comprehensive insight into the effects of incineration on teeth. It was observed during the study that deciduous teeth underwent a post-incineration decrease in their weight as the experimental temperature increased due to the distillation of the organic components of the tooth. A statistical evaluation of this loss of weight would be important to correlate the temperatures and condition of the remains. It is also recommended that further research employ appropriate computer software such as Corel Photo Paint 12.0 (Corel Company, Canada) for the colorimetric analysis such as the methodology of Fereira *et al.*[17] This will reduce subjectivity and ensure the availability of an extensive range of colours.

Table 1: A summary of post-incineration changes in deciduous teeth.

Temperature	Colour	Stereomicroscopic and SEM Characteristics
100°C	Crown  Pale yellow  Very pale brown Root  Yellowish brown	Crown fracture in anterior teeth due to vertical fissures. Surface crazing. Melting of the external surface.
200°C	Crown  Pale yellow  Gray Cervical patches  Very dark brown Root  Shiny black	Surface bubbling and vesicle formation on the root surface. Silvery deposits on the root surface.
300°C	Crown  Light gray Patches  Very dark gray Root  Shiny black	Surface bubbling on the root surface. Globular knob like formations on the predentinal surface.
400°C	Enamel  Very pale brown Patches  Very dark brown Dentin  Very dark gray Cementum  Light yellowish brown	Enamel and dentin begin separation. Very deep fissures on the root surface and through the dentin.

500°C	Enamel  Light gray Patches  Dark gray Dentin  Dark bluish gray Cementum  Grayish brown  Light grayish brown	Crown-root separated. Complete separation of the enamel and dentin. Loss of enamel lustre. Reduction in the tubular diameter, especially near the dentino-enamel junction.
600°C	Enamel  Light gray Cervical patches  Very dark gray Dentin  Dark bluish gray Cementum  Bluish black	Extremely fragile remains with deep fissures in the dentin and cementum.
700°C	Enamel  Light bluish gray Dentin  Dark bluish gray Cementum Light bluish gray	Extreme reduction in the dentinal tubule diameter.
800°C	Enamel  Bluish gray Dentin  Very dark bluish gray Cementum  Very dark bluish gray	Predentinal surface had a spicular appearance.

900°C	Enamel Neutral white Dentin Light bluish gray Cementum Light bluish gray Patches Very dark bluish gray	Star-shaped fibrillar structures emerging from the intertubular dentin matrix. Cementum was unidentifiable at this temperature and had a granular appearance.
1000°C	Enamel Light bluish gray Dentin Light bluish gray Cementum-external surface Bluish black Cementum- subsurface Light bluish gray	Obliteration of the dentinal tubules was observed. Prismatic structure of the enamel and tubular morphology of the dentin remained identifiable.
1100°C	Enamel, dentin and cementum Light bluish gray Predentin Light greenish gray Pink discolouration of the crown.	Enamel and dentin remained identifiable. Granular appearance of intertubular dentin. Cementum was unidentifiable and had a granular appearance.

ACKNOWLEDGEMENTS

The authors acknowledge the facilities, scientific and technical assistance of the Australian Microscopy & Microanalysis Research Facility at the Centre for Microscopy, Characterisation & Analysis, The University of Western Australia, a facility funded by The University, State and Commonwealth Governments. We also thank the Department of Pedodontia at the Government Dental College and Hospital, Nagpur and Sharad Pawar Dental College and Hospital, Sawangi.

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Address for correspondence:
Shalmira Karkhanis
Centre for Forensic Science,
The University of Western Australia
M420, 35 Stirling Highway, Crawley,
Western Australia - 6009
Ph: *+61 6488 7286*
Email: *karkhs01@student.uwa.edu.au*