DIPLOMARBEIT

MICRO-CT ANALYSIS OF HUMAN TEETH AFTER EXPOSURE TO CONTROLLED THERMAL STRESS

angestrebter akademischer Grad

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Burned human remains have been the focus of study

by anthropologists and archaeologists for over 60 years.

One might therefore surmise that we know

a great deal about this subject: we do not.'

T.J.U. Thompson (2009)

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Summary

By now, only a few methods have been published dealing with the visualization of heat-induced cracks and fissures inside bones and teeth. During the last few decades the focus of interest shifted from the macroscopic to the microscopic level of heat-induced changes. In this diploma thesis, the method of non-invasive x-ray micro tomography (micro-CT) was used as a novel approach for volume analysis of heat-induced fissures and cracks to study the reaction of human teeth to various levels of thermal stress.

In total, 18 isolated human teeth were divided into three groups and burned under controlled temperatures of 400°C, 650°C and 800°C in an electric furnace. After the heating, a series of high-resolution scans (voxel-size 17.7 - 27.0 µm) were made with a SkyScan 1174 compact micro-CT scanner. Following reconstruction and conversion of the raw data, the obtained images were segmented. In the pilot study manual segmentation of the 15 burned molars and premolars was a simple classification of voxel data on the basis of grey scale differences, using Visage Imaging Amira 5.2. In contrast, the main study (14 scans of third molars) used automated segmentation based on a complex image analysis algorithm (Definiens XD Developer 1.2) to evaluate the volume of the cracks, the dental pulp and the dental tissue. To qualitatively analyze heat-induced changes, in both studies three-dimensional models were computed with Amira 5.2.2.

The image data of the pilot study were only used for three-dimensional models. The statistical evaluation of the main study image data shows temperature-dependent increase of heat-induced cracks and fissures between the three temperature groups. In addition, the distribution, direction and shape of the heat-induced cracks and fissures could be classified due to the computed three-dimensional models.

In contrast to earlier publications, this novel method allows to visualize the entire three-dimensional expansion of heat-induced cracks and fissures inside hard dental tissue. Therefore the pilot and main study used the same experimental conditions proposed in literature, revealing novel insight into heat-induced changes of teeth, confirming previous results and offering new perspectives for forensic investigations as well as archaeological excavation material.

Zusammenfassung

Bis zum heutigen Tag wurden nur vereinzelt Methoden mit dem Ziel publiziert, Risse und Fissuren innerhalb von Knochen und Zähnen zu visualisieren, welche durch hohe Temperaturen verursacht werden. Während der letzten Jahrzehnte änderte sich der Fokus der Forschung von den makroskopischen hin zu den mikroskopischen Hitze-induzierten Veränderungen. In der vorliegenden Arbeit wurde erstmals die nicht invasive mikro-CT Technologie verwendet, um das Hitze-induzierte Rissvolumen zu analysieren und die Veränderungen bei verschiedenen Temperaturstufen bei menschlichen Zähnen zu beobachten.

Insgesamt wurden 18 isolierte menschliche Zähne wurden in drei Gruppen eingeteilt und in einem elektrischen Muffelofen bei kontrollierten Temperaturen (400°C, 650°C und 800°C) verbrannt. Anschließend wurde eine Reihe von hochauflösenden Scans (Voxel Größe: 17.7 – 27.0 µm) mittels eines SkyScan 1174 kompakt mikro-CT Scanners gemacht. Nach der Rekonstruktion und Konvertierung der Rohdaten wurden die daraus resultierenden Bilddaten segmentiert. In der Pilotstudie basierte die manuelle Segmentierung der 15 verbrannten Molaren und Prämolaren auf der Klassifizierung der Voxeldaten aufgrund ihrer Grauwertunterschiede (unter Verwendung von Visage Imaging Amira 5.2). Im Gegensatz dazu wurde bei der Hauptstudie (14 Scans von Weisheitszähnen) eine automatisierte Segmentierung verwendet, welche auf einem komplexen Bildverarbeitungsalgorithmus basiert (Definiens XD Developer 1.2) um das Volumen der Risse, der Pulpa und des Zahngewebes zu evaluieren. Für eine qualitative Beschreibung der Hitze-induzierten Veränderungen wurden in beiden Studien dreidimensionalen Modelle mit Amira 5.2.2 erstellt.

Die Bilddaten der Pilotstudien wurden nur für die Erstellung von dreidimensionalen Modellen verwendet. Die statistische Auswertung der Bilddaten der Hauptstudie zeigte einen statistisch signifikanten Temperatur-abhängigen Anstieg von Hitze-induzierten Rissen und Fissuren zwischen den drei Temperaturgruppen. Zusätzlich konnten Klassifizierungen bezüglich der Verteilung, Richtung und Form von Hitze-induzierten Veränderungen aufgrund der dreidimensionalen Modelle vorgenommen werden.

Im Gegensatz zu bisher publizierten wissenschaftlichen Arbeiten, kann mit dieser neuartigen Methode die gesamte dreidimensionale Ausbreitung von Hitze-induzierten Rissen und Fissuren innerhalb von Zähnen präsentiert werden. Die Pilot- und Hauptstudie wurde unter den in der Literatur vorgeschlagenen experimentellen Umständen durchgeführt und offenbarten neue Einblicke in die Hitze-induzierte Veränderungen von Zähnen, welche die bisherigen Resultate stützen, aber auch neue Perspektiven bei forensischen Untersuchungen und archäologischem Ausgrabungsmaterial eröffnen.

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A. Analysis of burned human remains

Introduction

The main aims of forensic odontology are the identification and age estimation of living and dead humans. Teeth structures, like fingerprints, are unique, and can therefore clearly identify a person. Besides DNA studies, the comparative dental radiography of human dentition is the most widely used method in forensic odontology (Wood 2006). There are 160 surfaces of diagnostic value for adult human teeth - combined with evidence of dental treatments, specific tooth positions or individual root formation, this is a perfect method for cost effective identification.

Various events can lead to burned human remains, e.g. aircraft accidents (Taylor et al. 2002), explosions, natural disasters (Schuller-Gotzburg and Suchanek 2007) or house fires (Sledzik et al. 2002). In rare cases, fire is used for suicides (Schmidt et al. 2000; Shkrum and Johnston 1992) or for homicides to destroy forensic evidence and prevent clear identification and recovery (Fairgrieve 2008). In forensic cases involving fire the comparative dental radiography combined with the visual dental record is currently the most commonly used identification method.

Evidence of heat-induced changes of skeletal remains is also present in archaeological material, in the majority of the cases due to ritual cremations (Daghighi 2006; Fereira et al. 2008; Großkopf 2004; Lange et al. 1987; McKinley 1994; Ubelaker and Rife 2007; Wahl 1982). The oldest archaeological finding of cremation is dated back 26000 – 20000 years. The burned female skeleton (also known as 'Mungo Lady' or LM-1) was found in 1969 at Lake Mungo (New South Wales, Australia). The remains of LM-1 showed a noticeable burning pattern: After death the body was burned, smashed and then burned for a second time. Archaeologists interpret this as an early burning ritual to ensure, that her spirit will not return and haunt her descendants (Bowler et al. 1970; Jones 1988).

During the last decades researchers mostly concentrated on the macroscopic heat-induced changes of bones and teeth (Bachmann et al. 2004; Beach et al. 2008; Devlin and Herrmann 2008; Herrmann 1976; Moreno et al. 2009; Muller et al. 1998; Schmidt 2008b; Shipman et al. 1984; Wahl 1982; Walker et al. 2008). However, macroscopic changes can be influenced by a large number of external factors (e.g. oxygen availability, environmental conditions) and material properties and therefore are not the ideal tool for temperature estimation (Thompson 2009; Walker et al. 2008).

With reference to microscopic changes, only few methods have been published dealing with the visualization of heat-induced cracks and fissures inside bones (Thompson and Chudek 2007) and teeth (Fereira et al. 2008; Savio et al. 2006).

With the novel approach using x-ray microtomography (micro-CT) for computing a non-invasive 3-D model, even thinnest fissures inside a tooth can be visualized. A temperature-dependent increase of multiple fissures and cracks was found inside teeth, and was used to draw conclusions about the degree of high temperature exposure. The purpose of this diploma thesis, consisting of a pilot and a main study, was the quantitative and qualitative volume analysis of cracks resulting from high temperatures. This novel technique might be used like-wise for forensic investigations and archaeological purpose.

In the first section of this diploma thesis, an overview of the morphology and terminology of human dentition, dental identification process and heat-induced changes in teeth is given. Ad-ditionally, a detailed summary of the methods and materials used for the studies is presented. In the second section, the pilot study and the main study, as well as future prospects are given.

1.1. Morphology and terminology of human teeth

To understand the heat-induced changes in dental tissue the knowledge of histological and morphological properties of human teeth is crucial. Adults have 32 permanent teeth, which are in general histologically identical with the 20 deciduous teeth that are completely replaced at the age of 11±2 years (Ubelaker 2008).

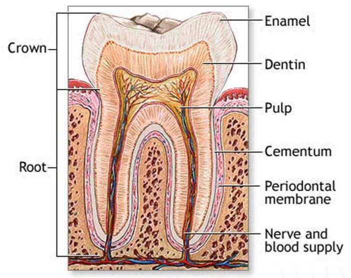

Figure 1: Structure and composition of a human tooth

A human tooth (see Figure 1) can be subdivided into a crown (Corona dentis) and a root (Radix dentis). Root canals (Canalis radicis dentis) are arising from the dental pulp cavity (Cavum dentis) for nerves and blood supply. The number of root canals is specific for each tooth type. While incisive, canines and second premolars have one root canal, first premolars and lower first and second molars have two. The upper first and second molars have three, and third molars have up to five root canals. Human teeth mainly consist of dentin (Substantia eburnae). The crown which is exposed above the jaw is covered by a thin layer of dental enamel (Substantia adamantine), which consists of 96% inorganic salts and therefore is the hardest tissue in the human body. The root is anchored inside the alveolar jaw bone by periodontal ligament/membrane. It is composed of dentin that is encased into cementum (Substantia ossea dentis). The dental pulp cavity is found inside the dentin layer and is filled with organic connective tissue. (Gratzl 2005; Wachtler 2000)

The exact knowledge of the terms labeling surfaces and orientation of teeth, shown in Figure 2, is essential to understand the structure and method of anthropological finding sheets as well as the computer based dental identification system (e.g. WINID3, http://www.winid.com). The permanent dentition can be subdivided into four quadrants with eight teeth each. The Fédération Dentaire Internationale (FDI) system uses a two digit system – the first digit indicates the quadrant and the second the tooth (Hillson 1996). Accordingly, in dentistry the fillings are named after the surfaces filled.

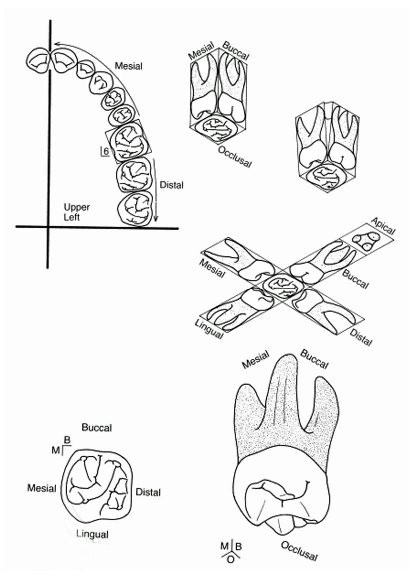

Figure 2: Labeling surfaces and orientation of teeth (Hillson 1996)

1.2. Dental identification process

An unknown body can be identified based on characteristics of the body like estimated age, sex, weight, height, hair, eye color and dentition. Fingerprints can be analyzed by specialized software, and images of the deceased can be compared. Additionally, many bodies do have individual jewelry, tattoos, implanted surgical devices or surgical/traumatic scars (Dolinak and Matshes 2005). The practice shows, that a clear identification of victims can be 100% successful like the ones at the cable-car accident in Kaprun in 2000, where all 155 victims were identified within 19 days (Meyer 2003). In other mass fatalities in recent history like the Tsunami in Southern Asia in 2004, the positive identification rate of foreign victims was 90,36%, whereas due to missing medical records only 74,42% of the known Thai victims could be identified during the first 15 months after the catastrophe (Schuller-Gotzburg and Suchanek 2007). But the identification rate of victims is not always that high. Haglund (2002) gives the impressive example of a mass grave from the 1994 Rwandan Genocide. Out of 500 individuals only 17 could be identified correctly with the help of their identity cards or clothes. The forensic investigation teams at other mass graves around the world are confronted with similar problems.
Especially when soft tissue is lost by environmental, human, chemical or physical force, forensic odontology, forensic radiology and forensic anthropology, besides DNA comparison can help to clearly identify individuals (Cox et al. 2008) .

The main aim of forensic odontology is the determination of identity on the basis of photo- and radiographic analysis of dentition, jaws, and craniofacial bones (Bernstein 1997; Bernstein 1998; Schmidt 2008a). Bite mark analysis (Sweet 1998) as well as application of dental evidence for jurisprudence are also part of the scope.

There are 160 surfaces of diagnostic value for adult human teeth - combined with evidence of dental treatments, specific tooth positions or individual root formation, these characteristics allow personal identification. Especially dental restorations and ante mortem dental records, e.g. dental radiographs or dental impressions, are very helpful (see Figure 3 and 4). Other evidence like dental prosthesis, dental brace, and diagnostic models can secure positive identification. Sometimes the patient's name or initials are printed onto the denture or crowns. In the last years individual bleaching trays and customized fashion accessory became more and more important (Bowers 2004).

The visual dental record combined with computer based dental identification systems still is the gold standard in the field of forensic odontology. In cases of mass fatalities, dental identification is usually faster and less expensive than DNA analysis (Savio et al. 2006; Souviron 2005; Yoshida et al. 2005).

An individual identification is possible via comparison of ante mortem and post mortem dental X-rays (comparative dental radiography) (Bernstein 1998; Raitz et al. 2005; Souviron 2005). The first forensic case, in which comparative dental radiography was applied as an identification method, was in 1949, when 72 of 119 victims of a fire on a steamship could be identified. Since that time, comparative dental radiography has been routinely used for the individual identification of unknown decedents. However, there are also limitations of this method. A dental X-ray or orthopantomography (OPG) only shows a two-dimensional shadow of a three-dimensional filling, whereas the site of the restoration cannot be distinguished. Also the various metals and fillings cannot be differentiated, because all are radiopaque. (Bernstein 1998)

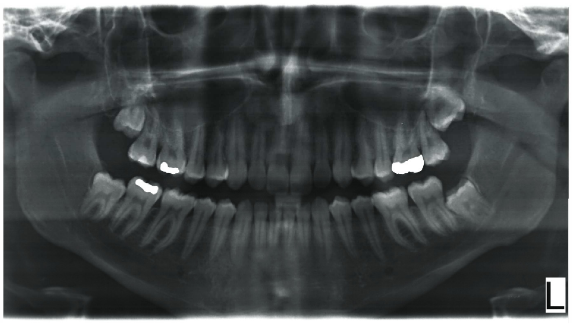

Figure 3: Standard orthopantomography (23 years, male)

Figure 4: Dental impression of lower dentition

In Figure 3 a standard OPG of human dentition (male, 23 years, caucasian) is shown (73kV, 15mA; Orthopos XG Plus, Sirona Dental Systems, Bensheim, Germany). This kind of dental x-ray allows a two-dimensional overview of mandible, maxilla and the complete dentition.

Clearly visible are the different kinds of dental treatments, fillings and the state of development of the third molars, which would allow a positive identification in a forensic case. Additionally, during the dental treatment of the upper left first molar (26) a dental impression of the same patient's lower dentition was made (see Figure 4), which could be used in the forensic bite-mark analysis. The pictures were kindly provided by Dr. Andreas Werner and the Primary Care Clinic of the Bernhard-Gottlieb University Clinic of Dentistry, both located in Vienna, Austria.

Kirchhoff et al. (2008) compared the effectiveness of visual dental record and post mortem computed tomography (PMCT) for identification process. With a standard 64-slice computed tomography the dental fillings/inlays, especially synthetic were only hardly detectable. Though, if the ante mortem x-rays are missing (Taylor et al. 2002), or to provide a fast documentation of the entire dentition (Jackowski et al. 2006) the method of computed tomography can be very helpful.

If ante mortem dental records are missing or difficult to locate photographs of missing persons smiling on them can also help in the identification process. These photographs can easily be obtained from e.g. social networks, friends or family. At the Medical Examiner Department of Miami-Dade County approximately 30 percent of positive visual dental identifications are preformed with smiling photographs. The comparison obviously cannot be performed in cases of missing, putrefactive or severe burned anterior teeth (Souviron 2005).

However, in spite of all the technical advances, it is very important to remember, that not every forensic dental examination is leads to a positive identification.

1.3. Historical background of investigation of burned human remains

Exact knowledge of human osteology and a lot of experience is of fundamental importance for the anthropological and forensic investigation of burned human remains. One of the first experimental approaches on the alteration of the skull subjected to heat was done in 1875 by Eduard v. Hofmann, an Austrian professor in legal medicine (Hofmann 1875). Throughout many decades it was believed that burned human bones and teeth from forensic and archaeological sites would not provide enough evidence for a trial or scientific study and had been reburied in many cases. In 1928, Wrzosek considered burned bone fragments as adequate research material and tried to find out factors for identification of age, height and number of graves. During the 1930's, Krumbein and Thieme were the first anthropologists, who did extensive research on burned human remains (Trautmann 2006; Wahl 1982). Waller (1934, cited by Wahl 1982) developed guidelines for the treatment of burned human remains. Another important study was performed in 1939 by the Austrian anthropologist Ämilian Kloiber, who published his research on metrical data of various bone fragments for cremated remains (Kloiber 1939). Wahl (1982) published a color and burn stage scheme, which is still widely used in a slightly modified version for the analysis of burned human remains. Detailed reviews on the anthropological and forensic methods used for the investigation of burned human remains (including age and sex estimation, shrinkage and temperature determination and histological methods) were published by Mayne-Correira (1997), Großkopf (2004), Daghighi (2006), Trautmann (2006) and Schmidt (2008a).

An important basic question in this context is the origin of the remains – if they are non-human or human. Therefore, burned bone fragments are a big challenge, even though there are possibilities to determine the different types of human and other species' bones (e.g. cranial bones from post-cranial bones) (Hincak et al. 2007; Whyte 2003). Confusion of human and non-human teeth is nearly impossible if they are complete. The specific thickness of 1-2mm of human dental enamel distinguishes non-human from human teeth (Grine 2005). The third molar of the polar bear seems to be the only tooth of a mammal to be similar to the structure of a human tooth (Schmidt 2008a). The arising problem to determine crown or root fragments can be solved by examining the tooth on the microscopic level. In cross sections human enamel has a keyhole pattern. Any animal with teeth of similar size does not have this pattern (Hillson 1996).

1.4. Thermal properties of teeth and heat-induced changes in teeth

1.4.1. Heat-induced transformation

Table 1: Terminology for cremated remains after Eckert et al. 1988, cited by Mayne-Correira (1997)	
Type of Cremation	**Tissue Survival**
Charred	Interal organs
Partial	Soft Tissue
Incomplete	Bone pieces
Complete	Ashes only

Cremation of human remains means the practice of disposing of a corpse by burning it. Shipman et al. (1984) distinguished a wide spectrum ranging from unburned, completely incinerated to calcined bone material. In Table 1 a general classification of types of cremation introduced by Eckert et al. in 1988 is shown (Mayne-Correira 1997). The German phrase 'Leichenbrand' is used in archaeological context to refer to the rite of cremation but mostly to refer to fragments of the human skeleton that could not be burned entirely. Normally, there are only mineralized fragments of bones/teeth remaining.

Mayne-Correira (1997) also categorized four transformation stages in the process of cremation (shown in Table 2) that were modified by Thompson (2004). At the first stage, the so called dehydration, the water inside the bone and dental tissue is lost due to the breaking of hydroxyl bonds. Organic components and carbonates are lost at the second and third stage, respectively (Devlin and Herrmann 2008).

Table 2: The four stages of heat-induced transformation in bone (Thompson 2004)

Stage of transformation	Evidence	Temperature range (Mayne-Correira 1997)	Temperature range (Thompson 2004)
Dehydration	Fracture patterns; weight loss	100°-600°C	100°-600°C
Decomposition	Color change; weight loss; reduction in mechanical strength; changes in porosity	500°C-600°C	300°-800°C
Inversion	Increase in crystal size	700°-1100°C	500°-1100°C
Fusion	Increase in mechanical strength; reduction in dimensions; increase in crystal size; changes in porosity	1000 °C +	700°C +

In 23.4% of the forensic cases involving extreme heat the soft tissue of the skull is affected, whereas in almost every case the soft tissue of the lower extremities is destroyed by the heat (Gerling et al. 2000).

If a body is subjected to extreme heat, the teeth are not affected at first. Teeth are primarily protected by the soft tissue for about 10-24 minutes and the temperature inside the oral cavity does not rise over 87°C, depending on the nutritional state, age, temperature and time of exposure (Eichenhofer 1980, cited by Madea and Schmidt 2000).

The protection against direct heat of the anterior teeth is caused by the swelling of the lips and protrusion of the tongue. If even more heat is induced, the lips and the tongue retract and the heat affects the anterior teeth (Madea and Schmidt 2000). Bohnert et al. (1998) listed the effects of fire on the skull more specifically, based on observations of cremations carried out at 670°-810°C. After 8-10 minutes the soft tissues of the face is charred and only sparse soft tissue remains are visible after 20 minutes.

Figure 5: Fragments of a lower jaw burned for 30 minutes at 400°C. The anterior teeth are partially destroyed and carbonized, while molars only show some fissures. The dentin is colored black, changing to white color. (Rötzscher et al. 2004)

In case of burned human teeth resulting from homicide, accidents, or aircraft crashes, etc., the remains are very fragile and have to be handled with great care. To preserve them from shattering and thus to secure a positive identification they can be fixed with cyanoacrylate or hair spray (Griffiths and Bellamy 1993; Schmidt 2008a). Myers (1999) and Feriera (2008) concluded that teeth subjected to gradual heat are more brittle, while direct heat often leads to complete fragmentation of the teeth.

Brown et al. (1970) and Lloyd et al. (1978) were the first physicists who did profound experiments about thermal properties of human teeth. They used a thermal cycling apparatus to simulate thermal stress and to find out the density and specific heat of dentin and enamel. Additionally, Brown et al. (1970) published a differential equation for calculating the thermal conductivity of dentin and enamel. Braden (1985) did experiments on the different properties of restorative materials, so that mathematical models on conductivity can be computed. These experiments are very important for implantology and filling material research (Gale and Darvell 1999; Kodonas et al. 2009), as well as for the explanations of the behavior of human teeth exposed to extreme thermal stress (Hughes and White 2009; Muller et al. 1998).

1.4.2. Analysis of macroscopic and microscopic heat-induced changes of teeth

The focus of interest in the analysis of heat-induced changes shifted from the macroscopic to the microscopic level during the last few decades (Thompson 2009).

The surface color of bone and teeth is one of the main macroscopic features analyzing of heat-induced changes. Concerning bone, the change of surface color is mainly related to the temperature rise and time of exposure. A shift from natural color to light yellow, brown, grayish-blue, white and finally pink is often reported in literature (Devlin and Herrmann 2008; Merlati et al. 2004; Moreno et al. 2009; Muller et al. 1998; Schmidt 2008b). Shipman et al. (1984) introduced the 'Munsell Soil color charts' to standardize the description of surface color of burned human remains following experiments with bones and teeth from sheep and goats. Various experiments showed that environmental conditions, time, temperature and oxygen availability are also key factors that influence the color of cremated bones (Walker et al. 2008).

Specific research on heat-induced changes of teeth color is rare (Bachmann et al. 2004; Beach et al. 2008; Moreno et al. 2008; Moreno et al. 2009; Muller et al. 1998; Myers et al. 1999; Schmidt et al. 2005). In general, teeth react in a quite similar manner as bones do (see Figure I (p.32) for molar teeth; Figure B (p.25) for premolar teeth), although enamel has a much lower organic content than bone (Beach et al. 2008). However, surface color changes can be influenced by a large number of external factors (e.g. oxygen availability, environmental conditions) as well as material properties, and is therefore not the ideal tool for temperature estimation (Thompson 2009).

The behavior of human teeth subjected to high temperatures (200° - 1200°C) was recently studied by Moreno et al. (2008; 2009) using 200 extracted human teeth. The study proved that dental tissues as well as dental filling materials (e.g. amalgam, composite, etc.) resist extreme thermal stress. Small changes in dimensional expansion, fissures, cracks, fractures and color changes of the tissue and dental filling materials were present. Studies by Rossouw et al. (1999), Merlati et al.(2002; 2004), Bush et al. (2006), Savio et al. (2006), Brandao et al. (2007) and Bonavilla et al. (2008) showed that the preservation of modern dental filling materials during heat may also help for the identification process. Microscopic changes of burned human and animal bones and teeth were often examined using classical histology (Fereira et al. 2008; Hughes and White 2009; Muller et al. 1998; Myers et al. 1999).

The pioneer study concerning heat-induced changes of ultrastructure of bone and teeth using scanning electron microscope (SEM) was published by Shipman et al. (Shipman et al. 1984). This destructive and intricate method has been used throughout many decades, and is useful for the estimation of burning temperature and characterization of dental filling materials, but entire teeth or bone fragments cannot be analyzed (Carr et al. 1986; Fairgrieve 1994; Holden et al. 1995b; Merlati et al. 2004; Muller et al. 1998; Quatrehomme et al. 1998; Shipman et al. 1984; Thompson 2005; Wilson and Massey 1987).

Two other methods, X-ray Diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR), concentrate on the Crystallinity Index (Enzo et al. 2007; Holden et al. 1995a; Piga et al. 2008; Piga et al. 2009; Rogers and Daniels 2002; Shipman et al. 1984), which “is a measure of the order of the crystal structure and composition with bone” (Thompson et al. 2009) are reported. Changes of this Crystallinity Index are the result of thermal stress. XRD is based on “the conclusion that the hydroxyapatite (…) is altered in structure between 700 to 1000°C to resemble β tricalcium phosphate” (Mayne-Correira 1997). Shipman et al. (1984) compared this transaction of crystals with the production of ceramics.

Hiller et al. (2003) used small-angle X-ray scattering (SAXS) to determine the actual crystallite size and shape, providing a more accurate determination method and very promising results for the prediction of burning temperature. Disadvantages of these methods are that they also are destructive and intricate.

However, the destruction of the material is not acceptable in archaeological excavation material or evidence from forensic cases. Therefore, non-invasive and non-destructive methods had to be found in order to be able to analyze heat-induced changes of bones and teeth.

1.5. Visualization of heat-induced changes inside teeth

Fereira et al. (2008) used photomicroscopy (100x magnification) on histological sections to visualize the variations of cracks and fissures in young and old teeth. In total 30 human teeth were either heated directly with a gas burner or heated constantly with a muffle furnace (increase of 18.8°C/minute) up to 1150°C.For further analysis they were embedded in Polymethylmetacrilate. The teeth which were heated constantly, showed less structural damage than those submitted to direct heat.

Savio et al. (2006) evaluated the effects of high temperatures on human teeth using periapical radiographs. The study used 90 human teeth divided into two groups (unrestored vs. endodontically treated teeth) and exposed them to six different temperatures (200°C, 400°C, 600°C, 800°C, 1000°C, 1100°C) using an electric furnace with a constant increase of 30°C/minute. At lower temperatures (200°C) no or only small fissures (400°C) were present in the crown. Fissures within the crown and root were observed at a temperature of 600°C and 800°C. At a temperature of 1000°C and 1100°C the crown was reduced into fragments, while the root showed large fractures through the dentin, and all significant radiographic details (e.g. fillings) were conserved.

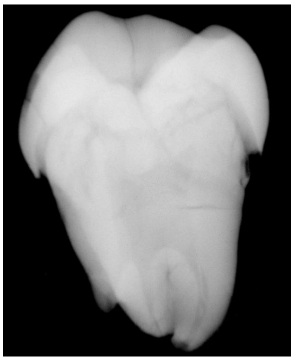

Figure 6: Radiograph (tooth 6 main study, 800°C)

Another important study was performed by Thompson and Chudek (2007) using Magnetic Resonance Imaging (MRI) to visualize heat-induced changes in bone. The authors used 1.5cm thick sections of sheep bone and exposed them two times for 30 minutes at 700°C using an electric furnace. This was the first time three dimensional imaging methods were used for the imaging of burned bone.

Combining the basic ideas and methods of Fereira et al. (2008), Savio et al. (2006) and Thompson and Chudek (2007) the next logical step was using micro-CT for the visualization of heat-induced changes inside teeth and bones. With micro-CT images a non-invasive 3-D model of entire teeth or bone fragments can be computed and the microscopic structure can be statistically analyzed. While the MRI technique uses a voxel size of 230µm (Thompson and Chudek 2007), standard micro-CT provides a voxel size as small as 6 µm, allowing to visualize smallest fissures within the teeth or bones, normally only visible in microscopic sections.

1.6. Hypothesis

The main aim of this diploma thesis is to introduce a novel approach in the imaging of burned human remains. By now, there are no scientific publications about the usage of the technology of micro-CT in the forensic or archaeological context for burned human teeth or bones. Micro-CT provides a non-destructive, three-dimensional reconstruction of very high resolution.

The pilot study, which primarily served for the testing of methods, as well as the main study, tried to verify the following hypothesis:

> *Thermal stress leads to a temperature-specific volume of cracks in human teeth, which can help to estimate the temperature at the time of heat exposure.*

The three main aims of these studies can be summarized with the following questions:

1. How do isolated healthy young human third molars react to thermal stress induced by high temperatures?
2. What is the visible, measurable and statistically relevant data that can be elicited from burned human third molars?
3. Is the 3-D micro-CT comparison of burned human teeth an appropriate alternative method for determining temperatures of burned human remains (e.g. from forensic investigations or archaeological excavations)?

1.7. Materials and Methods

1.7.1. Background

Human third molars

Compared to frist and second molars, third molars show large variation in anatomy compared to other permanent molars. There often are just two of them present or they are congenitally completely missing (Schumacher and Schmidt, 1990). In general, third molars can be distinguished into right and left maxillary/mandibular third molars. Although there is a large variation these tooth type was chosen in this studies for four reasons: 1) Because of their position in the jaw they most likely tend to survive severe heat, 2) they rarely show damages/fillings, 3) they are the teeth that are most commonly extracted, and 4) they are extracted in a certain age group

Heat treatment

Experimental approaches with a pyre of dry hardwood (Whyte 2003) as well as a direct fire source (e.g. gas burner) were used to simulate extreme thermal stress (Fereira et al. 2008), but do not produce satisfying and repeatable results. Therefore, the usage of an electric furnace to simulate the thermal stress on human bones and teeth is widely used in experimental forensic science (Bush et al. 2006; Fereira et al. 2008; Hiller et al. 2003; Holden et al. 1995b; Merlati et al. 2002; Merlati et al. 2004; Moreno et al. 2008; Moreno et al. 2009; Myers et al. 1999; Rossouw et al. 1999; Savio et al. 2006; Shipman et al. 1984; Thompson 2005; Thompson and Chudek 2007; Thompson et al. 2009).

However, there is a high variability in time of exposure, final temperature and temperature increase (ranging from 10°C to 30°C increase per minute) in previously published studies.

Gerling et al. (2000) stated that the majority (104 out of 106 cases) of forensic cases involving fire do happen in closed rooms. The model of evolution of fire temperature inside rooms (EN1991-1-2:2002) shows that the temperature rises over 400°C in about five minutes (80°C increase per minute) during the flashover period (Karlsson and Qunitiere 2000). This extreme increase is unfortunately impossible to accomplish with an electric furnace (see Figure 9 (p.17) for a standard time/temperature curve of a muffle furnace). Shipman et al. (1984) stated that 400°C are reached by normal campfire (rarely reaching 700°C), an apartment fire reaches about 650°C (American Society of Forensic Odontology 2007) and modern crematoria 800°C to 1000°C (Madea and Schmidt 2000). Therefore, three temperature groups (400°C, 650°C and 800°C) have been chosen for the studies to simulate typical situations of cremation.

Micro-CT

The micro-CT is basically a miniaturized version of cone-beam CTs, which are normally used in medical diagnostics. In the last few years the micro-CT scanning technology, which is based on x-ray transmission images, became more commonly used in the field of biology and medicine. Micro-CT provides a non-destructive, three-dimensional reconstruction of small objects in a very high resolution. The specimens are placed on a rotating disk, which is controlled by a step motor inside the lead-shielded chamber (Buzug 2008; De Witte et al. 2008). This study used a SkyScan 1174 (SkyScan, Kontich, Belgium) micro-CT scanner, which is currently the most compact micro-CT scanner worldwide. It has a cooled 1.3 megapixel x-ray camera, a tungsten x-ray source and a spatial resolution as small as 6µm, and allows an object size of 5-30 mm in diameter and 50mm in length, which is ideal for teeth and bone fragments.

Figure 7: Micro-CT SkyScan 1174

1.7.2. Human third molars

The 23 teeth (including molars and premolars from various age groups) used in the pilot study (see chapter 2.1) were donations and partially derived from cadaver extractions at the Department for Systematic Anatomy, Center for Anatomy and Cell Biology, Medical University of Vienna, Austria. The 37 third molars used in the main study were donations derived from endodontic dental treatments at several Austrian medical offices and departments (Dr. Michael Griss, Rankweil; Department of Oral Surgery, Landeskrankenhaus Feldkirch; Department of Oral Surgery, Sozialmedizinisches Zentrum Ost – Donauspital, Vienna; Dr. Mathias Kränzl, Vienna; Dr. Klaus Wurzinger, Höchst). A declaration of consent was filled out by the donors involved in the study. In total 18 third molars (9 female: 9 male, average: 23.06 ± 2.15 years) are included in the main study, 19 teeth were excluded from the study due to unknown age, damages (e.g. broken roots), endodontic treatments, dental restorations or caries.

In the run-up to the main study all third molars were photographed for documentation in buccal, lingual, apical and occlusal position (see Figure 8). The pictures were taken with a Panasonic™ Lumix TZ7 compact digital camera (1/125s, f 11, ISO 80, automatic white balance) fixed at a reproduction stand.

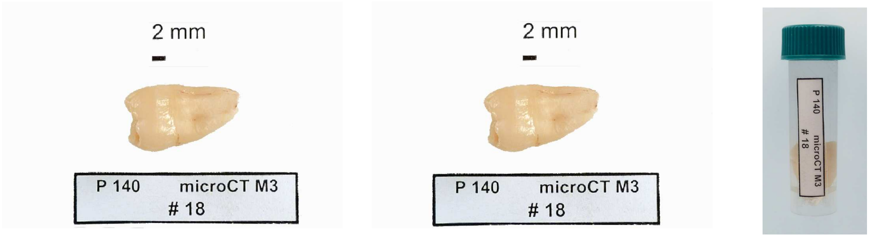

Figure 8: Photo-documentation of third molar in buccal (a) and lingual (b) view, storage tube (c)

Each extracted tooth was cleaned, disinfected and afterwards stored in a conical 5ml centrifuge tube with a stand (VWR SuperClear™). Rehydration and removal of formaldehyde (when derived from cadaver extractions) of the teeth was achieved by prepared phosphate buffered saline (PBS) storage for 30minutes at room temperature using the following receipt (De Angelis 2007):

- *8 g of NaCl*
- *0.2 g of KCl*
- *1.44 g of* Na_2HPO_4
- *0.24 g of* KH_2PO_4
- *Dissolving in 800 ml of distilled* H_2O
- *Adjust the pH to 7.4 using HCl or NaOH*
- *Add distilled* H_2O *to 1 liter. Store at room temperature*

At the next step the teeth were stored in commercially purchasable artificial saliva (Sigma Sialin, Sigmapharm Vienna), which consists of:

- *500mg of Carboxymethyl cellulose Natrium*
- *85 mg of NaCl*
- *120 mg of KCl*
- *35 mg of* KH_2PO_4
- *22 mg of CaCl x6* H_2O
- *3 mg of* $MgCl_2$
- *3000 mg of* $C_6H_{14}O_6$
- *100 mg of* $C_8H_8O_3$

The artificial saliva, which is normally used in cases of Xerostomia, should simulate the existing environment in the oral cavity and save the teeth from drying out. Until the teeth were burned they had been stored in this fluid (exchanged every two weeks) at 4°C in a commercial refrigerator.

1.7.3. Electric Furnace and Temperature Adjustment

The heat treatment of the teeth was performed with an electric muffle furnace (Medlin-Naber N3R, Vienna, Austria) at the Department of Chemical Ecology and Ecosystem Science, University of Vienna, Austria. In a furnace test heating on the 11.5.2010, starting at room temperature (20°C), 400°C was reached within 13,25 minutes; 650°C in 23,25 minutes and 800°C in 31,5 minutes. In Figure 9 a time/temperature curve of the used electric furnace is shown. The heating protocol can be seen as a linear curve with 25°C mean increase per minute.

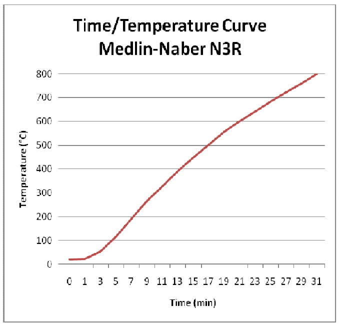

Figure 9: Time/temperature curve of muffle furnace Medlin-Naber N3R

After the thermal stress (especially at 650°C and 800°C) the teeth were very fragile and brittle. After the second micro-CT scan they were fixed as suggested in literature with commercial hair spray for storage (Griffiths and Bellamy 1993; Souviron 2005).

1.7.4. Micro-CT

Depending on the size of the tooth the pixel-size and exposure time was chosen (see Appendix). The general adjustment for the scans was 17,7 µm and 3500-4500 ms (800µA, 50 kV), 180° rotation (with 3 pictures per degree), six averaging steps and random movement resulting in a mean scanning time of 5-6 hours per tooth. The resulting slices were reconstructed using NSRECON (SkyScan, Kontich, Belgium).

The reconstruction software helped to reduce typical 2-D scanning artifacts, e.g. ring artifacts, misalignment and beam-hardening. While the original scanning files (1024x1024 pixels) were stored in 16-bit TIFF format, the reconstructed files were saved as 8-bit JPGs for easier handling during the 3-D reconstruction.

1.7.5. 3-D reconstruction

Manual classification

Following the reconstruction and conversion of the raw data (Adobe® Photoshop Creative Suite 4) the scanning files of the pilot study were manually segmented and 3-D reconstructions were made. The manual segmentation of the burned teeth (see Figure 10) was a classification of voxel data into objects representing the spatial location of dental pulp (blue), cracks (green) and dental tissue (red) on the basis of differences in grey scale values and shape.

The segmentation and 3-D modeling was done with Visage Imaging Amira 5.2 (Visage Imaging Inc., San Diego, USA) at the Department of Oral Surgery, Medical University of Vienna, Austria.

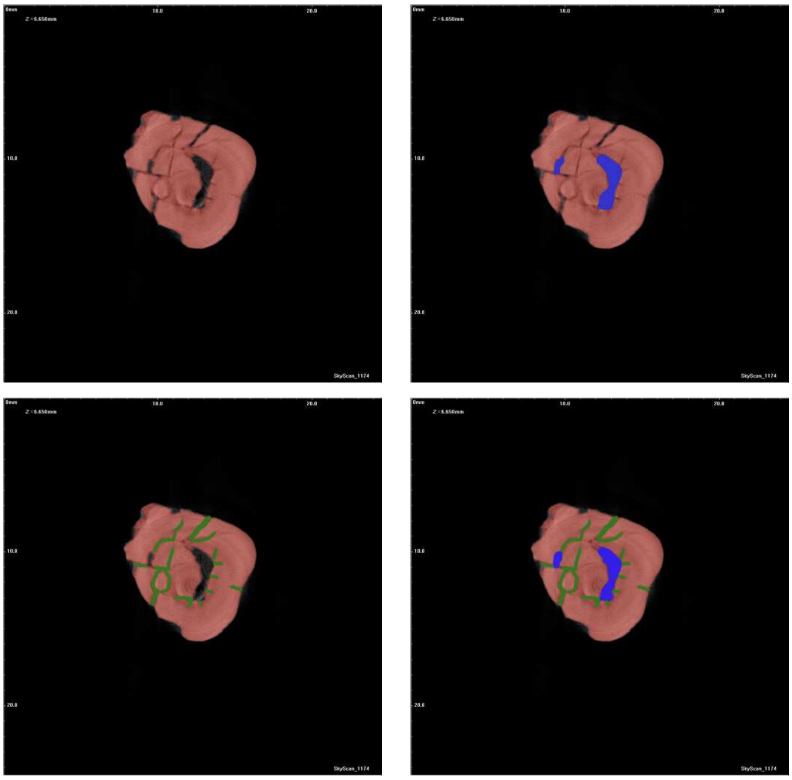

Figure 10: Manual segmentation using Visage Imaging Amira 5.2.2

Automated classification

In contrast to the pilot study, following the reconstruction and conversion of the raw data (Adobe® Photoshop Creative Suite 4) the scanning files of the main study were automatically segmented using Definiens XD Developer 1.2 software package (Definiens AG, Munich, Germany) at the Department of Oral Surgery, Medical University of Vienna, Austria.

Definiens XD Developer 1.2 (Definiens AG, Munich, Germany) was originally developed for the analysis of satellite images. Therefore, special image pattern recognition algorithms are needed. The cracks and fissures inside the hard dental tissue also show a specific pattern and therefore can be analyzed using a specially developed algorithm. A flowchart of this algorithm used for the automated segmentation is shown in Figure 11A. After down sampling the micro-CT images, they are subdivided into smaller squares if the color variability in its pixels is greater than a set threshold value (Quadtree Segmentation, see Figure 11B). Following this step, which helps to distinguish between the uniform black background and the tooth, an initial classification (cracks, dental dental pulp, dental tissue, and background) is made (see Figure 11C). At the next step, the cracks and the background have to be exactly distinguished from dental tissue (see Figure 11D). Following this, small cracks (fissures) can be classified and added to the class of cracks, shown in Figure 11E, if certain requirements are fulfilled (e.g. continuity, grey scale value, shape). The area between the roots, which might be erroneously classified as cracks, is found at the next step and added to the background class. At the last classification step, the dental dental pulp is found based on the shape, grey scale value and continuity within the image stack. After all, unwanted clutter is removed and the classification synchronized to the original image data (see Figure 11F). The classification values (number of pixel belonging to a certain class) are exported to a CSV file for the statistical analysis with SPSS 16 (SPSS Inc., Chicago, USA).

This method is much more time effective compared to the manual segmentation, although sometimes false positive results in the classification that had to be removed manually before exporting the data. Afterwards, 3-D models were computed using Visage Imagin Amira 5.2.2 (Visage Imaging Inc., San Diego, USA) at the Department of Oral Surgery, Medical University of Vienna, Austria.

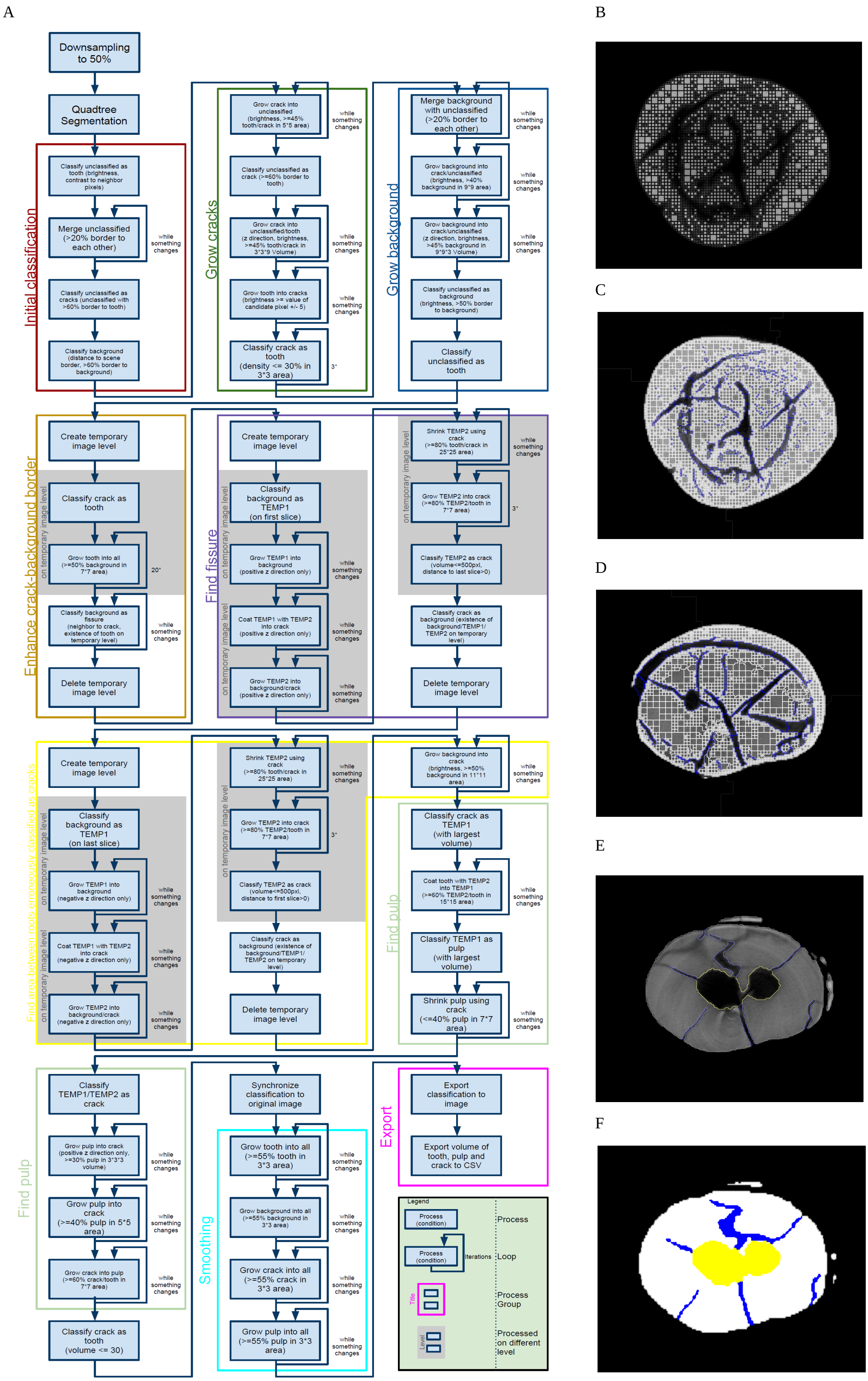

Figure 11: Flowchart and main steps of automated segmentation using Definiens XD Developer 1.2

B. Micro-CT analysis of heat-induced changes in human molars

2.1. Pilot Study

The pilot study was primarily performed to test the reliability of the novel approach of micro-CT in the analysis of burned human teeth. One of the main aims was to find out if any qualitative relevant data could be elicited from the image data set. Therefore a lot of experience had to be gained concerning temperature adjustments, storage of specimens, micro-CT scanner setup, and thermal properties of human teeth. These first tests should produce the basis of the main study. The experience gained during this pilot study should furthermore help to produce not only qualitative results but also quantitative results for statistical analysis.
My collaboration partner of the pilot study was Ms. Katharina Baron. She is also co-author of the poster 'Micro-CT analysis of adult human molars and premolars after exposure to controlled high temperatures' that presented first results of the pilot study at the 'Vth International Anthropological Congress of Ales Hrdlicka' (2. – 5. September 2009, Prague, Czech Republic). Additionally the final results of the pilot study were presented at the '4th Meeting of Junior Scientists in Anthropology' (25. – 28. March 2010, Freiburg im Breisgau, Germany). The following chapter is printed in excerpts in the Conference Proceedings (Sandholzer 2010).

2.1.1. Introduction

The main aims of forensic odontology are the identification and age estimation of living and dead humans. Teeth structures, like fingerprints, are unique and can therefore clearly identify a person. Postmortem radiographic analysis is one of the most widely used identification methods in forensic odontology. Human teeth subjected to high temperatures are able to resist fire exposure up to 1400°C due to protection given by soft tissue of the head. There are 160 surfaces of adult human teeth - combined with evidence of dental treatments, specific tooth positions or individual root formation, this means a perfect method for cost effective identification. The visual dental record combined with radiographic analysis and computer based dental identification systems are still the golden standard in the field of forensic odontology (Savio et al. 2006; Souviron 2005).

Various events can lead to burned human remains, e.g. aircraft accidents, explosions, earthquakes or house fires (Sledzik et al. 2002). In rare cases fire is used for suicides (Shkrum and Johnston 1992) or in homicides to destroy forensic evidence and prevent clear identification and recovery (Fairgrieve 2008). In archaeological material evidence of thermal stress is also present (Baier 2009; Daghighi 2006; Großkopf 2004; McKinley 1994; Wahl 1982). Exact knowledge of human dentition and extensive experience are of particular importance for the investigation of burned human remains. For many decades burned human remains from archaeological sites were reburied, because it was believed that no information is provided from burned bones or teeth. In 1928 Wrzosek considered burned bone fragments as adequate research material and tried to find indications for identification of age, height and number of graves. Since the early days of the analysis of burned human remains many scientific articles and reviews have been published (Ubelaker 2009), but mainly isolated and pilot studies as well as case reports.

Besides the classical methods for the temperature estimation of burned human remains (reviewed by Großkopf 2004), various scientifically complex approaches using trace element analysis, x-ray diffraction (including small-angle version), infrared spectrometry, mercury-intrusion porositometry, electron microscopy and many other techniques were developed or improved during the last few decades (Thompson 2004).

The behavior of human teeth subjected to high temperatures (200°C, 400°C, 600°C, 800°C, 1000°C, 1200°C) was recently macroscopically studied by Moreno et al. (2008) using 199 extracted human teeth. The study proved that dental tissues as well as dental materials (e.g. amalgam fillings, composite, etc.) resist extreme thermal stress. Small changes in dimensional expansion, fissures, cracks, fractures and color changes of the tissue and dental materials were reported. Another approach by Walker et al. (2008) was to estimate the burning temperature of human remains based on color changes of the material. The authors indicate that the most important factors for color changes are oxygen availability, the duration of heat exposure, and the actual fire temperature. Schmidt (2008b) and Beach et al. (2008) found out that the color changes in teeth are similar to those documented in bone, but the technique is quite uncertain due to different external factors (e.g. metal surrounding the dead body) and lighting conditions during data acquisition. The studies by Piga et al. (2009) using x-ray diffraction and Thompson et al. (2009) using Fourier Transform Infrared Spectroscopy showed very promising results for the prediction of burning temperature. Disadvantages of these methods are that they are mainly destructive and intricate.

Note: The part about visualization of heat-induced changes in bones and teeth, which is included in the Conference Proceedings, is already mentioned in chapter 1.5.and therefore left out in this section.

However, the destruction of the material is not acceptable in archaeological excavation material or evidence from forensic cases. Therefore, non-invasive and non-destructive methods had to be found in order to be able to analyze heat-induced changes of bones and teeth. In this micro-CT pilot study the appearance of cracks and fissures inside human teeth tried to be used to draw conclusions on the high temperature exposure. The purpose of this pilot study was the testing of methods and the basic qualitative analysis of cracks and fissures resulting from high temperatures.

2.1.2. Materials and Methods

In total, 23 molars and premolars of various age groups from dental treatments and human cadaver extractions were collected and categorized according to Hillson (1996). Of the 23 adult human teeth used for this pilot study, due to different scanner and furnace adjustments that had to be made, only 15 were later included in the data analysis. After treatment with phosphate buffered saline (PBS) and rehydration, the specimens were stored in synthetic saliva (Sialin-Sigma Solution, Sigmapharm Vienna) at 4°C. First, high resolution micro-CT scans of teeth were made with a SkyScan 1174 compact micro-CT scanner (SkyScan, Kontich, Belgium) at the Department of Theoretical Biology, University of Vienna, Austria. A series of longitudinal high-resolution images was generated using a pixel size of 24-27 μm. The teeth were divided into three temperature groups and burned under controlled temperatures of 400°C, 650°C and 800°C in an electric furnace (Medlin Naber N3R, 25°C increase/minute) starting at 20°C (room temperature). After the controlled heating the specimens were scanned for a second time with similar scanning parameters as before.

Following the reconstruction and conversion of the raw data (SkyScan NRECON Reconstruction, Adobe® Photoshop Creative Suite 4) the scans were segmented and reconstructed. The segmentation and 3-D modeling was done with Visage Imaging Amira 5.2 (Visage Imaging Inc., San Diego, USA) at the Department of Oral Surgery, Medical University of Vienna, Austria. The manual segmentation of burned teeth was a classification of image data into objects representing the spatial location of dental pulp, cracks and dental tissue by assigning these voxels to a particular material on the basis of grey scale differences.

2.1.3. Results of pilot study

General color changes as reported in literature (from blue grayish at 650°C to chalky white at 800°C) are visible (see Figure A and B). There are also color structures in Figure A (female M3, 800°C). In general the color first turns dark (blue grayish) and then to lighter colors (ash-grey to white). In the left part of Figure B, a female first premolar burned with 650°C and a male first premolar burned with 800°C are shown.

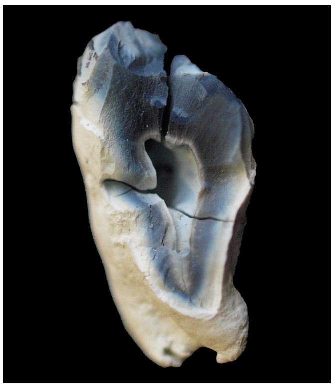

Figure A: Unrestored third molar (female, 26 yrs) after the thermal stress (800°C). Presence of many fractures within the dentin arising from the dental pulp.

Figure B: Comparison of unrestored teeth (left: female, 89 yrs, 650°C; right: male, 65 yrs, 800°C) after different degree of thermal stress. Presence of many fractures within the dentin arising from the dental pulp. The premolars have a grayish color in the enamel and a chalky-white in the cementum.

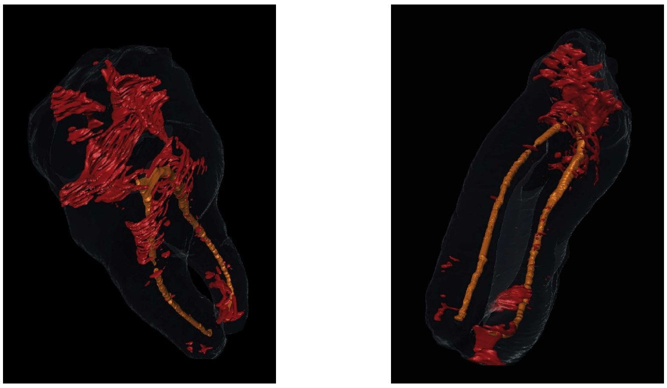

Figure C: 3-D models of burned premolar (male, 65 yrs; 800°C) of Figure B. The tooth shows an increased number of cracks in the upper region due to loss of crown.

Figure D: Premolar comparison of conventional X-ray (longitudinal view) and corresponding micro-CT scan (cross section)

In the 3-D models (Figure C-F) cracks are shown in red, the dental pulp in gold and the hard dental tissue transparent in blue. In contrast to the teeth burned at 400°C the molars and premolars burned at 650°C and 800°C have clearly visible differences in linear fragmentations in micro-CT scans, in varying dimensions.

Figure C (male premolar, 65yrs; 800°C) shows an increased number of fissures and cracks in the amelodential limit due to the fragmentation of the crown. In the case of a male molar (65yrs, 650°C) sporadic cracks arising from the dental pulp and some longitudinal fractures deriving from the cementum are visible (see Figure E).

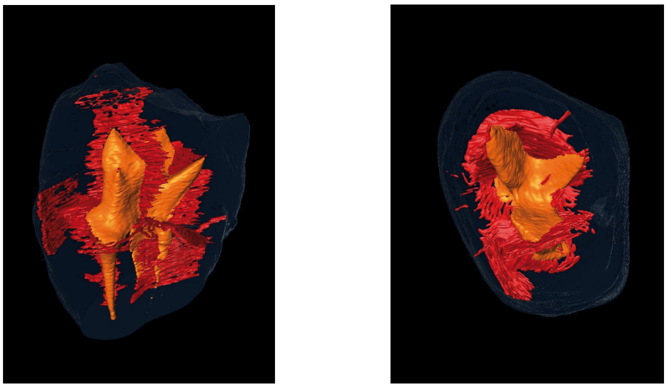

Figure E: 3-D models of burned molar (male, 65 yrs; 800°C). Shows sporadic cracks and fissures arising from the dental pulp and some longitudinal fractures deriving the cementum.

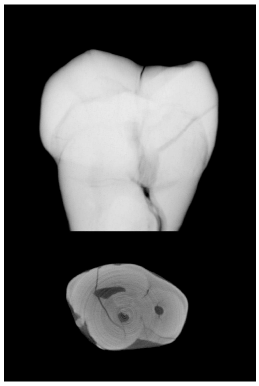

Figure F: Comparison of conventional X-ray (longitudinal view) and corresponding micro-CT scan (cross section)

2.1.4. Discussion

Statistical analysis and comparison of the total volume, maximal length and width of the cracks were not possible due to the reduced sample size and heterogeneity (age and teeth type) in this pilot study. This study, like the vast majority of previous studies did not take some important variables in account, which are present under real circumstances. Usually teeth are not burned isolated but within the jaws of the victims covered by facial soft tissue. Another factor that was not considered is the fact of the fast increase of temperature (up to 400°C in 5 minutes) in a real house burning, the duration of heat exposure and the thermal shock (fast cooling due to quench water) expected to cause slightly different results (Lindemaier 2009, personal communication). A comparison of these heating curves is given in Diagram 1. The blue line illustrates the temperature increase used in crematoria for complete destruction of the human body (2-3 hours, 800-1000°C depending on body size) and the green lines shows the different heating protocols used by the authors mentioned in this manuscript.

The orange line represents the approximation of the modality of development of the fire in a house burning (Eurocode EN1991-1-2:2002) used in fire protection engineering. When looking at the overview of heating protocols previously used in literature the discrepancy between the “real” and previously used protocols can be seen. Although, this pilot study used the same experimental conditions of a muffle furnace proposed in the literature.

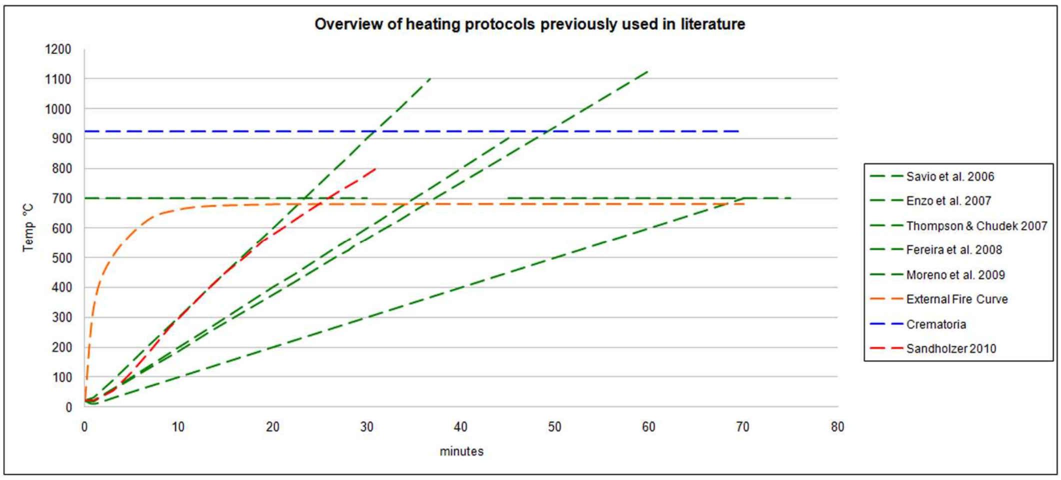

Figure G: Overview of heating protocols previously used in literature

However, it should be kept in mind that these findings were the first results of a novel experimental approach in the analysis of burned human teeth. The pilot study showed interesting observations confirming previous two-dimensional results from current literature. Micro-CT was demonstrated to be a good method for the non-invasive analysis of burned human teeth and therefore these new insights should be included in another more detailed study.

For this follow-up study a homogenous sample of healthy human teeth should be used. Consequently only molars from clinical extractions (not cadaver extractions) of a young age group should be collected. Especially technical adjustment refinement as well as additional approaches in the quantitative and statistical analysis of the image data should be included in the follow-up study to provide even more representative results.

2.2. Main Study

2.2.1. Abstract

MICRO-CT EVALUATION OF HEAT-INDUCED CHANGES IN HUMAN MOLARS

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[3-D]epartment of Theoretical Biology, University of Vienna, Austria

Only a few methods have been published dealing with the visualization of heat-induced cracks and fissures inside bones and teeth. As a novel approach this study used non-destructive x-ray microtomography (micro-CT) for volume analysis of heat-induced fissures and cracks to observe human molars' reaction to various levels of thermal stress.

Eighteen clinically extracted young third molars were rehydrated and burned under controlled temperatures (400°C, 650°C, 800°C) using an electric furnace (Medlin-Naber N3R, Vienna, Austria) adjusted with 25°C increase/minute. The subsequent high-resolution 3-D images (voxel-size 17.7 µm) were made with a compact micro-CT scanner (SkyScan 1174, Kontich, Belgium). In total, 14 scans were automatically segmented with Definiens XD Developer 1.2 (Definiens AG, Munich, Germany), three-dimensional models were computed with Amira 5.2.2 (Visage Imaging Inc., San Diego, USA) and statistically analyzed with SPSS 16 (SPSS Inc., Chicago, USA).

A statistically significant ($p<0.05$, ANOVA post hoc LSD) temperature-dependent increase of heat-induced cracks and fissures was observed between the three temperature groups. In addition the distribution and shape of the heat-induced changes could be classified using the computed three-dimensional models.

The macroscopic heat-induced changes observed in this study correspond with previous observations of unrestored human teeth but do also take into account the entire microscopic three-dimensional expansions of heat-induced cracks and fissures inside hard dental tissue. Using the same experimental conditions proposed in the literature, this study shows interesting observations confirming previous results and offering new perspectives in forensic investigations as well as in the analysis of archaeological excavation material.

2.2.2. Introduction

Positive identification and investigation of burned human remains require a lot of experience and great care (Griffiths and Bellamy 1993). Burned human remains can be the results of direct contact with open flames or the exposure to high temperatures (American Society of Forensic Odontology 2007). Various events can lead to burned human remains, e.g. aircraft accidents (Taylor et al. 2002), natural disasters (Schuller-Gotzburg and Suchanek 2007) or house fires (Sledzik et al. 2002). In some cases fire is used for suicides (Schmidt et al. 2000; Shkrum and Johnston 1992) or for homicides to destroy forensic evidence and prevent clear identification and recovery (Fairgrieve 2008).

Bohnert et al. (1998) listed the effects of fire on the skull based on observations of cremations carried out at 670°-810°C. Teeth are primarily protected by the soft tissue, muscles and fat for 10-24 minutes, and consequently the temperature inside the oral cavity does not rise over 87°C, depending on the nutritional state, age, temperature and time of exposure (Eichenhofer 1980). In 23.4% of the incomplete cremations in forensic cases the soft tissue of the skull is affected and the heat impairs the oral cavity (Gerling et al. 2000). However, the posterior teeth are more likely to be preserved due to the layers of skin, muscle and fatty tissue and therefore, are more likely to be used for the identification process of deceased (American Society of Forensic Odontology 2007). A general estimation of burning temperature could provide important information of ritual habits in past societies as well secure the possibility to reconstruct a criminal act. The results provided in this study are of high importance for the fields of forensic science and archaeology providing a more detailed understanding of thermal stress induced three-dimensional alteriations in human teeth.

In the last decades the focus of interest shifted from the analysis of macroscopic heat-induced changes to the microscopic changes. In general, macroscopic changes can be influenced by a large number of external factors such as time and temperature of heat exposure, availability of oxygen as well as material properties and therefore do not seem to be the ideal tool for temperature estimation (Thompson 2009; Walker et al. 2008). So far, microstructure and ultrastructure of burned bones and teeth were mainly examined by using classical histology (Fereira et al. 2008; Hughes and White 2009; Muller et al. 1998; Myers et al. 1999), X-ray Diffraction (XRD) (Enzo et al. 2007; Piga et al. 2008; Piga et al. 2009; Rogers and Daniels 2002), Fourier Transform Infrared Spectroscopy (FTIR) (Thompson et al. 2009), small-angle X-ray scattering (SAXS) (Hiller et al. 2003) and scanning electron microscope (SEM) (Carr et al.

1986; Fairgrieve 1994; Holden et al. 1995b; Merlati et al. 2004; Muller et al. 1998; Quatre-homme et al. 1998; Shipman et al. 1984; Thompson 2005; Wilson and Massey 1987).

All of these destructive and intricate methods have been used throughout many decades, and can be useful for the estimation of burning temperature of bones and teeth as well as for the characterization of dental filling materials.

However, the destruction of the material is not acceptable in archaeological excavation material or evidence from forensic cases. Therefore, non-invasive and non-destructive methods had to be found in order to analyze heat-induced changes of bones and teeth.

Visualization of heat-induced changes

Savio et al. (2006) evaluated the effects of high temperatures on human teeth using periapical radiographs. In total, 90 unrestored and endodontically treated human teeth exposed to temperatures from 200°C-1100°C using an electric furnace with a constant increase of 30°C/minute, were analyzed.

Another important study was performed by Thompson and Chudek (2007) using Magnetic Resonance Imaging (MRI) to visualize heat-induced changes in bone. The authors used 1.5cm thick sections of sheep bone and exposed them two times for 30 minutes at 700°C using an electric furnace. This was the first time three dimensional imaging methods were used for the purpose of burned bones.

Aim of micro-CT study

For a better understanding of the heat-induced three-dimensional changes inside teeth the non-invasive technology of x-ray microtomography (micro-CT) is used for the first time. This experimental study provides image data for a qualitative three-dimensional reconstruction as well as quantitative results. This novel approach of evaluation of burned human teeth presents results for a more detailed understanding of thermal stress induced three-dimensional alterations in human teeth as well as useful statistical data for interpretation and estimation of burning temperatures.

2.2.3. Material and Methods

Human teeth sampling and preparation

A total of 37 human third molars, derived from clinical extractions were cleaned to eliminate blood residues, and disinfected in a 5% sodium hypochlorite solution for 30 minutes. A declaration of consent was filled out by every donor involved in this study. Nineteen teeth were excluded from the study because of unknown patients' age, damages (e.g. broken roots), endodontic treatments, dental restorations or caries. The 18 intact third molars (nine female, nine male, mean age: 23.06 ± 2.15 years) were rehydrated by using phosphate buffered saline for 30 minutes at room temperature and afterwards, each specimen was stored in a conical 5ml tube in synthetic saliva (Sialin-Sigma Solution, Sigmapharm Vienna) at 4°C for two to eight month. The solution was changed every two weeks.

Thermal treatment

The 18 teeth were randomly divided into three groups before subjecting them to the thermal stress. The six teeth of each temperature group (400°C, 650°C, 800°C) were put in crucibles and burned at the same time in an electric furnace (Medlin Naber N3R, Vienna, Austria) with 25°C increase/min starting at room temperature. The time of thermal stress exposure for each group was on average 13.25 min to reach 400°C; 23.25 min to reach 650°C and 31.5 min to reach 800°C. As soon as the desired temperature was reached the specimens were removed from the furnace, cooled to room temperature and then stored in conical tubes stowed with cotton. The macroscopic changes of the teeth were described and documented by direct vision of the samples and photographs using a compact digital camera (Canon Ixus 80IS).

Micro-CT study

This study used a SkyScan 1174 compact micro-CT scanner (SkyScan, Kontich, Belgium) located at the Department of Theoretical Biology, University of Vienna, Austria. The general adjustment for the scans was a voxel size of 17.7 µm and 3500-4500 ms (800µA, 50 kV), 180° rotation (with 3 pictures per degree), 0.5mm Aluminium Filter and random movement resulting in a mean scanning time of 5-6 hours. The resulting slices were reconstructed using NSRECON (SkyScan, Kontich, Belgium). The original scanning files (1024x1024 pixels) were stored in 16-bit TIFF format; the reconstructed files were saved as 8-bit JPGs for easier handling during the 3-D reconstruction.

3-D Reconstruction

Following the reconstruction (SkyScan NRECON Reconstruction) and conversion of the raw data (Adobe® Photoshop Creative Suite 4) the scans were segmented, measured and reconstructed. The automated segmentation was done by using Definiens XD Developer 1.2 (Definiens AG, Munich, Germany) based on a specially developed image analysis algorithm, allowing a classification of voxel data into objects representing the dental pulp, cracks and dental tissue on the basis of grey scale and shape differences. To control the results of the automated segmentation additional manual segmentation was carried out in five cases using Visage Imaging Amira 5.2.2 (Visage Imaging Inc., San Diego, USA) at the Department of Oral Surgery, Medical University of Vienna, Austria. The resulting data (number of pixels of the three classes) of the automated segmentation were analyzed with SPSS 16 (SPSS Inc., Chicago, USA) software package. The final 3-D reconstruction was also performed with Visage Imaging Amira 5.2.2.

Statistical Analysis

The results of the automated segmentation were analyzed with analysis of variance (ANOVA) and uncorrected post hoc LSD tests using SPSS 16 (SPSS Inc., Chicago, USA) software package. A probability level of $p<0.05$ was used as an index of statistical significance.

2.2.4. Results

Heat-induced Changes

Three teeth completely broke apart during the heating process and one tooth during the subsequent handling. In total, 14 teeth (400°C group n=5; 650°C group n=5; 800°C n=4) were included in the statistical data analysis.

Figure I: Comparison of heat-induced changes in tooth color (from left to right: tooth 7: 400°C, tooth 5: 650°C, tooth 12: 800°C)

400°C Group

At 400°C the teeth showed a loss of brightness of the crown and root. In two cases the root acquired a brown color. A partial or full disintegration of crown was observed in none of the specimens. In the micro-CT images (see Figure II) only small fissures were visible in the crown region (Figure II A), whereas multiple fissures and small cracks were in the root region (Figure II B). These fissures were frequently located in the root and usually did not reach the dentin-enamel junction. In the 3-D models, the 400°C group showed mostly fissures in longitudinal, in rare cases also in transverse direction. The mean volume of cracks and fissures was 2.95% (SD: ± 1.83%, values ranging from 1.61% – 4.38%) of the total tooth volume.

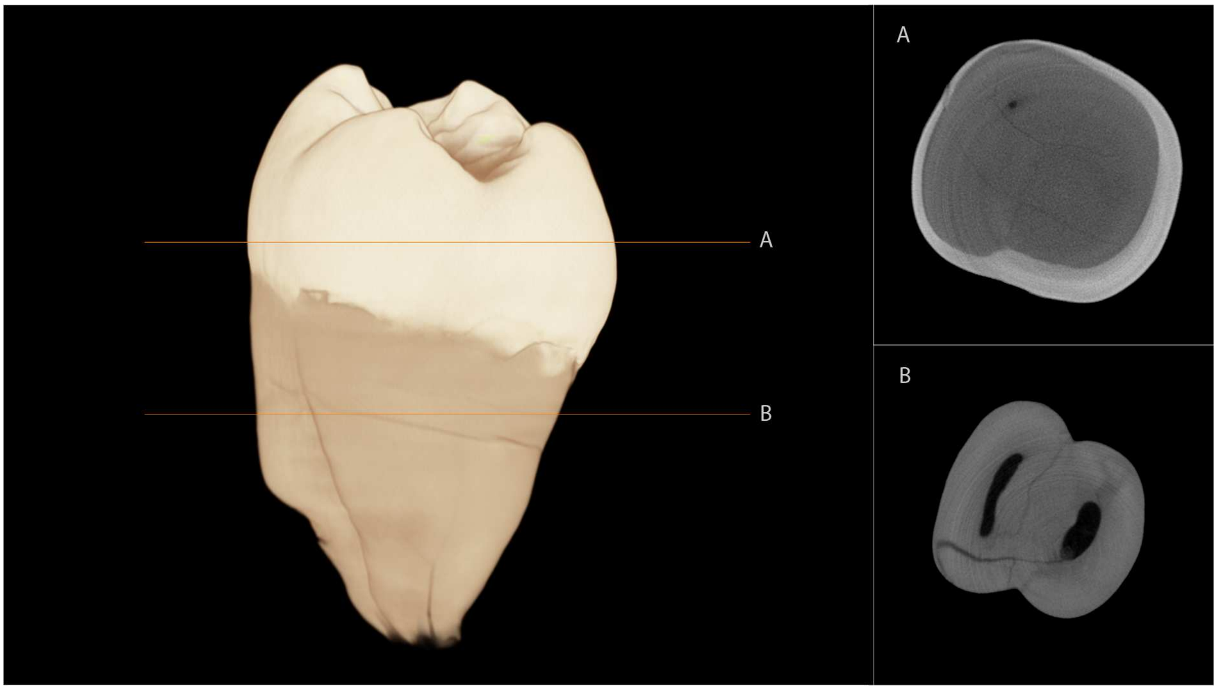

Figure II: 3-D surface rendering (400°C group, tooth7)

650°C Group

The teeth subjected to 650°C became fragile and showed grayish-black discoloration of the whole tooth. A partial or full disintegration of the crown was observed in all specimens. Fragmentation after the separation of the crown could be observed in two specimens. In the micro-CT images (see Figure III) a variety of fissures and cracks was identified. Deep cracks were evident in the root (Figure III B), whereas the enamel surface showed small fissures. The vast majority of the cracks were present in the area of the dentin-enamel junction (Figure III A). In general, this region showed a small amount of cracks accompanied by many fissures around the dentin-enamel border. The cracks did also affect the enamel but only rarely propagated out through the enamel. In the 3-D models, the 650°C group mostly showed cracks in longitudinal and transverse direction arising from the dental pulp, and a complex contour pattern could be found. The mean volume of cracks and fissures was 7.75% (SD: ± 2.65%, values ranging from 5.02% – 11.83%) of the total tooth volume.

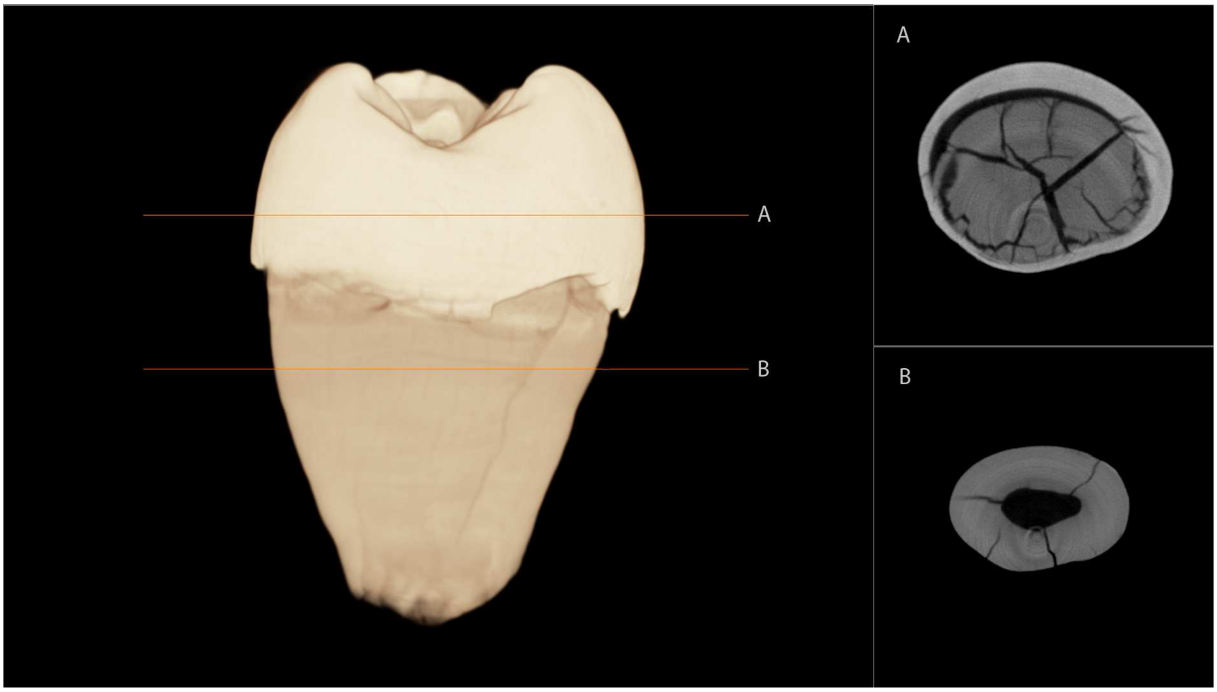

Figure III: 3-D model (650°C group, tooth5)

800°C Group

In general, the teeth subjected to 800°C became very fragile and showed chalky white discoloration of the root and slightly grayish color variations of the crown. A partial or full disintegration of the crown was observed in all specimens. Fragmentation after the separation of the crown could be observed in two specimens. In the micro-CT images (see Figure IV) a high variation of fissures and cracks was observed. The vast majority of the cracks were present in the area of the dentin-enamel junction (Figure IV A). In general, this region showed massive cracks with many fissures around the dentin-enamel border. The cracks did also affect the enamel and did propagate out through the enamel. In the 3-D models, the 800°C group mostly showed cracks in longitudinal and transverse direction arising from the dental pulp, and a complex contour pattern could be found. The mean volume of cracks and fissures was 9.65% (SD: ± 5.65%, values ranging from 4.03% – 14.54%) of the total tooth volume.

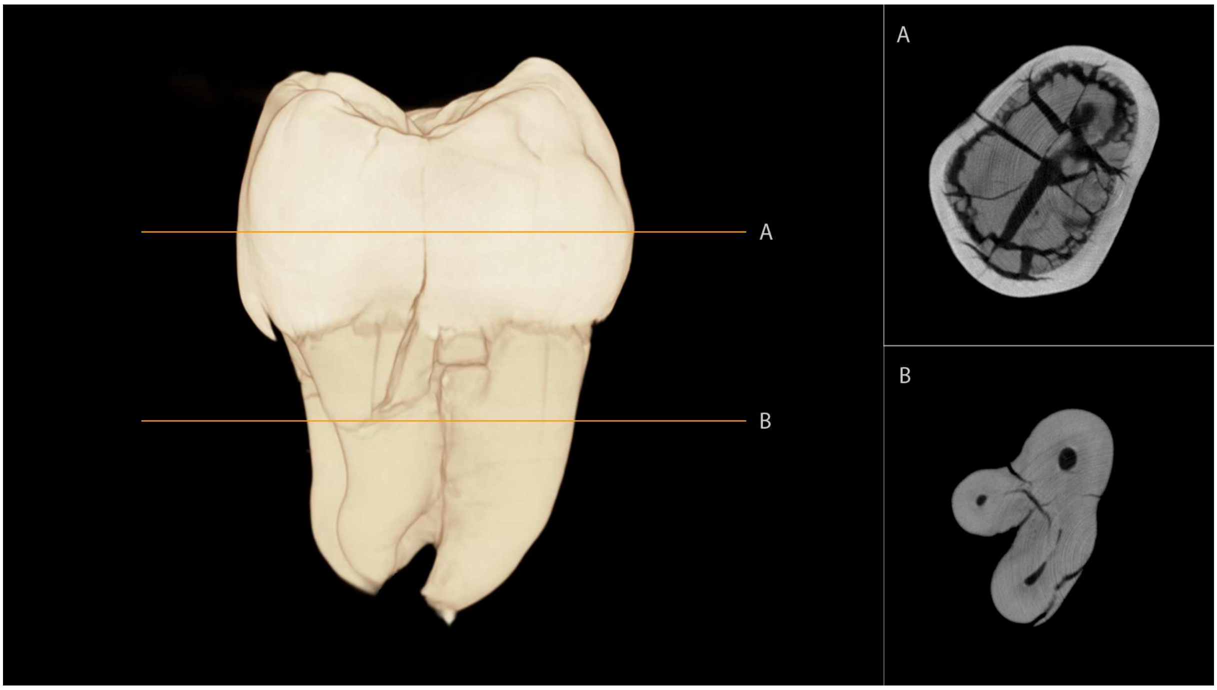

Figure IV: 3-D model (800°C group, tooth12)

Statistical Analysis

The results of the volume data elicited from the automated segmentation, which were analyzed with SPSS 16 (SPSS Inc., Chicago, USA) software package, are given in Table I. The resulting data from Table I were used for a multiple comparison shown in Table II.

Table I: Volume elicited from automated segmentation data (TEMP: temperature group; VC: Volume Cracks; VDP: Volume Dental Dental pulp; VDM: Volume Dental Material; TV: Total Volume; PC: Percentage Cracks; PDM: Percentage Dental Material; PDP: Percentage Dental Dental pulp)

	TEMP	VC	VDP	VDM	TV	PC	PDM	PDP
tooth13	400°C	260268 px	498989 px	15373563 px	16132820 px	1.61%	95.29%	3.09%
tooth4	400°C	297555 px	710144 px	12393600 px	13401299 px	2.22%	92.48%	5.30%
tooth10	400°C	283950 px	424204 px	10637190 px	11345343 px	2.50%	93.76%	3.74%
tooth18	400°C	523613 px	365249 px	12030155 px	12919017 px	4.05%	93.12%	2.83%
tooth7	400°C	664903 px	374927 px	14132696 px	15172525 px	4.38%	93.15%	2.47%
tooth11	650°C	836294 px	884915 px	14946493 px	16667700 px	5.02%	89.76%	5.31%
tooth17	650°C	726109 px	344335 px	10025293 px	11095737 px	6.54%	90.35%	3.10%
tooth14	650°C	354368 px	186035 px	4856763 px	5397165 px	6.57%	89.99%	3.45%
tooth5	650°C	712821 px	327098 px	7054513 px	8094431 px	8.81%	87.15%	4.04%
tooth2	650°C	1872007 px	831219 px	13122695 px	15825920 px	11.83%	82.92%	5.25%
tooth15	800°C	347491 px	362227 px	7916578 px	8626296 px	4.03%	91.77%	4.20%
tooth9	800°C	493011 px	428694 px	7964024 px	8885729 px	5.55%	89.63%	4.82%
tooth12	800°C	1982607 px	575634 px	11109772 px	13668013 px	14.51%	81.28%	4.21%
tooth6	800°C	1258431 px	195683 px	7203777 px	8657890 px	14.54%	83.20%	2.26%

Table II: Multiple Comparisons (ANOVA post hoc LSD test)

Multiple Comparisons						
Volume of Cracks LSD						
(I) Temperature	(J) Temperature	Mean Difference (I-J)	Standard Error	Significance	95% Confidence Interval	
					Lower Bound	Upper Bound
400	650	-4.79812%*	2.17196%	.049	-9.5786%	-.0177%
	800	-6.69994%*	2.30372%	.014	-11.7704%	-1.6295%
650	400	4.79812%*	2.17196%	.049	.0177%	9.5786%
	800	-1.90182%	2.30372%	.427	-6.9723%	3.1686%
800	400	6.69994%*	2.30372%	.014	1.6295%	11.7704%
	650	1.90182%	2.30372%	.427	-3.1686%	6.9723%
*. The mean difference is significant at the 0.05 level.						

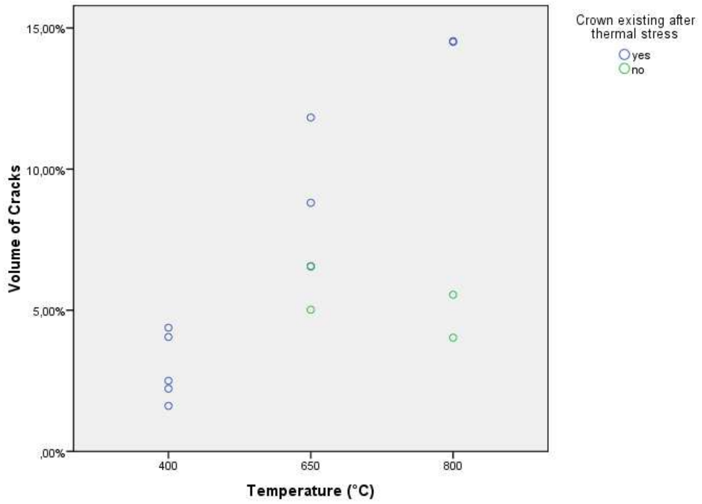

Figure V: Scatter Plot of group classifications. Blue circles indicate the presence of the crown, while green circles indicate its' absence after thermal stress. In the 400°C group all crowns were preserved, while the crown was often missing in the 650°C and 800°C temperature groups.

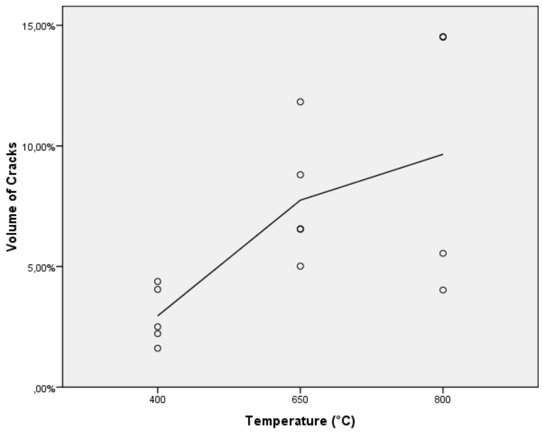

Figure VI: Scatter Plot including interpolation line. The interpolation line indicates a steep increase of the crack volume between 400°C and 650°C. In contrast there is a less steep increase between 650°C and 800°C.

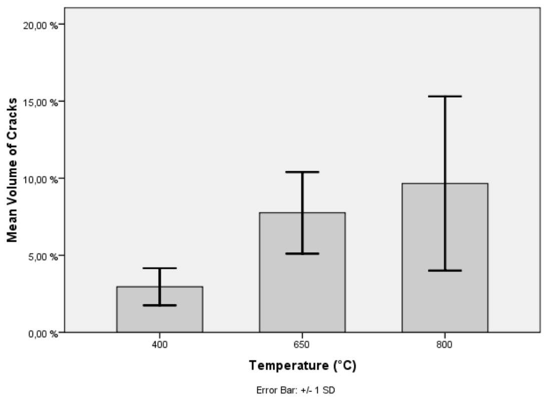

Figure VII: Mean volume of cracks is shown (± 1 SD). The error bars indicate a small variation of crack volume in the 400°C group, whereas in the 650°C and 800°C group the variation of crack volume was much higher due to presence/absence of crown.

2.2.5. Discussion

Macroscopic Results

The macroscopic heat-induced color changes of this study correspond with previous observations of unrestored human teeth (Merlati et al. 2002; Merlati et al. 2004; Moreno et al. 2009). The different material properties of dentin and enamel cause a seperation and fragmentation of the crown between 400°C and 500°C (Myers et al. 1999). The partial or full disintegration of the crown is strongly related to the final temperature. Hughes and White (2009) indicate that in general, postmortem teeth undergo dehydration which makes the dentin material more brittle and dentin-enamel junction weaker. Additionally, the location of the origin of the cracks near the dental pulp cavity is caused by the intertubular tensile stress, allowing the crack to propagate through the structurally modified dentin and enamel (Hughes and White 2009).Therefore, artificial saliva represents a very good storage to prevent teeth from drying out and a certain hydration level was maintained. There was no statistical correlation of the duration of storage and the total volume of cracks could be found. In total, four specimens of the 650°C and 800°C temperature group had a completely fragmented crown, what led to a reduced volume of cracks, similar to teeth exposed to 400°C with complete crowns (see Figure V).

The water content inside the dentin, which is unlike higher in young teeth, supports the rapid evaporation of the stored water, leading to a more complex crack pattern (Myers et al. 1999). In addition, in the pilot study with molars and premolars of various age groups similar results were observed (Sandholzer 2010). Because of the young mean age of the specimens in this study, further studies are necessary to observe and compare the three-dimensional changes inside older teeth.

Segmentation Results

The automated segmentation with Definiens XD Developer 1.2 (Definiens AG, Munich, Germany) showed accurate and repeatable results. All measurements were computed using the same image algorithm; therefore, an intra-examiner error is not present. Manual control of the segmentation results were additionally carried out in five cases but showed no major influence on the originally elicited data. The statical results have primarily been influenced by two factors. First, by the small sample size (n=14) and second, the seperation of the crown influenced the results, leading to a big variance in the total volume of cracks (Figure V-VII).

A statistically significant ($p<0.05$, ANOVA post hoc LSD) temperature-dependent increase of heat-induced cracks and fissures was observed between 400°C and 650°C ($p = 0.049$), as well as 400°C and 800°C ($p = 0.014$). ANOVA tests ($p<0.05$) also showed that in this study the total volume of the tooth as well as the volume of the dental pulp do not influence the total volume of cracks.

Temperature Adjustments

A comparison of electric furnace heating protocols previously used in literature shows a wide range of time of exposure and temperature increase per minute (Enzo et al. 2007; Fereira et al. 2008; Moreno et al. 2009; Savio et al. 2006; Thompson and Chudek 2007). An important factor, generally not taken into account, but which would be present in an actual house burning, is the fast increase of temperature, usage of fire accelerants, the long duration of heat exposure and the fast cooling due to quench water. These facts are expected to lead to modified results. This micro-CT study used a 25°C gradual increase per minute, comparable to other previous experimental studies dealing with heat-induced changes inside teeth (Fereira et al. 2008; Savio et al. 2006).

micro-CT

The high resolution of micro-CT compared to MRI and CT is the most important advantage. While the MRI uses a voxel size of 230µm (Thompson and Chudek 2007), micro-CT provides a maximum resolution as small as 6µm, allowing visualization of fine fissures within teeth or bone, normally only visible in light microscopic observations. However, as micro-CT is based on x-ray transmission images, teeth fillings with metal compositions might lead to artefacts influencing the image data analysis (Buzug 2008).

Clearly, this novel approach has advantages in comparsion with previously proposed methods. In contrast to histological sections, SEM, XRD, SAXS and FTIR, micro-CT is non-invasive and can therefore also be used in forensic and archaeological investigations without destroying the specimen. Additonally, not only small pieces or sections can be considered, but also whole jaws or longbones could be handled with modern micro-CTs in a sufficent resolution.

2.2.6. Conclusion

In the last few years the micro-CT scanning technology became commonly used in various fields of biology and medicine. Currently this technology also gets more and more used for forensic purposes (Thali et al. 2003).

This study clearly visualized how isolated healthy teeth of young humans react to extreme temperatures and that thermal stress can lead to a temperature-specific volume of cracks in human teeth, which can help to estimate the temperature at the time of heat exposure. Relevant data of the cracks and fissures could be effectively elicited from the burned human third molars using modern techniques and complex analysis methods. Due to the small sample size it is too early to say, that micro-CT is *the* appropriate alternative method for determining temperatures of burned human remains (e.g. from forensic investigations or archaeological excavations). However, the results of this study are not meant to provide results, which would have occurred in an intact human body in a real fire. Therefore further research involving more specimens, complete human mandibles or skulls and different heating protocols (e.g. duration of heat exposure) is necessary to confirm the results from this study. The usage of micro-CT proved to have many advantages compared to earlier, mostly invasive or destructive, methods.

However, this study used the same experimental conditions proposed in literature, revealing novel insight into heat-induced changes of teeth, revealing novel insight into heat-induced changes of teeth, confirming previous results and offering new perspectives for forensic investigations as well as archaeological excavation material.

C. Appendix

3.1. Study survey

	Gender	Age	Donor	Group	Saliva Storage	Burning
# 1	female	21	SK	400	21.9.2009	18.5.2010
# 2	female	21	SK	650	21.9.2009	18.5.2010
# 3	female	21	SK	800	21.9.2009	20.5.2010
# 4	female	21	SK	400	21.9.2009	18.5.2010
# 5	female	23	CM	650	16.9.2009	18.5.2010
# 6	female	23	CM	800	16.9.2009	20.5.2010
# 7	female	23	CM	400	16.9.2009	18.5.2010
# 8	female	23	CM	650	16.9.2009	18.5.2010
# 9	male	22	MU	800	15.1.2010	20.5.2010
# 10	male	22	MU	400	15.1.2010	18.5.2010
# 11	male	22	MU	650	15.1.2010	18.5.2010
# 12	male	28	RB	800	21.9.2009	20.5.2010
# 13	male	28	RB	400	26.1.2010	18.5.2010
# 14	male	25	MG	650	25.9.2009	18.5.2010
# 15	male	23	MG	800	26.1.2010	20.5.2010
# 16	female	21	DW	800	26.1.2010	20.5.2010
# 17	male	24	MG	650	28.3.2010	18.5.2010
# 18	male	24	MG	400	28.3.2010	18.5.2010

	Start Tempera-ture	Increase/min	End temperature	Duration of Expo-sure	micro-CT scan
# 1	20°C	25°C/min	400°C	13.25 min	18.5.2010
# 2	20°C	25°C/min	650°C	23.25 min	19.5.2010
# 3	20°C	25°C/min	800°C	31.50 min	20.5.2010
# 4	20°C	25°C/min	400°C	13.25 min	25.5.2010
# 5	20°C	25°C/min	650°C	23.25 min	27.5.2010
# 6	20°C	25°C/min	800°C	31.50 min	28.5.2010
# 7	20°C	25°C/min	400°C	13.25 min	28.5.2010
# 8	20°C	25°C/min	650°C	23.25 min	31.5.2010
# 9	20°C	25°C/min	800°C	31.50 min	31.5.2010
# 10	20°C	25°C/min	400°C	13.25 min	31.5.2010
# 11	20°C	25°C/min	650°C	23.25 min	1.6.2010
# 12	20°C	25°C/min	800°C	31.50 min	1.6.2010
# 13	20°C	25°C/min	400°C	13.25 min	2.6.2010
# 14	20°C	25°C/min	650°C	23.25 min	2.6.2010
# 15	20°C	25°C/min	800°C	31.50 min	2.6.2010
# 16	20°C	25°C/min	800°C	31.50 min	4.6.2010
# 17	20°C	25°C/min	650°C	23.25 min	4.6.2010
# 18	20°C	25°C/min	400°C	13.25 min	4.6.2010

female	9	**mean age**
male	9	23.06 (± 2,15) yrs

Scanning Settings and Calibration (SkyScan 1174)

	Pixel size	Exposure	Filter
# 1	21 µm	4500 ms	0,5mm Al
# 2	21 µm	4500 ms	0,5mm Al
# 3	21 µm	5000 ms	0,5mm Al
# 4	21 µm	3500 ms	0,5mm Al
# 5	21 µm	4000 ms	0,5mm Al
# 6	21 µm	4500 ms	0,5mm Al
# 7	21 µm	4500 ms	0,5mm Al
# 8	21 µm	4500 ms	0,5mm Al
# 9	21 µm	4500 ms	0,5mm Al
# 10	21 µm	4500 ms	0,5mm Al
# 11	21 µm	4500 ms	0,5mm Al
# 12	21 µm	4500 ms	0,5mm Al
# 13	21 µm	4500 ms	0,5mm Al
# 14	21 µm	4500 ms	0,5mm Al
# 15	21 µm	4500 ms	0,5mm Al
# 16	21 µm	4500 ms	0,5mm Al
# 17	21 µm	4500 ms	0,5mm Al
# 18	21 µm	4500 ms	0,5mm Al

Nominal pixel size	Measured pixel size
6 µm	6.3 µm
9 µm	7.4 µm
12 µm	9.4 µm
15 µm	11.9 µm
18 µm	14.8 µm
21 µm	17.7 µm
24 µm	21.2 µm
27 µm	24.6 µm
30 µm	28.3 µm
33 µm	32.0 µm

3.2. Figures

Analysis of burned human remains

Figure 1: Structure and composition of human tooth
http://medicalimages.allrefer.com/large/tooth-anatomy.jpg DOR: 25.10.2009

Figure 2: Labeling surfaces and orientation of teeth,
Hillson S. 1996. Dental anthropology. Cambridge University Press, p.10

Figure 3: Standard orthopantomography (23 years, male)

Figure 4: Dental impression of lower dentition

Figure 5: Fragments of a lower jaw burned for 30 minutes at 400°C.
Rötzscher K, Grundmann C, and Benthaus S. 2004. The effects of high temperatures on human teeth and dentures. International Poster Journal of Dentistry and Oral Medicine 6(1):213.

Figure 6: Radiograph of burned third molar (tooth 6, 800°C)

Figure 7: Micro-CT SkyScan 1174 compact micro-CT scanner,
http://www.skyscan.be/products/1174.htm DOR: 2.11.2009

Figure 8: Photo-documentation of third molar in buccal and lingual view, storage tube

Figure 9: Time/temperature curve Medlin-Naber N3R

Figure 10: Manual segmentation using Visage Imaging Amira 5.2.2

Figure 11: Flowchart and main steps of automated segmentation using Definiens XD Developer 1.2

Pilot study

Figure A: Unrestored third molar 800°C

Figure B: Comparison of unrestored teeth 650°C/800°C

Figure C: 3-D models of burned premolar 800°C

Figure D: Premolar comparison of X-ray and corresponding micro-CT scan

Figure E: 3-D models of burned molar 800°C

Figure F: Molar comparison of conventional X-ray and corresponding micro-CT scan

Figure G: Overview of heating protocols previously used in literature

3.3. Tables

3.4. References

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3.5. Curriculum Vitae

Personal Information

Name	Michael Sandholzer
Date of birth	04.05.1986
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Education

12/2009 – 08/2010	Master Thesis 'Micro-CT analysis of human teeth after exposure to controlled thermal stress' (Department of Anthropology, University of Vienna)
06/2007 –	University of Vienna: Physical Anthropology (A442) and Genetics and Microbiology (A441) (Mag.rer.nat. program) Specialization: Osteology, Human Genetics
03/2006 – 09/2010	Medical University of Vienna: Medicine (Co-registration)
10/2005 – 06/2007	University of Vienna: Biology (A437, Mag.rer.nat. program)
09/1996 – 06/2004	High School Bundesgymnasium Rebberggasse (Feldkirch, Austria)

Scientific activities

Publications

1. **Sandholzer M** (2010)
ANALYSIS OF BURNED HUMAN REMAINS: NOVEL APPROACHES WITH HIGH RESOLUTION IMAGING TECHNIQUES.
In: Buhl C, Engel F, Hartung L, Kaestner M, Ruedell A, and Weisshaar C: Proceedings of the 4th Meeting of Junior Scientists in Anthropology - Beiträge zum 4. Kongress des Wissenschaftlichen Nachwuchses der Anthropologie (Permalink: http://www.freidok.uni-freiburg.de/volltexte/7603/) p. 56-65.

Conference presentations
Podium presentations (as presenter)

2. **Sandholzer M**, Baron K, Heimel P, Metscher B (2010)
MICRO-CT EVALUATION OF HEAT-INDUCED ALTERATIONS IN HUMAN TEETH
12th Annual BABAO Conference, University of Cambridge (United Kingdom) 17.9-19.9.2010

3. **Sandholzer M** (2010)
ANALYSIS OF BURNED HUMAN REMAINS: APPROACHES WITH CLASSICAL (X-RAY, MRI) AND HIGH-RESOLUTION (MICRO-CT) IMAGING TECHNIQUES
4th Meeting of Junior Scientists in Anthropology, Freiburg im Breisgau (Germany) 25.3.-28.3.2010

Posters

4. **Sandholzer M**, Baron K, Metscher B (2010)
MICRO-CT ANALYSIS OF HUMAN MOLARS BEFORE AND AFTER EXPOSURE TO CONTROLLED THERMAL STRESS
4th Meeting of Junior Scientists in Anthropology, Freiburg im Breisgau (Germany) 25.3.-28.3.2010

5. Baron K, **Sandholzer M**, Metscher B (2009)
MICRO-CT ANALYSIS OF ADULT HUMAN MOLARS AND PREMOLARS BEFORE AND AFTER EXPOSURE TO CONTROLLED HIGH TEMPERATURES
Vth International Anthropological Congress of Ales Hrdlicka, Prague (Czech Republic), 2.9-5.9.2009

6. **Sandholzer M**, Nagl IM, Grof K, Daghighi S, Patzak B, Grossschmidt K, Salaberger D, Tangl S (2009)
COMBINATION OF CLASSICAL (CT, X-RAY) AND HIGH-RESOLUTION (MICRO-CT) IMAGING TECHNIQUES FOR PRECISE DIFFERENTIAL DIAGNOSIS OF CHRONIC OSTEOMYELITIS OF THE JAWS IN THREE HISTORICAL SPECIMENS
36th Annual Meeting of the Paleopathology Association, Chicago (USA), 31.3.-1.4.2009

Working experience (2008-2010)

08/2010	Volunteer technical assistant at the 18th European Meeting of Paleopathology Association (Natural History Museum Vienna, Austria)
06/2010 –	Voluntary co-worker at the Department of Radiology (General Hospital, Medical University of Vienna), Project title 'Micro-CT versus Histology of pediatric bone tumors'
10/2009 – 01/2010	Co-lecturer 'Biophysical Phenomena' (Studies Program Biology) with Ao.Univ.-Prof. Dr. Armin Fuith (Institute of Nonlinear Physics)
01/2009 –	Research assistant Bone Research Group at the Department of Oral Surgery (Bernhard Gottlieb University Clinic of Dentistry, Vienna, Austria)
05/2006 –	Student Counselor (ÖH, Austrian National Union of Students) Member and Deputy Chairman of Biology (since 07/2007) at the University of Vienna.
11/2008 – 12/2008	Research assistant at the 'Ludwig-Boltzmann-Institute for experimental and clinical Traumatology' (Austrian Cluster of Tissue Regeneration)
04/2008 – 08/2008	Medical adviser at ‚Bodies – The Exhibition' (Vienna, Austria)

Memberships

American Board of Forensic Odontologists (ABFO); Physicans for Human Rights (PHR);
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Personal Interests

Osteology, Pathology, Traveling, Reading, Black & White Photography

3.6. Eidesstattliche Erklärung

Ich erkläre an Eides statt, dass ich diese Diplomarbeit selbständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe. Die Stellen meiner Arbeit, die dem Wortlaut oder dem Sinn nach anderen Werken entnommen sind, habe ich in jedem Fall unter Angabe der Quelle als Entlehnung kenntlich gemacht. Dasselbe gilt sinngemäß für Tabellen, Karten und Abbildungen. Diese Arbeit hat in dieser oder einer ähnlichen Form noch nicht im Rahmen einer anderen Prüfung vorgelegen.

Wien, im August 2010 Unterschrift