TECHNICAL NOTE

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Experimental Single Controlled Study of Burned Bones: Contribution of Scanning Electron Microscopy

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ABSTRACT: Burned bones were studied using Scanning Electron Microscopy. The samples were cut from a maxillary-mandibular block taken during an autopsy. These fragments were heated in a furnace under controlled temperature conditions for 60 minutes. The temperatures ranged from 150 to 1150 degrees Celsius. The results are as following: (i) there are significant alterations of the bone, more and more obvious as the temperature increases, (ii) it appears to be difficult to establish a precise correlation between the temperature and the scanning electron microscopy patterns.

KEYWORDS: forensic science, identification, burned bone, fire victim, scanning electron microscopy, forensic pathology

Forensic pathologists more and more examine burned or carbonized human remains (1,2), and sometimes have to cope with only burned bones, usually in the form of fragments (3). According to Bass and Driscoll (4), the number of burned skeletons has doubled within one decade, and is increasing from year to year. The depth of a burn on the soft tissues depends on the degree of heat, the duration of the exposure, and the region of the body in contact with the heat. The chronology of cremation is explicit in some works: for example at a temperature of 680 degrees Celsius, skeletonization of the face and arms occurs after a period of 15 mn (2). The degree of destruction and fragmentation is roughly proportional to the heat of the fire (5). Cremation is complete when the temperature is above 700–800 degrees Celsius (6). This can occur in domestic house fires (7). Morphologic and microscopic alterations of burned bones have been studied on various samples (8). Macroscopy allows for the observation of color changes depending on temperature (5,9–11). Shrinkage, distortion and fragmentation of bone tissue obviously complicate identification, and cremation of dried bone produces patterns that are different from fleshed bone (12). Histological heat modifications in bone structure have also been studied (6,13). An increase in the diameter of the canals, and a decrease in the diameter of the osteons have been demonstrated in burned bones (14). The ultrastructure of normal and pathological bone tissue is well documented (15). Scanning Electron Microscopy has been used for estimating age in nonburned archaeological samples from vertebrae and normal ribs (16), dating of bone remains (17–19), but rarely studying burned bones (10,20,21). Shipman (10) noted some alterations under the Scanning Electron Microscopy, that were correlated with temperature. Holden et al. (21) showed in experimental controlled heat bones that the ultrastructural features were quite specific to the associated temperature transitions (within 200 degrees Celsius), and age of the deceased within three categories of age (young, adult, old). Only two authors bring up descriptions and pictures of burned bones studied in SEM (10,21). In one of these works (10) the conditions of experiments (origin of the bones, duration of cremation) were not clearly defined. In the other one (21) the authors dealt with femoral samples representing various age groups, ranging from 1-year to 97-year-old of age, in both sexes. Therefore it appears necessary to complete the documentation in this field, especially with skull and face bones, because the face is often targeted in murders to avoid identification. The aim of this work is to study the effects of different temperatures on bone fragments taken from a maxillary-mandibular block observed in Scanning Electron Microscopy under standardized experimental conditions.

Methodology

All the bone samples were part of a maxillary-mandibular block taken by temporo-mandibular disjointing, during the autopsy of a

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72-year woman. The whole block was cold stored (−20 degrees Celsius) until it was used. The samples taken from this block consisted of several mandibular and maxillar specimens of about 1 cm in width, vertically cut from both sides of an anterior tooth (incisives and canines). Furthermore, fragments of palatine bone and of mandibular symphysis were also tested. Each sample was exposed to a controlled temperature under reproducible conditions (Kavo furnace type 5645 for dental prostheses; controlled temperature and duration). The applied temperature varied from 150 to 1150 degrees Celsius according to the sample. The maximum available temperature in furnace was 1150 degrees. The duration of the treatment was 60 mn. After cooling (from the morning to the afternoon), the sample was machine cut horizontally. Above 400 degrees Celsius, the bone became too friable to use machine cutting, and a piece of bone was simply broken by hand. Bone tissue was then observed at the macroscopic and microscopic levels. Each sample was coated with metal (gold), then immediately examined by Scanning Electron Microscopy (JEOL JSM 35).

Results

Nine samples were prepared as described above and studied at ultrastructural level in Scanning Electron Microscopy. Temperatures ranged from 150 to 1150 degrees Celsius, with 60 minutes exposure for each sample.

First Sample (150 Degrees Celsius)—This sample is a fragment of mandibular bone cut from both sides of the right central incisor. The material does not seem to have been damaged from the macroscopic point of view. Its color is yellowish. In Scanning Electron Microscopy, at ×100 magnification (Fig. 1), a polycyclical pattern is observed, a little cloudy. The canals are undamaged. We can observe a few small irregular deposits, of about 10 micrometers (μm) in diameter.

Second Sample (300 Degrees Celsius)—This fragment was taken at the level of the right lateral incisor of the mandible. When placed at 300 degrees Celsius, it takes a dark gray color. Under the microscope (×100), it exhibits small 10 to 100 μm particles, deposited at random on the surface, which itself is not altered.

Third Sample (400 Degrees Celsius)—This is a maxillary sample cut from both sides of the left canine. When placed at 400 degrees Celsius, the bone becomes blackish gray. It is very friable and can no longer be machine cut. Therefore, it is simply fractured by the hand. In scanning electron microscopy (×100), many cracks are visible and even 30 to 50 μm crevices. Some 20 to 100 μm glomerular aspects are noted, which represent either extraneous products of combustion or a phenomenon of bone tissue coagulation. Numerous spheroid, round or oval, sometimes polygonal, images, measuring 10 to 100 μm, are observed.

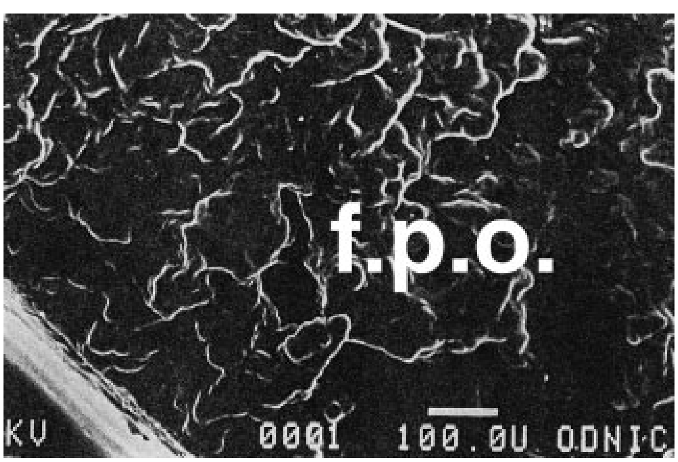

FIG. 1—*Polycyclical pattern. (Mandibular bone) (150℃, ×100.)*

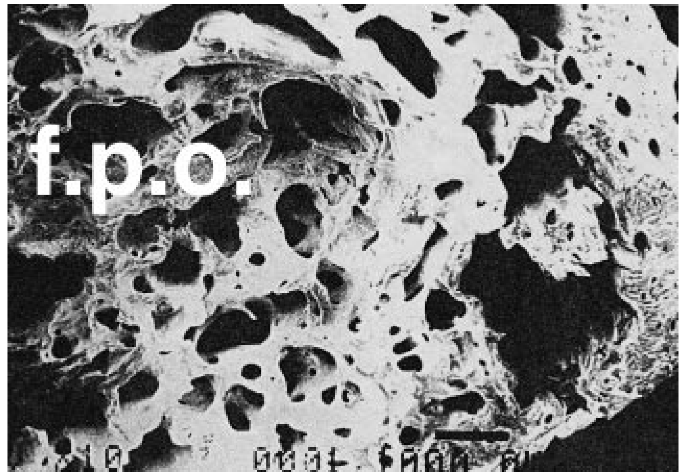

FIG. 2—*No particular alteration. (Mandibular symphysis) (600℃, ×10.)*

Fourth Sample (500 Degrees Celsius)—It is a mandibular fragment corresponding to the left central incisor. After 60 mn combustion, the bone becomes black. At the microscopic level (×100), the canals are undamaged. There appears rare 1 μm wide fissures. A few glomerular areas are visible.

Fifth Sample (600 Degrees Celsius)—This sample is different by nature, as it relates to the mandibular symphysis. After being treated at 600 degrees Celsius for 60 mn, the sample, black-colored, observed in Scanning Electron Microscopy (×10) shows no special alteration (Fig. 2). At higher magnification (×390), we can enter into a cavity of about 100 μm, with an apparently little structure alteration, except perhaps for some irregularity of the edges (Fig. 3).

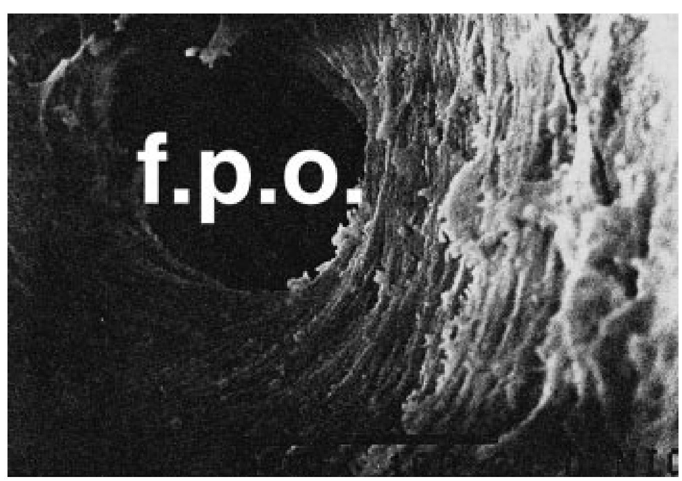

FIG. 3—*Haversian canal of 100 μm in diameter. Irregularity of the edges (mandibular symphysis, same sample as Fig. 2) (600℃, ×390).*

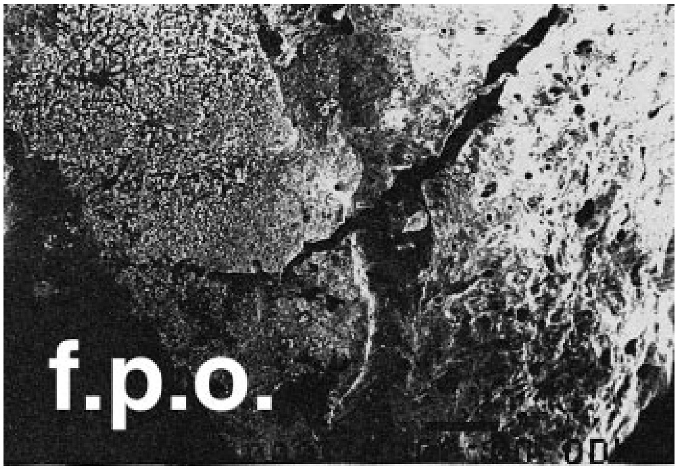

FIG. 4—*Clean fissure and surface of dusty aspect (palatine bone) (700℃, ×10).*

FIG. 6—*Raised-relief whitish deposit, with spicular pattern on background of crumbly structure (same sample as Figs. 4 and 5) (700 ℃, ×1600).*

Sixth Sample (700 Degrees Celsius)—It is a fragment of light-gray palatine bone. At low magnification (×10, Fig. 4), the bone tissue presents a clean fissure due to heat, showing up on a somewhat dusty and crumbly area. This latter appearance corresponds to the presence of small raised-relief elements that are separated from one to another. At higher magnification (×160, Fig. 5), the surface appears to be slightly irregular and crumbly. At high magnification (×1600), a raised-relief, whitish deposit stands out on a background of a crumbly structure. It presents a spicular appearance, which can probably be refered to products of combustion deposited during carbonization (Fig. 6).

Seventh Sample (900 Degrees Celsius)—A maxillar fragment including the right canine took a white color. At ×250 magnification (Fig. 7), it has a very altered, appearance. The surface seems to be covered with deposits mingled with apparent coagulation, in expanses of 10 to 100 μm, and even more. There is also an appearance of cells, whose average size is 10 to 20 μm. A canal of about 50 μm is visible. Its edges are irregular and covered with deposits.

Eighth Sample (1000 Degrees Celsius)—It is a vertical white-colored section of the maxillar bone on both sides of the right central incisor. At × 100 magnification, the rearrangement is total. The completely irregular surface resembles a "field of cooled lava." It presents vestigial spaces forming pseudo-cavities (Fig. 8).

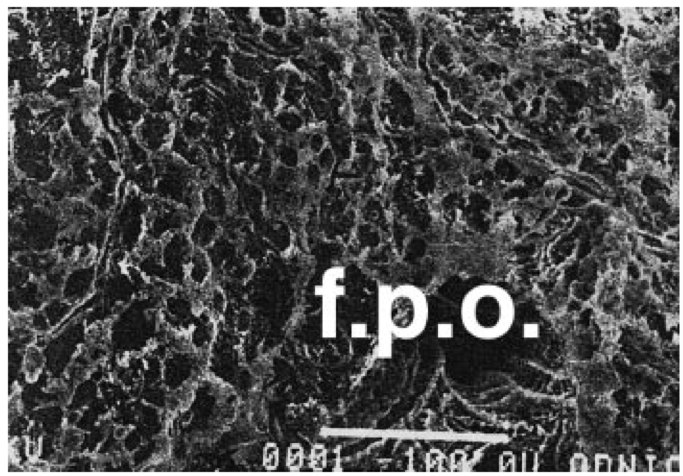

FIG. 7—*Surface of coagulated aspect, in 10 to 100 μm expanses. "Honeycomb" pattern, with alveoles of 10 to 20 μm in diameter, canal of 50 μm in diameter with irregular edges littered with deposits (Maxillar fragment) (900℃, ×250).*

FIG. 5—*Crumbly surface at higher magnification (palatine bone). (Same sample as Fig. 4) (700℃, ×160.)*

Ninth Sample (1150 Degrees Celsius)—It is a mandibular fragment taken from both sides of the left lateral incisor. When raised to 1150 degrees Celsius, it appears to be white. At low magnification (×10), multiple fissures are noted. Some very altered fragments are even detached (Fig. 9). At higher magnification (×100, Figs. 10 and 11; ×150, Fig. 12), this alteration is evident at the surface, which is extensively rearranged, with an appearance of "melting ice" or of "stalagite."

Discussion

The study of burned bones is necessary in forensic anthropology and pathology. Different disasters related in the literature have involved examination of burned bones and teeth for identification (22–24). According to Bass and Driscoll (4), out of 111 cases of skeleton identification in Tennessee over a period of 10 years,

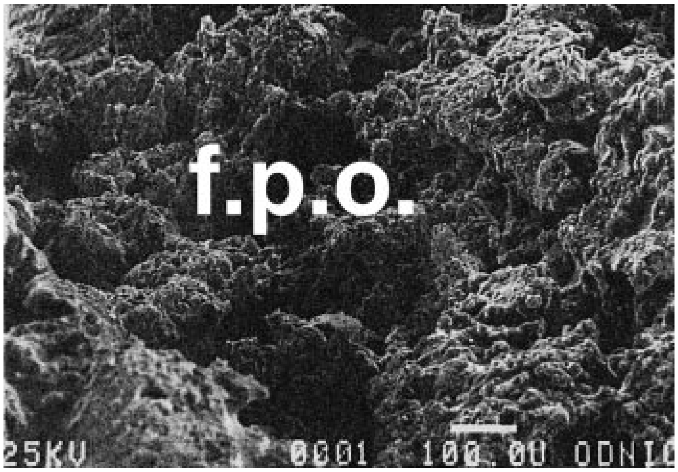

FIG. 8—*Completely rearranged surface: "field of cooled lava." (Maxillar fragment) (1000°C, ×100.)*

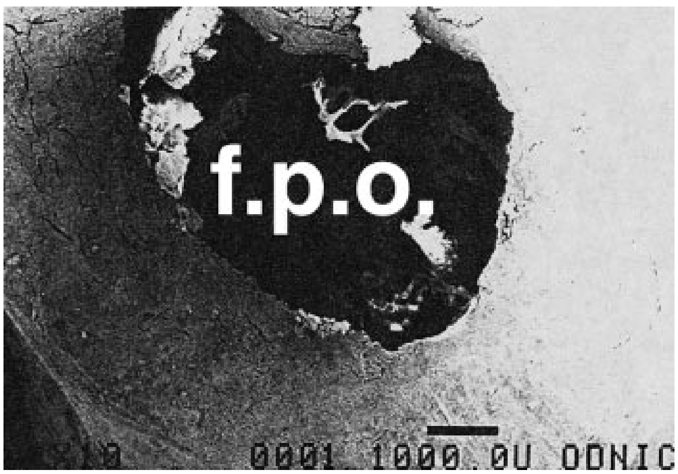

FIG. 9—*Multiple fissurations with some detached fragments. (Mandibular fragment) (1150°C, ×10.)*

FIG. 10—*Much rearranged surface: "Melting ice" pattern. (Mandibular fragment, same sample as in Fig. 9) (1150°C, ×100.)*

FIG. 11—*"Stalactite" pattern. (Mandibular fragment, same sample as in Figs. 8 and 9) (1150°C, ×100.)*

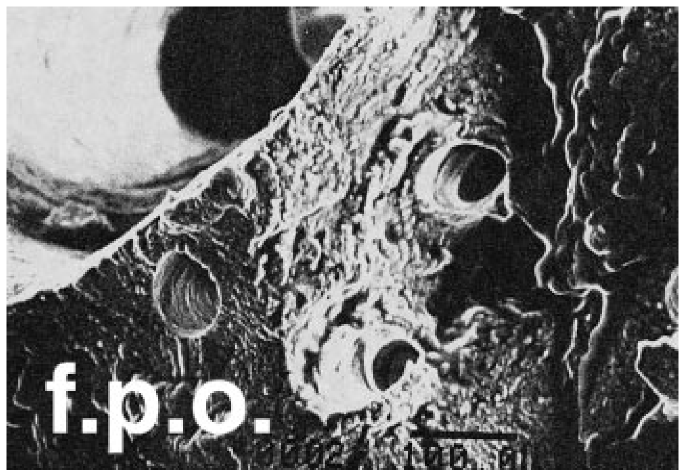

FIG. 12—*"Melting ice" pattern. (Mandibular fragment, same sample as in Figs. 8, 9, and 10) (1150°C, ×150.)*

20% concerned animals, the remainder concerned people, and the number of burned skeletons increased from year to year. Globally this author was able to identify 46% of remains with certainty and 13% with a high probability. In addition, observations on exposure of bone to fire often provide good evidence for criminal activity. For instance, the incompleteness of skeletal parts and their fragmentary nature, as well as variations in cremation, are typical of damage caused by small fires of relatively short duration. In contrast, a major house fire will reduce a body to small calcined fragments (but the body will not totally disappear). Fire may be applied in an attempt to disfigure the victim of a murder: head and face are usually targeted because of their assumed value of identification.

Color changes have been observed in this study, depending on temperature. Numerous studies have shown that regular changes in color occurred with increasing temperatures during the crematory process (10,12).

Microscopically bone has been studied by many authors, who have paid particular attention to defining its three functional areas: resting surfaces, forming surfaces, and resorbing surfaces (25), as well as the different pathological aspects in various diseases. In contrast, few temperature controlled experiments using scanning electron microscopy have been conducted. Even though a number of authors have been interested in the incineration of teeth, few works describe the microscopic appearance of burned bones. Thus Shipman et al. (10) noted some deformations under the scanning electron microscope, that were well correlated with temperature. Between 185 and 285 degrees Celsius, bone appeared to be more granular, covered with tiny asperities on the surface. Between 285 and 440 degrees Celsius, the surface looked vitrified. Between 440 and 800 degrees Celsius, it was frothy-looking. Between 800 and 940 degrees Celsius, the particles melted and mixed into ondulating polygonal structures in subchondral bone or into nodular structures in cortical bone. From 350 to 400 degrees Celsius all the organic material was destroyed, frequently bringing about fissures that tended to produce rectangular segments separated by deep clefts perpendicular to bone. At 750–800 degrees Celsius bone showed a marked change, where hydroxyapatite is reorganized into larger crystals. In this range, the organic matter is completely combusted, the bone crystals become more crystalline, merge into larger crystals and fuse together (6,10). Nevertheless bone mineral stabilizes the collagen against thermal denaturation and shrinkage (20). Holden et al. (21) observed in their experimental work on femur bone samples that the main original structural features of the mineralized bone tissue were unaltered when the specimens were heat-treated at any temperature in the range 200–600 degrees Celsius. Nevertheless the endosteum of the Haversian canal began to disintegrate at about 200 degrees Celsius. At a temperature of 600 degrees Celsius, this endosteum was completely destroyed and a new spherical crystal had formed; the diameter of these crystals decreased from about 12 to 97 years of age. Between 800 and 1400 degrees Celsius new crystals with an hexagonal morphology appeared, and the size of these crystals increased with the temperature. Above 1000 degrees Celsius fusion of the hexagonal crystals were found in localized areas, both on the Haversian canal walls and on the fractured surfaces, and between 1000 and 1400 degrees Celsius, new crystals, usually rhombohedral in shape, appeared in the majority of samples. Melting of the bone mineral and subsequent recrystallization of the bone mineral on cooling were considered to occur at 1600 degrees. These authors then applied these results to actual cremains, helping to determinate the temperature of fire and the age of the subject (26).

In the present work, we traced two types of modifications in the heated maxillary-mandibular bone that was studied: alteration of bone structure; deposition of extraneous (products of combustion) or osseous (particles of destroyed bone forming particular images) elements on bone surface. Obviously we encountered some difficulty in relating bone modification to temperature with precision. For instance, sample 3, raised to 400 degrees Celsius, looks more altered than sample 4, raised to 500 degrees Celsius. Up to 600 degrees Celsius, alterations usually are rather small, despite the progressive combustion of the organic portion of the bone tissue. Nevertheless, the quantity of deposits attached to the bone surface seems to increase from low temperatures (150 degrees Celsius) to the medium temperatures of this study (600 degrees Celsius). From 700–800 degrees Celsius, and mainly above 1000 degrees Celsius, bone structure is markedly reorganized, producing quite special appearances which we have called "field of cooled lava" or "ice solidified after melting." This could be explained by the recrystallization of the bone mineral beginning at 600 degrees, crystal growth beyond 600 degrees, fusion of crystals at 1000 (21).

Lastly, an important limit to this kind of study has to be underlined: although the experimentation is dully controlled with reproducible conditions (e.g., temperature and duration), these conditions do not correspond to a medico-legal reality. Therefore, an additional study utilizing archaeogical or forensic samples appears to be necessary, since such samples will correspond to actual conditions of cremation, within a ritual, accidental, or criminal environment. Nevertheless, in a previous study, forensic burned samples did not exhibit significant differences in the heat-induced ultrastructural changes in the fragments of incinerated bone, compared with the laboratory heat-treated bone (26).

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