Transformations of crystallographic phases in burned bones and teeth

Giampaolo Piga [1], David Gonçalves [2,3,4], T.J.U. Thompson [5], Calil Makhoul[3], Ana Amarante[3], Stefano Enzo [6]

[1] *Department of Political Science, Communication, Engineering and Information Technologies, University of Sassari. Viale Mancini 5, I–07100 Sassari (Italy).*

[2] *Research Centre for Anthropology and Health (CIAS) and Department of Life Sciences, Universidade de Coimbra. Calçada Martim Freitas, 3000-456, Coimbra, Portugal.*

[3] *Laboratory of Forensic Anthropology, Department of Life Sciences, Faculdade de Ciências e Tecnologia da Universidade de Coimbra, Calçada Martim Freitas, 3000-456, Coimbra, Portugal.*

[4] *Laboratório de Arqueociências, Direcção General do Património Cultural and LARC/CIBIO/InBIO, Rua da Bica do Marquês 2, 1300-087 Lisboa, Portugal.*

[5] *School of Science & Engineering, Teesside University, Borough Road, Middlesbrough, TS1 3BA, UK.*

[6] *Department of Chemistry and Pharmacy, University of Sassari. Via Vienna 2, I–07100 Sassari (Italy).*

ABSTRACT

We have applied spectroscopic and physico-chemical techniques to the osteometric study of four human skeletons and teeth, all the biomaterials experimentally burned in a furnace at 1100° C. This is an innovative way of tracking heat-induced changes in human bone, in terms of physico-chemical properties related with structure, which is expected to impact in forensic, bioanthropological and archaeological contexts.

INTRODUCTION

Archaeologists and Forensic anthropologists are frequently confronted with the need to study and interpret burned bones. There is a wide range of case types for which burnt bones are submitted to a forensic laboratory, including fire victims in vehicle accidents, from mass disasters and in house fires. In addition to accidental cases, we encounter homicides where the victim's body is purposely cremated and destroyed by the

perpetrator in order to obstruct the investigation.

Regardless of the context, one of the key factors for the correct interpretation of the remains and a reconstruction of the incidents leading to incineration is the estimation of the maximum exposure temperature. The recent years have seen an influx in experimental research focusing on temperature estimation, spanning from colour assessment, mechanical strength measurements, histology and structural observations, biochemical changes and crystallinity studies, vastly advancing the understanding of heat induced changes in bone, thus facilitating a more accurate interpretation.

The crucial change to occur to bone when heated is the recrystalisation of the inorganic phase. Micro-nano-structural and chemical properties of skeleton bones change drastically during burning; the micro- and nano-structural changes attending those phases manifest themselves, amongst others, in observable alterations to the bones colour, morphology, microstructure, mechanical strength and crystallinity, and it is in this area that much of the recent burned bone research has focussed.

Although still not fully understood, current research suggests that as burning intensity increases so does the extent of crystal change. This is exhibited as increasing crystal size, increasing crystalline organisation, and decreasing crystal strain within the bone.

The analysis of burned remains is a highly complex process, and with new technologies available a better insight can be gained. X-ray diffraction (XRD) and Infrared attenuated total reflectance (ATR-IR) techniques, originally developed for materials science and engineering research, are actually widely used to obtain primary material information in forensic and archaeological fields, such as the accuracy of temperature determination and the study of crystallinity. Bone which has been thermally altered shows an increase in crystallinity, exhibiting larger crystals and lower lattice strains. But crystallinity is not the only microstructural analysis that can be monitored. In fact when bioapatite is subjected to a thermal treatment, we can find also a multi–phase condition for the resultant product due to a transformation of a part of hydroxylapatite (HA) to the β–three-calcium-phosphate (β–TCP), portlandite and lime phases, detected in different percentages among bones and teeth.

The aim of this work is to show, through XRD and ATR-IR techniques, the various transformations of the apatite phase through the cremation of four human individuals at 1100° C for four hours of residence.

MATERIALS AND METHODS

In the present study, samples representative of whole body were collected from bones and teeth of four experimentally burned human skeletons (codes: *CEIXXI160*, *CCNI16*,

CCNI17, *CCNI18*), that are part of the 21st century collection of identified human skeletons of the Laboratory of Forensic Anthropology of the University of Coimbra (Portugal). This is a unique collection of experimentally burned human skeletons, that allowed us unmatched access to samples for the reported work. In total, 55 bones samples and 12 teeth were therefore collected for XRD and ATR-IR analyses.

The experimental burnings were carried out in an electric muffle (Barracha K-3, three-phased 14A model). The bones and teeth were all subjected to gradually increasing heating from room temperature to 1100° C for 4 hours of residence. The muffle was then allowed to cool down back to room temperature.

0.5 g of each sample was ball milled in a agate jar for one–minute using a SPEX mixer–mill model 8000. Our sample holder for XRD analysis is a circular cavity of 25 mm in diameter and 2 mm in depth, containing about 190 mg of powder bone. The XRD patterns were collected using Bruker D2-Phaser instrument working at a power of 30 kV and 10 mA in the Bragg–Brentano vertical alignment with a Cu–Ka tube emission (λ = 1.5418 Å).

The powder patterns were collected in the angular range 9°–140° in 2θ with a step size of 0.05°. The collection time of each pattern was pursued for 47 min.

Digitized diagrams were initially subjected to a pre–processing for qualitative phase recognition according to the programs Highscore® and Match® and then analyzed quantitatively according to the Rietveld method [1], using the programme MAUD [2].

FT–IR spectra were collected in ATR mode with a Bruker Alpha Platinum-ATR interferometer in terms of absorbance vs wavenumber ν in the range 370–4000 cm^{-1}, with a resolution of 4 cm^{-1}. Each spectrum was obtained by averaging 512 interferograms. The loose powder was dispersed inside a hole cavity of spheroidal shape with its surface aligned to the plate defining it.

RESULT AND CONCLUSIVE REMARKS

Thermally treated bones show interesting variability. On a total of 55 samples burned in a muffle at 1100° C the following mineralogical phases were found in addition to bioapatite, namely, portlandite [$Ca(OH)_2$], lime [CaO], β–tricalcium phosphate [β–TCP] and calcite [$CaCO_3$].

In detail, portlandite was found in 28 specimens (weigth fraction range from 2 to 12%), β–TCP in 14 specimens (from 2 to 32%), lime in 38 specimens (from 1 to 5%) and calcite in 7 specimens (from 1 to 4%). Only in six cases bones have remained unaltered, bioapatite 100%.

As regards teeth, only three mineralogical phases were detected in addition to bioapatite: β–TCP in 11 specimens (from 2 to 44 wt%),

lime in 4 specimens (from 1 to 4wt%) and magnesium oxide [MgO] in 4 specimens (from 1 to 3.wt%).

The ν_4 band of the phosphates present in the ATR-IR spectrum can provide a variety of support information to XRD analysis, due to numerous deformations and displacements which can suffer the shape of the band and the numerical value of the splitting factor SF, index of the bone sample crystallinity. Particularly, when the presence of β–TCP is massive (≥ 20%) the ν_4 band appears strongly deformed due to the overlap with the ν_4 band of bioapatite (see the deformation of the peak at 554 cm^{-1}), and also the SF calculation is problematic. Therefore in these cases the numerical value is very low if compared to other burned bone samples.

The presence of β–TCP phase from bones appears to be sporadic and seems to occur at high temperatures, around 1100 °C [3], and it seems to be related to the different availability of carbonates in the various samples. Conversely, in teeth we have observed a more systematic occurrence of β–TCP at temperatures as low as 750 °C [4].

The reason why β–TCP appears at relatively moderate temperature in teeth here examined with comparison to bones remains still obscure, and further studies need to be addressed acquiring information about chemical species and following the crystal structure parameters.

REFERENCES

[1] H.M. Rietveld, “Line profiles of neutron powder-diffraction peaks for structure refinement”, *Acta Crystallographica* vol. 22, 1967, pp.151–152.

[2] L. Lutterotti, “Total pattern fitting for the combined size-strain-stress-texture determination in thin film diffraction”, Nuclear Inst. and Methods in Physics Research B, vol. 268, 2010, pp. 334–340.

[3] G. Piga, G. Solinas, T.J.U. Thompson, A. Brunetti, A. Malgosa, S.Enzo, “Is X-Ray Diffraction able to distinguish between animal and human bones?” J. Archaeol. Sci., vol. 40, 2013, pp. 778–785.

[4] G. Piga, D. Gonçalves, T.J.U. Thompson, A. Brunetti, A. Malgosa, S. Enzo,. “Understanding the crystallinity indices behavior of burned bones and teeth by ATR–IR and XRD in the presence of bioapatite mixed with other phosphate and carbonate phases”, Int. J. Spectrosc., Volume 2016, Article ID 4810149 (doi: 10.1155/2016/4810149).