

CHARACTERIZATION OF ARCHAEOLOGICAL BONES FROM THE AL KHIDAY CEMETERY (CENTRAL SUDAN): STRUCTURE AND MICROSTRUCTURE OF DIAGENETICALLY ALTERED BIOAPATITE

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INTRODUCTION

Human bones recovered from archaeological contexts represent a valuable source of information about the past. Bioarchaeological, anthropological, chemical and isotopic analyses can be carried out in order to retrieve information on health, demography, age, diet, and mobility of ancient populations, as well as on the experienced environmental conditions.

The large amount of data resulted from these studies enables scientists to investigate the social, economic, and cultural transformations within human groups over time. However, the reliability of retrieved information depends on the preservation state of bone material and of its constituents.

Bone is a composite material, formed by an organic matrix (mainly type-I collagen) and a mineral phase, named bioapatite, constituted by nanocrystals of non-stoichiometric hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$), as several types of ionic substitutions in the crystal structure occur (LeGeros, 1981; Elliot, 2002). Among them, the most significant is the substitution of phosphate ions, and to a lesser extent of hydroxyl ions, by carbonates, which enter the crystal structure of bioapatite as the result of metabolic processes.

When buried, bones undergo several taphonomic and diagenetic processes that may severely alter their macro- and micro-structure as well as the chemical and isotopic composition of their constituents (Hedges, 2002). Therefore, a diagenetic study on archaeological bones, aiming to accurately determine their preservation state, is essential in order to assess the reliability of information retrieved by chemical or isotopic analyses. Moreover, the study of the alteration processes provides a better understanding of the diagenetic history of bones, of the mechanisms involved in bone alteration, as well as of the archaeological and palaeoenvironmental context.

Based on this perspective, the main aim of this research was the radiocarbon dating of archaeological human bones recovered at Al Khiday 2 - 16D4 site (Khartoum, Sudan) and to assess the reliability of results.

Radiocarbon dating is generally performed on the organic fraction of bones, but when collagen is not preserved, such as in this case study, structural carbonate incorporated in the bioapatite crystal lattice represents a valuable source of ^{14}C for bone dating (Cherkisky, 2009; Zazzo & Saliège, 2011). However, diagenetic alteration of bioapatite can significantly affect the radiocarbon determination on bones, as secondary carbonates, as well as carbon isotope exchange between bioapatite and the burial environment, occur.

In order to assess the reliability of radiocarbon dating of bioapatite, this research was developed through subsequent steps, aiming to characterize the bones preservation state under a microstructural, mineralogical, and geochemical viewpoint, and taking into account local burial and environmental conditions. Once established the model for the diagenetic alteration of bones, radiocarbon dating of bioapatite of selected samples was performed and reliability of results discussed.

The case study investigated in this research, the 16D4 site, is located near the Al Khiday village (Central Sudan), on the western bank of the White Nile, at 3.5 km from the present-day river course and 22 km South of its confluence with the Blue Nile (Fig. 1). In the Al Khiday area, a group of archaeological sites were excavated within the "El Salha archaeological project" (Usai *et al.*, 2010; Salvatori *et al.*, 2011). Under an archaeological viewpoint, the great relevance of these sites lies in the fact that they revealed the first and, so far, unique case of well-preserved stratigraphy referring to the Mesolithic period (7th millennium BC) in Central Sudan. Moreover

well-contextualized evidence of the Neolithic (second half of the 5th millennium BC), Meroitic and post Meroitic (1st century BC - 6th century AD) periods were also recovered.



Fig. 1 - Location of archaeological site 16D4, at Al Khiday, Khartoum, Sudan (from GOOGLE EARTH, version 7.1.2.2041; 15°25'27.80"N, 32°22'11.55"E, alt 106,82 km, Image Landsat, [11/02/2014]; 15°27'11.64"N, 32°24'24.81"E, alt 2,20 km, 2014 Digital Globe, [11/02/2014]).

At 16D4 site a multi-stratified cemetery has been excavated, revealing at least three burial phases belonging to different periods and covering a wide span of time during the Holocene. The oldest phase, the pre-Mesolithic, is characterized by bodies buried in a prone and elongated position, a rarely documented burial ritual, and without grave goods. A terminus *ante quem* for this burial phase was established at the first quarter of the 7th millennium BC, on the basis of the stratigraphic relationship with radiometrically dated archaeological features (Mesolithic pits); however a more precise age determination was not achieved. The site was subsequently reused as a burial ground during the Neolithic period (second half of the 5th millennium BC) and later on during the Meroitic period (1st century BC - 2nd century AD). The chronological attribution for each burial phase was indirectly determined; radiocarbon ages of the more recent burial phases were obtained on grave goods or associated material.

The archaeological record at the 16D4 site covers a wide chronological period corresponding to almost the entire Holocene. The current climate of the region is arid, however, significantly different climatic conditions, as well as several short periods characterized by rapid climatic changes at local and regional levels, occurred in the past (Williams, 2009).

The early Holocene was characterized by higher precipitation rates and environmental humidity than those currently observed, as well as higher flooding level of the White Nile, due to a northward expansion of the Indian monsoon domain. Since the middle Holocene, the progressive weakening of monsoon intensity led to rainfall decrease and overall reduction of water availability. In the late Holocene progressively drier environmental conditions occurred, up to the arid climate that now characterise central Sudan. Moreover, during the early Holocene wet phase, several short periods of rapid climatic changes, characterized by arid conditions, are attested (Williams, 2009).

Under a geoarchaeological viewpoint, 16D4 archaeological site is established on a late Pleistocene sandy ridge, corresponding to the remnants of a longitudinal sandy river bar, at the limit of alluvial sediments deposited during the very late Pleistocene and Holocene flooding of the White Nile (Zerboni, 2011). It rises few meters above the White Nile flooding plain. The area surrounding the site is marked out by dark sediments rich in

organic matter, deposited by former seasonal swamps contemporary to the Mesolithic occupation of the area (Zerboni, 2011; Williams *et al.*, 2015).

Progressive drier conditions characterized the Neolithic period, becoming more severe in the Meroitic. A further evidence of climatic changes is given also by the occurrence of a pedogenic calcrete horizon, occurring at about 30 cm under the current surface level of 16D4 site. The archaeological record is affected by the formation and development of calcrete, as some of the pre-Mesolithic graves are partially embedded in calcium carbonate concretions, whereas successive archaeological features and Neolithic and Meroitic graves are carved into the calcrete horizon. The well-preserved and accurately studied archaeological context provided a sound chronology for the anthropic activity in the area (on the basis of radiocarbon ages obtained on materials such as charcoal, wood and shell, suitable for dating), as well as a set of bone samples suitable to investigate the effects of diagenetic alteration on radiocarbon age determination.

In order to achieve this purpose, a multi-disciplinary approach to the study of bones and associated soil sediments was carried out, aiming to: *i*) define type and extent of diagenetic alteration; *ii*) assess the preservation state of bones, in particular the preservation of the biogenic composition of bioapatite; *iii*) define the interaction between bones and the burial environment, by the study of pedogenic processes occurring within soil sediments; *iv*) provide a model for the alteration process taking into account the wide chronological period covered by burial phases (almost the entire Holocene) as well as environmental and climatic changes occurring in Central Sudan along this wide span of time.

ANALYTICAL METHODS

A selected set of cortical bone samples (humeri and femurs) from each burial phase was analysed. Histological analysis and microstructural characterization of bone samples and their alteration were carried out by optical microscopy, scanning electron microscopy (SEM) and X-ray computed micro-tomography (μ -CT), in order to define, at the micro-scale, the variability of diagenetic alteration, in terms of types and extent, among different burial phases. Fourier transform infrared spectroscopy (FTIR) and micro-Raman imaging were performed on powdered and sectioned bone samples, respectively. Preservation state of collagen and of bioapatite was investigated by means of several parameters calculated from the spectra. In particular, the extent of diagenetic alteration of bioapatite was monitored by the relative carbonate content and crystallinity among burial phases. Moreover methodological issues concerning sample preparation and spectra acquisition had been tackled. As for bioapatite crystallinity, results obtained by infrared and μ -Raman spectroscopy were compared with those obtained by X-ray powder diffraction (XRPD). Rietveld refinement was applied to diffraction data in order to calculate the average size of bioapatite nano-crystals. In order to define the burial environment and the interaction between soil sediments and bones, a micromorphological study of calcitic nodules sampled from the calcrete horizon was carried out. Samples were thin-sectioned and analysed by optical and cathodoluminescence microscopy, SEM and XRPD. AMS- ^{14}C dating of selected calcrete samples was performed in order to better understand the timing of its formation and development as well as to provide a comparison with radiocarbon ages obtained on bioapatite. Once defined a model for bone diagenesis and alteration processes involved, bone samples were treated with acid solution in order to selectively remove secondary carbonates. The effectiveness of treatments was assessed by FTIR and XRPD. AMS- ^{14}C dating of bioapatite was then performed. Results are discussed in terms of reliability of radiocarbon ages, taking into account the information on bone alteration and burial environment acquired in this study as well as the information provided by archaeological, geomorphological and palaeoenvironmental investigations.

RESULTS AND DISCUSSION

Results show a poor preservation state of bones for all the burial phases. In particular collagen loss, microbially-mediated alterations (i.e., micro-tunnelling), bioapatite recrystallization, carbonate ions depletion

and secondary mineral phases precipitation were detected among samples (Fig. 2).

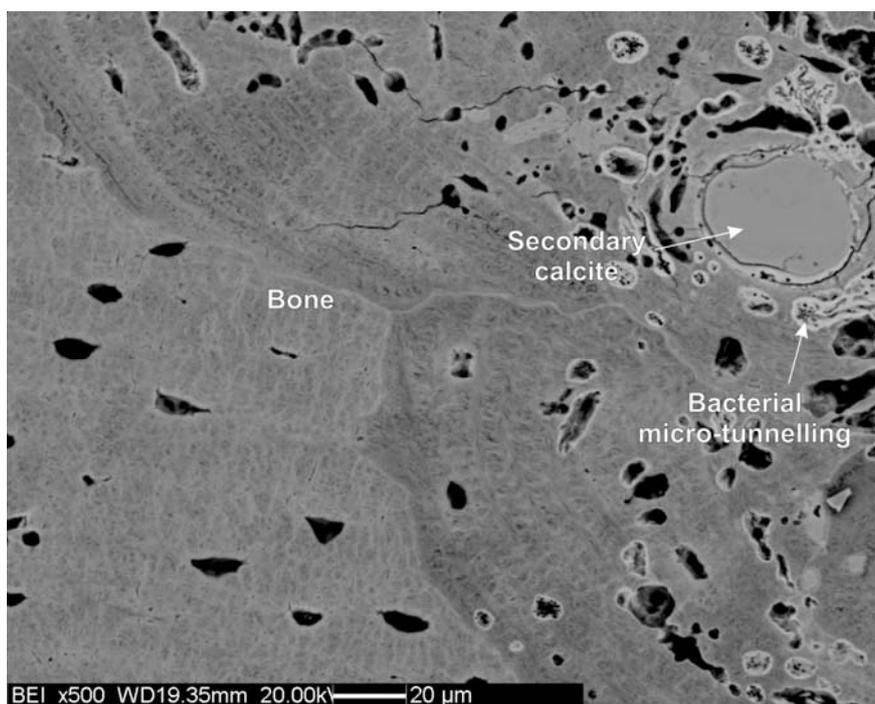


Fig. 2 - SEM-BSE image of a pre-Mesolithic femur section. Secondary calcite precipitated within the bone vascular system and micro-tunnelling due to bacterial activity are indicated.

Calcite is the most abundant secondary phase precipitated within bone micro- and nano-structures and was detected in pre-Mesolithic and Neolithic bones. Occurrence of secondary calcite within bones was related to the formation and development of the pedogenic calcrete horizon at Al Khiday. The micromorphological study of samples, coupled with AMS- ^{14}C dating of selected calcitic pedofeatures, proved that calcrete was characterised by alternate periods of growth due to dissolution and precipitation processes, and periods of quiescence or extremely slow growth rates. These processes are influenced by climatic changes as calcium carbonate accumulation in the sediments is caused by a long-lasting process characterized by subsequent events of calcite dissolution and precipitation, promoted by the progressive drying up of environmental conditions.

FTIR, μ -Raman and XRPD analyses on bones revealed extensive bioapatite recrystallization in samples belonging to different burial phases, the most recent of which are those characterized by higher recrystallization degree. Moreover, recrystallization degree is inversely proportional to the structural carbonate content in the bioapatite crystal structure.

Variations in terms of types and extent of diagenetic alteration detected among different burial phases were related to changes of climatic conditions experienced by bones during burial. In fact, the profound climatic changes, occurring in Central Sudan during the Holocene, determined significant variations over time in environmental and local burial conditions, especially in terms of humidity and amount of circulating pore water, thus involving different processes in bone diagenesis. Moreover, the detailed textural analysis of the alteration features and their relationship in terms of spatial distribution highlighted a chronological sequence of diagenetic events, which in turn were related to specific environmental burial conditions (Dal Sasso *et al.*, 2014). This provided valuable information on the interpretation of diagenetic history of bones in terms of palaeoenvironmental conditions experienced during burial, in accordance to palaeoenvironmental studies carried out in the area and in Central Sudan.

The results obtained from the study of diagenetic alteration of bones, buried during different periods in the same site, prove the stronger influence of climatic and local environmental conditions on the preservation state of bones rather than the age of burial. Moreover, this study proves the relevance of a multi-analytical approach to the study of the diagenetic history of bone material.

Based on these results, a selected set of bioapatite samples (pre-Mesolithic and Neolithic) was radiocarbon dated. The reliability of ^{14}C ages was assessed on the basis of the large amount of data supplied by the study of bone diagenesis and of burial environment, as well as by archaeological, geoarchaeological, geomorphological, and palaeoenvironmental investigations. In this case study, radiocarbon ages of bioapatite samples are not reliable, as they are much younger with respect to the reference ages for each burial phase. The main source of contaminants affecting the ^{14}C determination is, in this case, secondary calcite. Given the effectiveness of the chemical treatment applied to remove secondary carbonates (Fig. 3), contamination comes from exogenous carbonate exchanged with the biogenic one in the bioapatite structure.

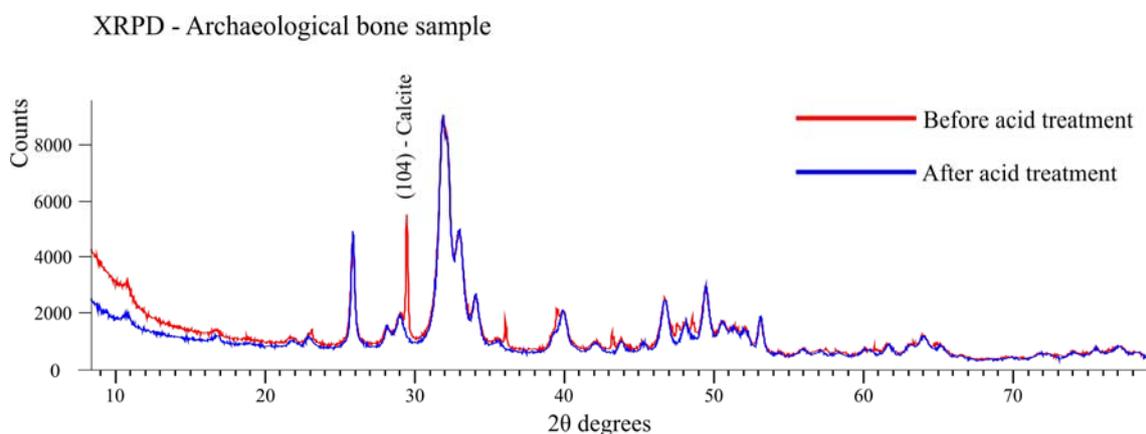


Fig. 3 - Diffractograms of an archaeological bone sample before (red) and after (blue) the acid treatment aiming to remove secondary calcite. Radiocarbon dating of bioapatite was performed after assessing the effectiveness of the acid treatment.

This is furthermore proved by the accordance between ^{14}C ages obtained on bioapatite and on pedogenetic carbonates samples.

As proven by previous studies, bioapatite is not a closed system in terms of ions exchange with the environment. In this specific case study, where bones are poorly preserved and bioapatite nanocrystals are exposed to the burial environment, as a consequence of collagen degradation, the biogenic ^{14}C activity was almost completely overprinted by that of exogenous carbonates, as carbonate-rich pore fluids circulated within soil sediments and bones.

Despite the failure of bioapatite radiocarbon dating, this research highlights the relevance and potentiality of a multidisciplinary approach to the study of the archaeological and palaeoenvironmental contexts. In fact, even if the chronological attribution of the pre-Mesolithic burial phase is still uncertain, several disciplines (mineralogy, geochemistry, histology, geoarchaeology, and geomorphology), provided valuable and consistent evidence, the analysis of which resulted in a plausible, although not certain, chronology for the pre-Mesolithic burial phase.

Therefore, taking into account evidence produced by the multi-disciplinary study on archaeological contexts, valuable information is provided not only to assess the reliability of radiocarbon ages, but also to constrain chronological hypothesis that radiocarbon dating methods cannot verify.

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