CHARACTERIZATION OF ARCHAEOLOGICAL BONES FROM CENTRAL SUDAN: STRUCTURE AND MICROSTRUCTURE OF DIAGENETIC BIO-APATITE

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Cycle: XXVII

Abstract

Bone is a composite material constituted by the association of an organic matrix and inorganic crystallites of hydroxyapatite (bio-apatite). When the organic fraction is not preserved, direct radiocarbon dating of human bones is performed on the mineral fraction; however \(^{14}C\) determinations on bio-apatite can be heavily affected by diagenetic alteration. In order to assess the reliability of radiocarbon determinations, a diagenetic study of human bones from the Al Khiday archaeological site (Khartoum, Sudan) was carried out. This case study provided a set of bone samples belonging to several burial phases, covering a wide span of time (at least 7000 years). Diagenetic alteration of bone microstructure and bio-apatite structure was investigated. Bio-apatite was successfully isolated from secondary mineral phases by chemical treatment; \(^{14}C\) determinations on bio-apatite will allow us to assess the reliability of this dating method and whether carbonate groups in bio-apatite crystal structure exchanged with the surrounding environment.

Introduction:

Bone material is constituted by an organic matrix (mainly collagen) associated with inorganic crystallites, which composition resemble the one of hydroxyapatite. Direct dating of bones by \(^{14}C\) determination is usually performed on the organic fraction but, in those cases in which collagen is not preserved, an alternative source of \(^{14}C\) can be found in the carbonate ions partially substituting phosphate ions in the hydroxyapatite crystal structure. This case study will analyze human bones coming from the archaeological site 16D4 (or Al Khiday 2) located near the village of Al Khiday (Khartoum, Sudan). The stratigraphic excavation revealed 190 graves belonging to at least three distinct burial phases: a “pre-Mesolithic” phase, which precedes a well-defined and radiometrically dated Mesolithic use of the site (6700-6300 cal BC); a Neolithic phase, dated between 4550 and 4250 cal BC; a Meroitic phase, dated between 50 BC and 210 cal AD (Salvatori et al. 2011; Zerboni 2011). A fourth burial phase identified during the archaeological excavation is not clearly chronologically defined; graves belonging to this phase were tentatively labelled “Mesolithic”. Since this unique archaeological site in Central Sudan revealed a preserved stratigraphic sequence, burial phases were indirectly dated (or chronologically constrained) by the study of associated materials and archaeological features. Given the failure of direct dating these bones on the organic matrix, as collagen deteriorated, \(^{14}C\) determination of the carbonate fraction of bio-apatite is the other approach for bone direct dating. This archaeological context provided a set of samples that suite this aim as information obtained from indirect dating can be useful to evaluate the reliability of results from direct dating. However diagenetic processes involved during burial can affect these results as alteration occurs at different scale level of the bone. Therefore a diagenetic study was carried out on these bones by defining the preservation state of bone microstructure and bio-apatite structure in order to validate the direct dating method. Moreover a one-meter section of soil from the archaeological site was sampled and studied in order to have a more complete comprehension of diagenetic processes involved, in particular to study the pedogenesis of calcium carbonate-rich horizon and its interaction with the diagenesis of bones.

Materials and methods:

Thirty graves belonging to the four different burial phases (12 pre-Mesolithic, 6 Mesolithic, 6 Neolithic, 6 Meroitic,) were selected for this study and one femur and one humerus for each grave was sampled (60 bone samples). Optical microscopy (OM), Scanning electron microscopy (SEM) and High resolution X-ray computed micro-tomography (µ-CT) analysis were performed in order to investigate the microstructure of bone sections. Fourier transform infrared spectroscopy (FTIR), by transmission and attenuated total reflectance (ATR) measurements, and X-ray powder diffraction (XRPD) were applied to
acquire information on bio-apatite structure. Rietveld refinement on XRPD data was performed with the MAUD software (Lutterotti, 2010) using the Popa model for anisotropic size-broadening of line profile in powder diffraction patterns (Popa, 1998). Three femurs were selected for radiocarbon dating of bio-apatite: samples were finely grinded and then treated with H$_2$O$_2$ (35%) for one hour in order to oxidise organic contaminants and with a solution 0.2 M of HCOOH for one hour in order to dissolve secondary calcite and carbonate contaminants and isolate the bio-apatite.

Calcium carbonate concretions, sampled from the soil section, were studied by OM, SEM and cathodoluminescence microscopy (CL) in thin section, and by XRPD. Samples were selected for radiocarbon dating in order to understand the formation process and to provide a constrain for $^{14}$C determination on bio-apatite.

**Results:**

OM, SEM and µ-CT analyses on 60 bone samples enabled us to assess the preservation state of bone microstructure and define alteration processes affecting the different burial phases. Several alteration types were identified:

- Microscopical focal destruction (MFD) caused by bacteria: bone portions characterised by intense micro-tunnelling, ranging from 0.1 to 2 µm in diameter, surrounded by hyper-mineralised rim due to dissolution and reprecipitation of hydroxyapatite are the remains of bacterial colonies;
- Microscopical focal destruction (MFD) caused by fungal activity is characterised by micro-tunnelling up to 8 µm in diameter and by the absence of hyper-mineralised rims;
- Deposition of manganese dendrites forming dark coatings (nearly 1 mm thick) on the external surface of bones, mainly constituted by birnessite and todorokite;
- Deposition of secondary calcite in bone vascular system and micro-fractures, related to the formation of calcium carbonate concretions in the soil, as a consequence of enhanced evapotranspiration during the aridification phases occurred in the region (the last phase started in Central Sudan at the end of the 7th millennium cal BC).

Observing textural relationship between those diagenetic features a chronological sequence of diagenetic events since the body deposition was established. MFD caused by bacterial and fungal activity is the first type of alteration affecting bones, presumably after few months/years after burial, and defined an early diagenetic process (Fernandez-Jalvo et al., 2010). The second event is the deposition of manganese dendrites that fill the micro-porosity of bone tissue and partially the micro-tunnelling left by bacterial activity. Subsequently calcite deposition occurs. The four burial phases here analysed cover a wide span of time, ranging from the early Holocene to the 1 millennium AD, therefore encompassing a period of profound climatic changes. In the early Holocene, North Africa was characterised by wetter climatic conditions, due to stronger monsoonal activity and increased rainfall. Numerous short dry periods occurred during the early Holocene Optimum Climaticum due to global climatic changes, leading to precipitation decrease in areas close to the tropics. By the middle Holocene gradual changes in the environmental conditions and weaker monsoonal activity lead to a progressive decrease in rainfall and water availability towards desert climatic conditions. In this study case, bodies were buried during different periods and, therefore, experienced different environmental conditions. Funeral practise of burying the dead in pits, preventing bone weathering from sub-aerial exposure and scavenging, is recorded for all burial phases. Therefore, the diachronic variation of diagenetic alterations here observed, in terms of abundance and type, is mainly due to environmental conditions during burial. Dark coatings due to manganese dendrites were observed in pre-Mesolithic and Mesolithic samples, alteration due to bacterial activity and calcite precipitation were detected in pre-Mesolithic, Mesolithic and Neolithic samples while these features are completely absent in Meroitic samples. Alteration due to fungal activity was detected in all samples. This study proved that environmental and climatic conditions strongly influence bone diagenesis. Besides these considerations, high variability in terms of extent of diagenetic alterations was observed in samples belonging to the same burial phase; nevertheless, results obtained for bones belonging to the same individual show a good agreement. Hence bone diagenesis is dependent both on the regional environmental and on the local burial conditions. Diagenetic alterations of bones belonging to the Mesolithic burial phase are more similar to those observed for the pre-Mesolithic one.
than for the Neolithic phase. This may confirm the correct identification of a fourth burial phase, possibly older than the Neolithic phase (Dal Sasso et al., 2014).

By FTIR analysis it is possible to distinguish between the contribution of secondary calcite and carbonate ions in the bio-apatite crystal structure to the IR spectra: $\nu_4$ (in-plane $\text{CO}_3^2-$ bend) vibration band is not observed in bio-apatite spectra while $\nu_2$ (out-of-plane $\text{CO}_3^2-$ bend) and $\nu_3$ (asymmetric $\text{CO}_3^2-$ stretch) vibration bands result from contribution of calcite and bio-apatite carbonate groups. Calibration curves based on calcite-bone mixtures of known weight percentage allowed us to quantify the secondary calcite content in archaeological bone samples. Moreover the contribution of calcite to the $\nu_3$ vibration band in IR spectra of bone-calcite mixture was determined. As for phosphate vibration bands, the resolution of the two peaks of the $\nu_4\text{PO}_4^{3-}$ vibration bands (605 and 565 cm$^{-1}$) expressed as infrared splitting factor (IRSF) as defined by Weiner and Bar-Yosef (1990) is related to the crystallinity of bio-apatite and it is used as a recrystallization index for bio-apatite; higher is the IRSF value, higher is the crystal size of bio-apatite crystallites. Results show a negative correlation between the bio-apatite carbonate content and IRSF, as the increased crystal size imply a more ordered crystal structure and therefore a decreased carbonate content. Archaeological bones here analysed show higher IRSF values for the Meroitic burial phase (more recent) than for the Neolithic phase, while the pre-Mesolithic (older) samples show the lowest values of IRSF, much more similar to the modern ones. This can be explained in terms of different diagenetic processes experienced by different burial phases and probably the presence of calcite prevented a stronger recrystallization of bio-apatite (Berna et al., 2004), as observed in Meroitic bones, that were buried during more arid environmental conditions. Correlation between IRSF values and crystallinity was confirmed by results obtained from Rietveld refinement of XRPD data. Higher IRSF values correspond to higher apparent crystallite size values obtained for the c axis crystallographic direction, which is the elongation direction of bio-apatite crystallites.

Two pre-Mesolithic and one Mesolithic femurs were selected for radiocarbon dating; samples were treated with $\text{H}_2\text{O}_2$ and HCOOH in order to remove organic and secondary carbonate contaminants, respectively. In particular secondary calcite has to be completely removed, as it can heavily affect $^{14}\text{C}$ determination on bio-apatite. The effectiveness of this treatment was verified by FTIR, $\mu$-Raman and XRPD. Results on bio-apatite radiocarbon dating will be discussed on the basis of the diagenetic study carried out during the PhD in order to assess the reliability of this dating method.

As for calcium carbonate concretions, OM, SEM and CL analyses show different textural features along the section: as a general trend at the bottom of the section carbonate concretions are more abundant and more compact then in the upper part. More in detail, some portions of these concretions are characterized by compact calcite with small-sized crystals (few $\mu$m, micritic-like), whereas other areas show a less dense deposition of larger-sized crystals of calcite (few tens of $\mu$m, sparite-like). CL analyses show the presence of several generations of calcite, marked by different luminescence intensity, and possibly linked to different fluid fluxes and evaporation rates through time. Preliminary radiocarbon dating of carbonate samples ranges between the 7th millennium cal. BC and the 3rd millennium cal BC, suggesting a diachronic formation of calcium carbonate concretions in the soil, in agreement with the general models of climate changes into dry conditions in North Africa. Seven selected samples of possibly different generations of calcite will be dated in order to better understand the pedogenesis of these concretions.
References


**SUMMARY OF ACTIVITY IN THIS YEAR**

**Courses:**


1st European Crystallography School, 31 August – 6 September 2014, Pavia, Italy.


**Communications:**

DAL SASSO G., ADDIS A., SECCO M., MARITAN L., ANGELINI I., USAI D., SALVATORI S., ARTIOLI G., Calcium carbonate concretions at Al Khiday site: interaction between soil, climate and archaeology, 3rd International Mortar Dating Workshop, 14-16 April 2014, Padova, Italy.

**Posters:**

DAL SASSO G., MARITAN L., ANGELINI I., ADDIS A., SECCO M., ARTIOLI G., SALVATORI S., USAI D., Bone diagenesis study on multiple burial phases at Al Khiday (Khartoum, Sudan) and interaction with soil and climate, 13th International Conference for Nubian Studies, 1-6 September 2014, Neuchâtel, Suisse.

**Publications:**

DAL SASSO G., MARITAN L., SALVATORI S., MAZZOLI C., ARTIOLI G., 2014. Discriminating pottery production by image analysis: a case study of Mesolithic and Neolithic pottery from Al Khiday (Khartoum, Sudan), *Journal of Archaeological Science*, 46, 125-143. DOI: 10.1016/j.jas.2014.03.004


**Internship:**

Kimmel Center for archaeological science – Weizmann Institute of Science, Rehovot, Israel. June 2014.