

PALEOHISTOLOGY AND THE STUDY OF HUMAN REMAINS: PAST, PRESENT AND FUTURE APPROACHES

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ABSTRACT The invention of the microscope revolutionized the course of human knowledge. This instrument changed the face of science and of previous beliefs, expanded the horizons of knowledge, and challenged philosophical and scientific thought, especially in the field of natural sciences and medicine. In the domain of bioarchaeology, the introduction of histological techniques was important; not only to identify body tissues and to diagnose diseases in mummified remains,

but also to understand bone and teeth microstructure, and associated patterns of response to environmental constraints. In this paper a critical review of the major contributions of histology to the growing body of knowledge in paleopathology and bioarchaeology will be presented, focusing on the current multiple applications of microscopy, its limitations, and its future challenges. *Rev Arg Antrop Biol* 18(2), 2016. doi:10.17139/raab.2016.0018.02.02

PALABRAS CLAVE microscopía, restos humanos, múltiples enfoques

RESUMEN La invención del microscopio ha revolucionado el curso del conocimiento humano. Este instrumento cambió la “cara” de la ciencia y de las creencias anteriores, amplió los horizontes del conocimiento y retó al pensamiento filosófico y científico, especialmente en el campo de las ciencias naturales y la medicina. En el dominio de la bioarqueología, la introducción de técnicas histológicas fue determinante, no sólo para identificar los tejidos del cuerpo y diagnosticar enfermedades en restos momificados, sino también para

comprender la microestructura y dinámica de los huesos y los dientes, y los patrones asociados de respuesta a las restricciones ambientales. En este trabajo se presenta una revisión crítica de las principales contribuciones de la histología al creciente cuerpo de conocimientos de la paleopatología y la bioarqueología, con énfasis en las actuales aplicaciones de la microscopía, sus limitaciones y los desafíos para el futuro. *Rev Arg Antrop Biol* 18(2), 2016. doi:10.17139/raab.2016.0018.02.02

Four centuries have passed since the invention of the composite microscope. With this fascinating instrument the inner face of nature, once closed off to both intuition and direct observation, was revealed to the human eye (Mayr, 1982; Wilson, 1995; Mazzarello, 1999). The microscope’s technical capabilities dramatically changed human concepts about the world, with substantial epistemological, metaphysical, and methodological implications to science (Wilson, 1995). By revealing layer after layer of very small articulated structures, the microscope gave sense to the idea of a non-occult interpretation of natural phenomena, leading to a truly remarkable “recalibration of human knowledge” (Wilson, 1995: 41). In agreement with Wilson (1995), the discovery of such a parallel microcosm offered metaphysicians the challenge to reconcile the ubiquity of life with contemporary anthropocentric views. Additionally, it revolutionized philosophical thought, shaking the foundations of previous beliefs (Mayr, 1982), and raising questions about the concepts of order and chaos, realism and the unrealistic world,

and the boundaries of human perception, as well as about the role of “instrumentally mediated knowledge” in the improvement of science (Wilson, 1995: 71).

The discovery of an entire world of microscopic living forms comprising algae, bacteria, protozoa, fungi, and viruses introduced new doubts about their origin, continuity, and possible relationship with human diseases (Mayr, 1982). In fact, it is almost impossible to talk about the findings of Gerhard Hansen (1841-1912, Norway), Robert Koch (1843-1919, Germany) and Louis Pasteur (1822-1895, France) without refer-

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ring to the value of the microscope (Kato, 1973; Ben-Menahem, 2009; Merrill, 2010). In the medical domain, the use of the microscope allowed the development of innovative methods for anatomical dissection, as complex body systems could be separated into smaller components -the tissues and the cells (Hogg, 1854; Mayr, 2004). Besides gross anatomy, physicians were now able to examine the physiology of organs and the interaction of cells (Mayr, 2004). For instance, we can mention the first descriptions of bone histology published by Antonie van Leeuwenhoek on November 20, 1720: "It is impossible for those, who have not seen this with their own eyes, to conceive the prodigious number of small vessels, of which the cortical part of the bone consists; which in some places lies no thicker upon the spongy part of the bone, than a thick hair of a man's head (...)" (p. 92). Microscopy has also made an important contribution to disease diagnosis (Hays, 2009; Merrill, 2010). Focusing on this issue, Hogg stated that "the smallest portion of a diseased structure, placed under a microscope, will tell more in one minute to the experienced eye than could be ascertained by many days' examination of the gross masses of disease in the ordinary method" (1854: vi). The usefulness of microscopy surpassed the frontiers of biology and medicine, being incorporated by other sciences. This was the case for paleopathology. In the bioarchaeological domain, the application of tissue microscopy is, by itself, deeply embedded in the history of paleopathology as a modern science (Aufderheide and Rodríguez-Martín, 1998). Presently, the microscope continues to be fundamental in the fields of biological and forensic anthropology.

This paper aims to review the role of paleohistology in the progression of the anthropological body of knowledge focusing on (1) the first incursions of microscopy into disease diagnosis; (2) the current applications of paleohistology to the study of past remains; and (3) the limits and future challenges of histology on bioarchaeological studies.

Paleohistology, disease diagnosis, and the emergence of new histological techniques

The first application of histology to the study

of ancient pathological remains -the scope of paleopathology- can be traced back to the nineteenth century. With respect to the analysis of mummified remains, this technique was pioneered by the Czech physician J. N. Czermak in 1879 to describe a case of arteriosclerosis in an Egyptian mummy (Aufderheide and Rodríguez-Martín, 1998; Aufderheide, 2003; Denton, 2008). In the beginning of the twentieth century, Sir Armand Ruffer, a professor of Bacteriology in Cairo (who is also considered the father of paleopathology), developed revolutionary methods for rehydrating mummified tissues for histological inspection (Sandison, 1967; Aufderheide and Rodríguez-Martín, 1998; Aufderheide, 2003; Denton, 2008). In order to restore the flexibility of tissues, Ruffer's method consisted in embedding sectioned material in several cycles of alkaline salts (sodium carbonate) mixed with alcohol for two to three days, followed by baths in pure reagents (alcohol, chloroform or paraffin) (Moodie, 1921). Through this innovative procedure, several pathological conditions were identified in mummified and bone remains, such as arterial diseases, diffuse anthracosis of lungs, kidney abscesses, spondylitis deformans of vertebrae, among others (Moodie, 1921). In his paper entitled "Histological studies on Egyptian mummies," presented at L'Institut Égyptien in 1911, Ruffer emphasized the importance of the systematic use of histology as a complement to the macroscopic observation of pathological changes (Moodie, 1921). Almost at the same time the eminent British pathologist S. G. Shattock studied histological sections of an aorta of an Egyptian pharaoh (Lovell, 2000). Since this early beginning, the contribution of histology to the analysis of disease in mummified bodies has been considerable (e.g. Sandison, 1955; Post and Daniels, 1969; Armitage and Clutton-Brock, 1981; Weinstein et al., 1981; Walker et al., 1987; Zimmerman et al., 1998; Ciranni et al., 1999). This growing interest was improved by new techniques for the preparation of mummified tissue (e.g. Graf, 1949; Sandison, 1955; Turner and Holtom, 1981; Hess et al., 1998; Aufderheide, 2003; Mekota and Vermehren, 2005).

Histological examination was also applied by Roy L. Moodie to the study of bone lesions on nonhuman fossil remains (Moodie, 1918a and

1918b), for example, a fracture in a rib of a reptile from the Permian of Texas (Moodie, 1918b); a hemangioma on two caudal vertebrae of an *Apatosaurus* from the Comanchian (Moodie, 1918a and 1918b); and a case of osteoperiostitis observed on a humerus of a *Mosasaur* from the Cretaceous (Moodie, 1918a and 1918b). Apart from applying this new diagnostic technique, Moodie also introduced the concept of paleohistology in 1926 (Garland, 1993). Nevertheless, it was not until 1949 that a proper definition was proposed by Wilhelm Graf, who described paleohistology as “the examination of microscopic sections of ancient human beings and the recognizing of tissues and cells in such sections” (1949:236). With regard to the study of archaeological human bone, the first histological analysis was undertaken by the American pathologist Theophil Mitchell Prudden in 1891 (Garland, 1993). Prudden used microscopy to confirm a possible case of syphilis observed in two adult tibiae exhumed from the prehistoric site of Animas River, Colorado, whose microstructure revealed chronic periostitis and osteomyelitis. Another historical reference concerning the application of histology to skeletonized remains is attributed to Carl Magnus Fürst who diagnosed, in 1920, a case of periostitis ossificans in the tibia of King Magnus of Sweden (thirteenth century AD) (Graf, 1949). In 1927, M. Weber developed a new microscopic technique to distinguish inflammatory bone lesions linked to presumed syphilitic cases (Graf, 1949; Schultz, 1997). In spite of Weber’s efforts to standardize diagnosis, little attention was paid to his work (Schultz, 1997). A year later, H. U. Williams described an experimental method for the study of osteoporotic bones that consisted in embedding fragile bones in celloidin and then decalcifying them in an acid solution (Graf, 1949). Focusing on the preservation of mummified and dry bone remains, Graf (1949) performed a comparative study on three Egyptian mummies and fifteen Swedish skeletons from different chronological periods, concluding that there were no parallels between the chronology and the microscopic preservation of remains (an assertion that is still valid today, see e.g. Stout and Teitelbaum, 1976; Schmidt-Schultz and Schultz, 2007). No pathological indicators were recorded by the author. Besides comparative histomorphology,

Graf also tested other methods. Regarding the preparation of dry bone specimens, he adopted the classical histopathological procedures used by clinicians that consisted in bone decalcification (nitric acid), paraffin-embedding and staining techniques (Graf, 1949).

Despite all the medical improvements associated with the use of light and polarized microscopy, as well as in techniques for the decalcification, paraffin wax embedding, and handgrinding of human bone, the histological analysis of archaeological bone and teeth remained underdeveloped until the mid-1990s (Garland, 1993). Two main reasons may explain this lack of interest: firstly, the emphasis placed, in bioanthropological studies during the first half of the twentieth century, for example, on the description of skull morphology and subsequent “racial” classification (Garland, 1993; Armelagos and Van Gerven, 2003); and, secondly, some technical limitations dictated by the nature of the samples. The physical properties of archaeological bone and teeth fall between those of fresh and fossil origin: they do not possess the elasticity that characterizes living tissues, nor the hardness of fossil remains; a fact that causes considerable difficulties during bone sectioning (Turner-Walker and Mays, 2008). The situation began to change in the 1950s with the introduction of plastic embedding techniques for the preparation of thin sections and new microscope devices (Herrmann, 1993; Turner-Walker and Mays, 2008). Shortly afterwards, the feasibility of plastic embedding media started to be investigated, with researchers trying to find the quickest and most advantageous method to produce bone samples (e.g. Arnold and Jee, 1954; Woodruff and Norris, 1955; Pugh and Savchuck, 1958). Focusing on the preparation of thin undecalcified bone sections, Frost published, in 1958, the guidelines for an innovative method characterized by its simplicity, cheapness, rapidity, and dependability. Instead of being fixed, embedded, dehydrated, and heated -as in the previous methods-, bone was simply ground on both sides using abrasive paper on a plate of glass gently moistened with water (Frost, 1958). In 1968, Frost and Hoyte published two distinct papers concerning the use of staining-labelling techniques (*in vivo*) to evaluate the rates of bone formation and resorption in undecalcified bone samples.

Although these innovations were first used in the field of medicine, they were rapidly adapted to the study of ancient remains. In 1969, the results of one of the first applications of the electron microscope to the analysis of pathological tissues from Egyptian and Peruvian mummies were presented by Macadam and Sandison (1969). Criticizing the pioneering methods of decalcification of ancient human bone, Stout and Teitelbaum (1976) proposed a new method using plastic embedding substances (methyl methacrylate). According to these authors, this technique provided excellent preservation of bone microstructure, allowing for the diagnosis of many systematic diseases and deficiencies (Stout and Teitelbaum, 1976). Two years later, Pawlicki (1978) described an alternative method combining grinding and staining techniques for the preparation of fossil bone for light and transmission electron microscopy. In 1988, a rapid method for producing stained undecalcified bone sections was presented by Emmanuel (1988). A similar work based on methyl methacrylate embedding and staining protocols was published by Sterchi and Eurell (1989). Two years later, Maat (1991) used a scanning electron microscope to compare the ultrastructure of both normal and pathological red blood cells with pseudopathological structures. The same technology was applied by Wakely et al. (1991) to the study of rib lesions. In 1995, Abou-Arab and coauthors published a technical note concerning the importance of staining in the study of secondary osteons. Caropreso et al. (2000) made available a simple method based on resin embedding (epoxy resin), cutting and mounting procedures that could be applied to both modern and archaeological remains. A year later, a revised and modified version of Frost's rapid manual method was proposed by Maat and coauthors (2001). In the same year, an innovative method for the morphological study of fungi and bacteria contaminating ancient human bone that combined resin embedding (Impex "three-phase" polyester resin) and staining techniques was presented by Dore et al. (2001). In the meantime, several studies were conducted to test the feasibility of the available methods for the study of dry bone samples (e.g. Beauchesne and Saunders, 2006; Martiniaková et al., 2006). Recently, a new method for embedding, saw-

ing, grinding, and staining was proposed by De Boer et al. (2012 and 2013a) in order to study undecalcified archaeological bone samples, especially when lesions were present. In addition to the improvements in the preparation of samples, other sophisticated microscope techniques were introduced to the study of archaeological remains—namely, the atomic force microscope (Thalhammer et al., 2001), the epifluorescence microscope, microscopic computerized tomography, and the confocal laser scanning microscope (Kuhn et al., 2007; Rühli et al., 2007; Maggiano et al., 2009; Šefčáková et al., 2001).

Paleohistological analysis: examples of its application

A review of the bioarchaeological literature permits the identification of several studies that base their methodological framework on histological techniques. In the following pages, a short overview of the multiple applications of histology will be provided.

Differentiation between human, nonhuman remains, and other structures

Histology is a useful tool to distinguish between human and nonhuman bone remains, especially when bone is fragmentary (e.g. Harsányi, 1993; Hillier and Bell, 2007; Croker et al., 2009; Greenlee and Dunnell, 2009; Mulhern and Ubelaker, 2012). This differentiation is possible because humans have a scattered distribution of cortical osteons, as well as of primary bone types, when compared with other mature mammals that have a plexiform pattern (e.g. Pfeiffer, 2000; Cuijpers, 2006; Pfeiffer, 2006; Cattaneo et al., 2009) and show osteon banding (Mulhern and Ubelaker, 2001; 2012). Apart from human and nonhuman primates (i.e. chimpanzees), which share a similar bone microstructure and age-related changes (Mulhern and Ubelaker, 2003), the distinguishing features of bone histomorphometry also allow for the taxonomic classification of zooarchaeological remains (e.g. Barnes et al., 2000; Miles, 2001; Horni, 2002; Dittmann, 2003; Dittmann et al., 2006; Martiniaková et al., 2007; Paral et al., 2007). Histology has also played a role in the identification of strange bodies re-

covered from human skeletal remains, such as renal and biliary calculi (e.g. Morris and Rodgers, 1989; Sanchez and Etxeberria, 1991), calcified tissues and organisms (e.g. Perry et al., 2008; Quintelier, 2009), fossilized body fluids and faecal deposits (Maat, 1991; Blondiaux and Charlier, 2008; Shillito et al., 2011), and parasites and contaminating substances (e.g. Oh et al., 2010).

Taphonomic processes and identification of burned remains

When bone is exposed to the burial environment it may experience structural changes induced by physical, chemical and biological agents (Stout, 1978; Garland, 1993; Bell et al., 1996; Schultz, 1997; Collins, 2002; Jans, 2008; Turner-Walker and Jans, 2008; Reiche et al., 2010). The study of postmortem alterations is important to differentiate decomposition phenomena from normal physiological processes and disease lesions (e.g. Stout, 1978; Lynne, 1990; Grupe and Dreses-Werringloer, 1993; Turner-Walker and Jans, 2008). In this area, microscopy has been used to evaluate the integrity of bone microstructure in different environmental contexts (e.g. Stout, 1978; Hermann, 1986; Hanson and Buikstra, 1987; Garland, 1993; Hedges et al., 1995; Bell et al., 1996; Nicholson, 1998; Nielsen-Marsh and Hedges, 2000; Roberts et al., 2002; Jans et al., 2004; Guarino et al., 2006; Schmidt-Schultz and Schultz, 2007; Tersigni, 2007; Monsalve et al., 2008; Jans, 2008; Turner-Walker, 2008; Turner-Walker and Jans, 2008; Bell, 2012; Hollund et al., 2012). In zooarchaeological studies it also contributes to understanding the diagenetic processes that affect buried bones and teeth (Haynes et al., 2002; Stutz, 2002). Microscopic methods are also of great value for examining pathological conditions and estimating age at death of cremated remains (e.g. Holden et al., 1995; Schultz, 1997; Hanson and Cain, 2007; Squires et al., 2011).

Estimation of age at death in skeletonized human remains

Several histological methods have been developed to estimate age at death in both ancient

and modern bone and dental remains. Bone growth, modeling, and remodeling are responsible for a mature cortex with particular features that can be quantified using histomorphometric analysis (Schultz, 1997; Pfeiffer, 2000; Robling and Stout, 2008; Stout and Crowder, 2012); that is, the quantitative study that consists in counting or measuring tissue components: cells or extracellular constituents or both (Boivin and Meunier, 1993: 137). Histological indicators of age are based on the grade of remodeling of osteons and their respective quantification in adult cortical bone (Simmons, 1985; Frost, 1987; Stout and Paine, 1992; Mulhern and Ubelaker, 2003; Streeter, 2012). Different skeletal elements have been considered in these studies, namely, ribs and clavicle (e.g. Stout and Paine, 1992; Stout et al., 1996; Crowder and Rosella, 2007; Kim et al., 2007; Pavón et al., 2010; Cho and Stout, 2011), long bones (e.g. Kerley, 1965; Singh and Gunberg, 1970; Kerley and Ubelaker, 1978; Pfeiffer, 1980; Stout and Gehlert, 1982; Frost, 1987; Stout and Stanley, 1991; Wallin et al., 1994; Ericksen, 1991 and 1997; Ericksen and Stix, 1991; Lynnerup et al., 2006; Maat et al., 2006b; Chan et al., 2007; Robling and Stout, 2008; De Donno et al., 2009; Han et al., 2009; Villa and Lynnerup, 2010), and ilium (e.g. Boel et al., 2007). The reliability of using weight-bearing bones, as well as the effect of intrinsic (sex and population variability) and extrinsic (adequate bone sampling) factors on age at death estimation, has also been discussed in the literature (e.g. Aiello and Molleson, 1993; Drusini, 1996; Iwaniec et al., 1998; Macho et al., 2005; Paine and Brenton, 2006; Robling and Stout, 2008; Henning and Cooper, 2011). Dental histological techniques have been developed on the basis of the study of secondary dentin formation, cementum annulation (e.g. Charles et al., 1986; Wittwer-Backofen et al., 2004; Maat et al., 2006a; Roksandic et al. 2009), striae of Retzius in enamel and daily cross striations (e.g. FitzGerald and Saunders, 2005; Martin et al., 2008), and root dentine translucency (e.g. Chandler and Fyfe, 1997). These methods have also been applied to the study of faunal remains (e.g. Beasley et al., 1992; Burke and Castanet, 1995; Wendy, 1998; Dirks et al., 2002). With regard to the cementum annulation technique, some publications question its reliability in the

estimation of age at death in human remains (e.g. Miller et al., 1988; Renz and Radlanski, 2006).

Ontogeny, phylogeny, and the skeletal response to biomechanical stress

During the individual's lifetime, the skeleton has the capacity to adapt to biomechanical stress, increasing bone mass when activity increases or reducing bone tissue as a result of inactivity (Schultz, 1997; Pearson and Lieberman, 2004; Peck and Stout, 2007). Through histomorphometric analysis it is possible to infer strain levels and bone dynamics within populations or among human groups not only of different chronologies and geographic provenance, but also with different modes of subsistence (i.e. hunter-gatherers vs. agriculturalists) (e.g. Martin and Armelagos, 1985; Martin et al., 1987; Mulhern, 2000; Cho et al., 2006; Drapeau and Streeter, 2006). Histology also provides insights into the dynamics of bone growth and remodeling over the course of human ontogeny and evolution (e.g. Martin and Armelagos, 1979; Oyen et al., 1979; Abbott et al., 1996; Gosman and Ketcham, 2009). Furthermore, it allows for the development of standards for comparison with other nonhuman primates (e.g. Schaffler and Burr, 1984; Havill, 2003; Mulhern and Ubelaker, 2003). A similar contribution is made by the histological study of dentition (e.g. Molnar et al., 1981; Hildebolt et al., 1986; Mann et al., 1991; Anemone et al., 1996; Hillson and Bond, 1997).

Mummy studies

The analysis of mummified tissues was one of the first applications of histology to the study of ancient remains. Nowadays it continues to fascinate researchers in a variety of subjects that range from the simple identification of soft tissues (e.g. Post and Daniels, 1969; Walker et al., 1987; Shin et al., 2003; Mekota et al., 2005; Kim et al., 200) to the study of abnormal lesions and taphonomic changes (e.g. Brothwell et al., 1969; Bellard and Cortés, 1991; Ciranni et al., 1999; Ciranni and Fornaciari, 2004; Bianucci et al., 2008; Aufderheide, 2011).

Bone paleopathology

The long partnership between paleopathology and histology is clear from the literature, and it seems to hold much promise for the improvement of disease diagnosis. Nevertheless, the application of histological techniques to the study of dry bone remains seems to be residual in comparison with other technical approaches. In a systematic review of published literature in English, De Boer and coauthors (2013) found that few studies (n= 57) have used histology as a paleopathological tool for differential diagnosis. Similar results were obtained by Assis (2013) in a literature review of articles written in English and French. Of a total of 5872 reference papers identified, only 94 focused on the paleohistological analysis of dry bone conditions (Assis, 2013). In most of the papers considered, histology was used as an auxiliary diagnostic tool, and not as a primary source for paleopathological evidence, mainly in the study of metabolic conditions, 34% or (32/94) (e.g. Stuart-Macadam, 1987; Bell and Jones, 1991; Aaron et al., 1992; Brickley and Ives, 2006; Brickley et al., 2007); benign and malignant tumors, 27% or (25/94) (e.g. Eshed et al., 2002; Luna et al., 2008); and specific and non-specific infectious diseases, 23% or (22/94) (e.g. Wakely et al., 1991; Von Hunnius et al., 2006; Weston, 2009). The lowest values were obtained in the analysis of combined pathologies (5.3%) and bone trauma (4.3%) (e.g. Kuhn et al., 2007; Rühli et al., 2007). The low specificity of some bone histomorphometric features (Weston, 2009; Van der Merwe et al., 2010), in addition to the invasive nature of most histological techniques (Bell and Piper, 2000; Ortner, 2003; Turner-Walker and Mays, 2008; Pfeiffer and Pinto, 2012), and the high level of scientific proficiency needed to interpret bone morphology at the microscopic level (Bell and Piper, 2000; Schultz, 2012) are the factors most frequently pinpointed to justify the reduced application of paleohistology as a diagnostic technique in paleopathology.

Tooth alterations and bone cut marks

Histological techniques have been applied to the analysis of tooth remains to make inferences about diet, behavior, and the impact of

physiological stress on an individual's development. Dental microwear texture is studied in order to assess and compare dietary habits and seasonal changes in food resources among fossil hominins (e.g. Puech and Albertini, 1984; Lalueza et al., 1996; Ungar et al., 2006; Estebarez et al., 2009), modern humans (e.g. Bullington, 1991; Teaford and Lytle, 1996; Schmidt, 2001; Mahoney, 2006; Patrick, 2006; Mahoney, 2007; Hogue and Melsheimer, 2008; Ma and Teaford, 2010), and nonhuman primates (e.g. Ryan, 1979; Gordon, 1982; Teaford and Runestad, 1992; Teaford et al., 1996; Nystrom et al., 2004; Scott et al., 2006). Certain patterns of microwear also reveal information about behavioral or "cultural" practices linked to the use of teeth as a third hand (e.g. Fox and Frayer, 1997; Ungar and Spencer, 1999; Minozzi et al., 2003; Lozano et al., 2008). Microscopic examination of linear enamel defects, such as Wilson bands, is also an important tool for understanding the physiological mechanisms responsible for growth disruptions during dental development (e.g. Rose, 1977; Rose et al., 1978; Marks and Rose, 1985; Goodman and Rose, 1990; King et al., 2005; Witzel et al., 2008).

Distinct microscopic techniques, such as scanning electron microscopy, have also been employed in the study of cut marks in human and nonhuman bone remains. These techniques are important to obtain evidence of hacking trauma and dismemberment in past populations and forensic contexts (Hutchinson, 1996; Ubelaker, 1998; Cox and Bell, 1999; Haverkort and Lubell, 1999; Bartelink et al., 2001; Tucker et al., 2001; Alunni-Perret et al., 2005); traces of defleshing in hominin fossil remains (e.g. White, 1986); and marks of butchering on faunal remains and their role in understanding the evolution of hominin handedness (e.g. Shipman and Rose, 1983; Bromage and Boyde, 1984; Bello and Soligo, 2008; Pickering and Hensley-Marschand, 2008). Cutmark micromorphology is also considered in the study of surgical or ritual bone incisions, such as trephinations, and their differentiation from taphonomic changes (e.g. Stevens and Wakely, 1993; Fabbri et al., 2012).

Forensic anthropology

As in past population studies, there is a con-

siderable body of knowledge in the literature concerning the use of microscopy in forensic research. In commingled, fragmented, and burned bone or teeth remains, histological analysis may be useful to distinguish human from nonhuman remains (e.g. Cattaneo et al., 1999, 2009; Hillier and Bell, 2007). Furthermore, histology can be pivotal to determine the presence of bone or teeth in remains thought not to be of skeletal origin and vice versa (Ubelaker, 1998). In the particular case of cremated remains recovered from civil or criminal contexts, the use of microscopy can also help in the identification of non-bone material, as well as in the evaluation of particle morphology to assess age at death and associated constraints (Ubelaker, 1998). The microscopic identification of bone inclusions, such as desiccated insect parts, may provide clues about time since death or postmortem events (Ubelaker, 1998). Other possible applications concern the study of taphonomic changes (e.g. Bell et al., 1996), the assessment of age-at-death estimation (e.g. Maat et al., 2006b; Crowder and Rosella, 2007; Kim et al., 2007; Han et al., 2009), and trauma analysis (e.g. Bartelink et al., 2001; Alunni-Perret et al., 2005). In the scope of trauma investigation, the microscope plays a key role in: (1) differentiating trauma lesions from postmortem phenomena and developmental defects; (2) classifying the origin of trauma signs; and (3) identifying the nature of bone inclusions (for a more comprehensive review see Ubelaker, 1998).

Paleontological studies

Since the pioneering works of Roy Moodie, the application of paleohistological techniques continues to be fundamental to the analysis of nonhuman fossil remains. In addition to the classic transmitted light approach (brightfield or polarization), other methodologies are currently being used to examine fossil material (e.g. bone, teeth, ossified tendons, and eggshells) and to explore questions regarding the physiology, ontogeny, pathology, functional anatomy, and behavior of extinct vertebrates (Lamm, 2007). Of the abovementioned issues, life history patterns, evolutionary mechanisms, and intra- and inter-skeletal variation in growth, aging, and maturation are the features most commonly ana-

lyzed in dinosaur *taxa* (e.g. Padian et al., 2001; Sander et al., 2004; Griebeler et al., 2013; Lee and O'Connor, 2013; Stein et al., 2013; Cullen et al., 2014).

Paleohistology: current limitations and future challenges

The contribution that the microscope has made to the development of science is enormous and difficult to express or quantify in numbers. Presently, cells, microorganisms, molecules, and nanoparticles are regarded as acquired evidence and used freely in the scientific discourse. The microscope changed the face of science and continues to be fundamental to the development of human knowledge. Year after year, new more sophisticated microscopes are being developed for use not only in biological and medical studies, but also in geology, paleontology, engineering, and physics. In medicine, histopathological analysis is crucial for the characterization of lesions and subsequent differential diagnosis. Nevertheless, the use of histological techniques in the study of past remains continues to be non-systematic. One of the common limitations attributed to paleohistology is its invasive and apparently destructive nature (Grupe and Garland, 1993; Ortner, 2003). Many museum curators and osteologists show an understandable reluctance to damage or allow the destruction of skeletonized remains in order to obtain samples (Turner-Walker and Mays, 2008). Bell and Piper (2000) explain this generalized feeling as the result of a misinterpretation of the meaning of the words “invasive” and “destructive”. In many museums’ sampling protocols, invasive analysis is used as a synonym for destructive handling, which is not completely true because a definitive sampling section can be preserved and archived indefinitely (Bell and Piper, 2000). Highlighting the role of histological collections, Spatola and coauthors (2012) have recently published a work describing the potential of using one of the largest bone slide collections in the world (with more than 10,000 slides of stained and undecalcified bone and joint specimens), housed at the Anatomical Division of the National Museum of Health and Medicine (NMHM) at the Armed Forces Institute of Pathology (AFIP) in Washington, DC, for research

in paleopathology, bioarchaeology, and forensic science. Similar histological collections are curated at European universities and museums, for example, the Collection of the Department of Legal Medicine at the University of Vienna, in Austria, and the Collection of the Department of Pathology at the University of Göttingen in Germany (Schultz, 2012). According to Pfeiffer (2000), the progress of paleohistology is dependent on a change in the curatorial rules. Before denying access to skeletal collections, curators and researchers should be aware that the preparation of thin sections involves the transformation of material rather than its destruction (Pfeiffer, 2000). Sampling bones showing slight postmortem damage and/or small bone elements such as ribs, metacarpals or metatarsals is regarded as a solution to expedite lab work and reduce the degree of disruption of curated material (Pfeiffer, 2000). However, this solution may impose some constraints on the research design since it limits the spectrum of analysis. The presence of postmortem damage at macroscopic level may eventually pose an additional problem. Nevertheless, some studies have shown that, despite the diagenetic changes, a considerable amount of information can be gathered from histological analysis (Bell and Piper, 2000). To compensate for bone sectioning, Schultz (2012) proposes the use of a true-to-life cast or a plaster complement to replace the removed bone pieces. This procedure was used by Wapler et al. (2004) after the extraction of skull samples that presented *cribra orbitalia*. In that case, the sample gaps were filled with pieces of tightly adjusted plaster (Wapler et al., 2004).

Another drawback concerns the highly time-consuming nature of histological techniques, which require expensive and sophisticated supplies (Schultz, 2012). This generalization is not entirely true because there are simple methods, like those published by Frost (1958) and Maat et al. (2001), that researchers can easily reproduce. The selection of a particular method is, of course, not random and depends on extrinsic factors (design and goals of the research) as well as on intrinsic ones (preservation of bone samples). Whenever bone samples are small or belong to fragile skeletonized pieces, the use of more time-consuming techniques in order to guarantee high quality thin-sections is preferred (Schultz, 2012).

The last and perhaps one of the greatest obstacles to paleohistological analysis is the researchers' lack of training. This limitation has been substantially discussed with regard to the reliable examination of paleopathological specimens (Bell and Piper, 2000; Pfeiffer and Pinto, 2012; Schultz, 2012), as well as in relation to the application of distinct methods for bone, teeth, and mummified tissue analysis. For instance, in a comparative study of seven methods for age-at-death assessment, Baccino and coauthors found that the histological "osteon" technique is more vulnerable to the experience of the observers, when compared with other approaches, such as the dental Lamendin method, concluding that the training and experience of the researchers is pivotal to reduce bias and inaccurate analysis. A deep understanding of the typical mosaic patterns found in normal and pathological cortical and trabecular bone (and in other preserved body tissues) throughout life could certainly make the interpretation of dry bone remains easier (Heuck, 1993).

The development of less invasive techniques is helping to reduce the main limitations frequently attributed to the histological study of skeletal remains. The synchrotron radiation X-ray microtomography, which is characterized by limiting the degree of direct contact with bones, is being applied to the study of fossil remains (e.g. Tafforeau et al., 2006; Anné et al., 2014; Sánchez et al., 2014). For example, Sanchez and colleagues (2012) have used phase-contrast synchrotron microtomography to provide new 3D insights into the submicron-scale histology of fossil and recent bones. With this analytic approach, it was possible to see numerous bone features, such as vascular spaces and bone cell lacunae and canaliculi housing the dendritic processes of the osteocytes in some extant fossils. Other embedded bone features imperceptible to the conventional micro-CT scan (e.g. growth arrest lines, resorption surfaces, and extrinsic fibers) were also revealed through 3D synchrotron analysis (Sanchez et al., 2012). In spite of being non-invasive, recent studies have reported bone and teeth color changes caused by exposure to synchrotron radiation. This is more frequent in diagenetic bones and it affects their mineral content (Richards et al., 2012). These observations indicate

that, to some extent, the synchrotron may cause some degree of physical damage (Richards et al., 2012). Another disadvantage of synchrotron microtomography is imposed by its size and the complexity of the infrastructure required to accommodate all the equipment. The use of circularly polarized microscopy (CPLM) may also greatly contribute to a better understanding of bone arrangement at a histological level. For instance, Goldman and colleagues (2008) have successfully applied circularly polarized light microscopy (CPLM) to study the pattern of collagen fiber orientation and its relation to biomechanical forces in a modern sample of mid-shaft human femurs. The portable confocal scanning light microscope (CSLM) constitutes another promising technique. As a non-destructive and portable technique, it has the advantage of not requiring sample preparation and it can be transported to the place where the subjects of study are stored (Bromage et al., 2005). This innovative technique has been used, for example, in the study of early hominin skeletal remains, such as the molar teeth of *Australopithecus africanus* and *Paranthropus robustus* (Lacruz et al., 2006).

In the future, a more frequent and systematic application of such techniques as portable confocal scanning light microscope (CSLM), circularly polarized light microscopy (CPLM) or even synchrotron radiation X-ray microtomography will possibly overcome some of the current limitations identified in the study of past remains, which may lead to a more extensive application of histology in bioarchaeology and allied disciplines.

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