Palaeogeography, Palaeoclimatology, Palaeoecology 310 (2011) 23–31

Contents lists available at ScienceDirect

Palaeogeography, Palaeoclimatology, Palaeoecology

journal homepage: www.elsevier.com/locate/palaeo

Estimating the chance of success of archaeometric analyses of bone: UV-induced bone fluorescence compared to histological screening

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ARTICLE INFO

Article history:
Received 6 April 2010
Received in revised form 2 March 2011
Accepted 18 March 2011
Available online 24 March 2011

Keywords:
UV fluorescence
Screening
Bone diagenesis
Histology
Biomolecules

ABSTRACT

For most archaeometric analyses on archaeological bone material, such as the determination of the isotopic composition or genetic approaches, an advanced degree of diagenetic alteration can make designated analysis impossible. Since the lack of a positive signal is mostly seen only after time consuming and cost intensive sample processing, the need for an easy-to-apply screening method that allows a pre-selection of samples containing well-preserved biomolecules is obvious.

In this study, we visually determined the UV-induced autofluorescence of 76 horse bone cross-sections, all from prehistoric archaeological sites of varying environmental and chronological background. In order to assess the screening potential of this method, the macroscopic fluorescence appearance of each sample was compared to its degree of histological preservation, a feature which is also commonly utilised as a marker for overall biomolecular preservation in bone. Collagen content and quality as well as PCR-success for DNA analysis were determined and evaluated with regard to the positive/negative predictive value of UV fluorescence and histological screening. The aim was to create a screening method designed not only for daily laboratory practice, but also for archaeologists with no access to elaborate machinery and who need to pre-select the most promising samples to send out to a contractor for archaeometric analyses.

1. Introduction

Archaeological skeletal remains are a major source for biomolecular and archaeometric analyses, such as ^{14}C-dating, isotope studies and ancient DNA research. In all cases, diagenetic alteration can have an impact on the reliability and reproducibility of the data obtained or even result in a complete lack of data — often only seen after a sample has been processed. This is very unfortunate, as most analytical methods are not only time and cost intensive but also destructive. Thus, an ex ante estimation with regard to the chance of successful analysis would be helpful in practice. An expedient pre-selection of specimens preceding the actual analysis and using an easy-to-determine indicator (screening) would provide an efficacious tool in archaeometry.

The influence of diagenetic processes on bone has been subject to much research work (e.g. Hedges, 2002; Tuross, 2002; Jans et al., 2004; Nielsen-Marsh et al., 2007; Smith et al., 2007). Also, several techniques have been suggested for assessing the potential of retrieving biomolecules (e.g. Poinar and Stankiewicz, 1999; Nielsen Marsh et al., 2000; Stutz, 2002; Hiller et al., 2004), and particularly many studies focus on an indicator that can increase the likelihood of recovering DNA effectively (e.g. Arroyo-Pardo et al., 2002; Smith et al., 2003; Burger et al., 2004; Guarino et al., 2006). However, the suggested techniques are often comparatively complex and expensive, for example the determination of the degree of amino acid racemisation (Poinar et al., 1996; Bada et al., 1999) or small angle X-ray scattering (Hiller et al., 2004). There have been numerous studies focusing on the evaluation and quantification of diagenetic bone alteration, using microstructural traits to depict the overall preservation of bone tissue (e.g. Jans et al., 2002; Turner-Walker et al., 2002; Turner-Walker and Syversen, 2002). Still, there is no recent study that offers a simple and reliable sample screening method with regard to the prediction of microstructural preservation without the use of a microscope. The microstructure as used in histological analyses is often applied for biological age determination (Robling and Stout, 2008), species determination (e.g. Cuijpers, 2006) and as a diagnostic tool in the case of paleopathology (Schultz, 2001). Even though histological analyses are not as expensive as the investigation of biomolecules, they are nonetheless quite time consuming, for the

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doi:10.1016/j.palaeo.2011.03.021

preparation of thin sections in most cases requires vacuum embedding in epoxy resin. This procedure might take up to 1 week before the sample can be cut or ground (e.g. Garland, 1989; Bell et al., 1996; Jackes et al., 2001; Jans et al., 2002, 2004). Consequently, a simple method to pre-select samples with a microstructure that still retains diagnostic features would be helpful to increase the success of the work. Therefore, we suggest UV-stimulated fluorescence as an indicator for microstructural and biomolecular preservation in bone. UV autofluorescence of bone tissue has been in the focus of research mainly from the 1950s to the 1970s. Looking for a simple method to date bone finds according to their post-mortem interval, UV fluorescence analysis was investigated in the context of applications in forensics (Berg and Specht, 1958; Berg, 1962; Knight and Lauder, 1969; Facchini and Pettener, 1977; Hunger and Leopold, 1978). After this time, only few publications took up the possible exploitation of fluorescent characteristics of bone tissue (Yoshino et al., 1991; Imaizumi, 2002), thus major literature sources are rather old.

Polished bone cross-sections fluoresce differentially when exposed to UV-light: modern samples display a light blue/whitish luminescence across the whole section (Bachman and Ellis, 1965), while older material additionally or exclusively shows brown, yellow, and grey fluorescence (e.g. Eyman, 1964). In order to understand the mechanisms that can change bone fluorescence it is necessary to explain why bone fluoresces at all and which of the bone components contribute to luminescence. However, little is known about what definitely causes bone fluorescence, but most likely it is triggered by the organic fraction (Prentice, 1967). Pulverised bone powder with a grain size<200 μm still retains the typical blue fluorescence which shows that luminescence is not a consequence of microstructural order (200 μm equals average human osteon size) but rather originates in bone ultrastructure (Doppler, 2008). Geological apatites also display strong luminescent features. Here, the colour of fluorescence is determined by the incorporation of rare earth elements (REE), which can also result in blue fluorescence (Waychunas, 2002). However, deproteination of fresh bone sections with 4% NaOCl does not change the original blue luminescent colour, while demineralization with HCl and EDTA often results in violet fluorescence (Doppler, 2008). Violet fluorescence is also observed in bones cremated at 500 °C or higher, temperatures at which the organic bone component is expelled from the composite material (Harbeck et al., 2011). Collagen contains several fluorophoric compounds triggering blue fluorescence: intermolecular hydroxypyridinium crosslinks and aromatic amino acids which are able to trigger strong fluorescence even in small fractions (Eyre et al., 1984a, 1984b; Fujimori, 1985, 1989; Lakowicz, 2006).

The observed modifications to bone fluorescence resulting in brown, yellow, grey or mixed colours are most likely due to defects in the crystal lattice caused by the infiltration with exogenous metal ions that frequently act as quenchers which can eliminate luminescence at a fractional amount of a percent (Lakowicz, 2006). This might be combined with major alterations of the collagen component resulting in a reduction of crosslinks and fluorophoric amino acids (for more details see Hoke et al., in press).

In contrast to the studies conducted in the middle of the last century, our unpublished results point to only a weak correlation between fluorescence of a bone and inhumation time. They rather suggest a link between the fluorescence properties and the individual degree of preservation of the bone. Thus, we chose to test whether the mere visual impression of fluorescence colour (without any measurement devices) can be related to the preservation of microstructure, collagen, and DNA.

In order to evaluate the significance of this method as a screening tool for biomolecular preservation, we compared its efficiency to the informative value given by screening the samples according to their microstructural preservation. Albeit somewhat more complex and time consuming than the determination of UV fluorescence, picturing microstructural traits by means of histological methods is less challenging than the analysis of biomolecules and so the degree of microstructural preservation also often serves as an indicator for the overall preservation of bones: several studies have postulated a possible link between DNA survival and the degree of histological preservation (e.g. Richards et al., 1995; Colson et al., 1997; Cipollaro et al., 1998; Haynes et al., 2002) and suggested that, based upon this relationship, bone histology could be used as a potential indicator for identifying samples that are most likely to yield DNA. Pfeiffer and Varney (2000), however, tested histological methods as a possible indicator for collagen content and found them not applicable.

Accordingly, in the presented study microstructural preservation was assessed for two purposes: first, we intended to test whether the UV fluorescence properties of a bone cross-section are applicable to estimate its microstructural preservation in order to pre-select samples suitable for histological analyses. Second, we compared the screening potential of linking microstructural integrity with biomolecular preservation to fluorescence screening.

2. Material and methods

A set of archaeological horse bones was used, consisting of 76 bone fragments taken from different skeletal elements (mainly from the extremities; exceptions: 4 scapulae and 1 pelvis). They were found at various sites dispersed throughout Central and Eastern Europe, including sites from Germany, Poland, Slovakia, Hungary, and Romania and showing a high diversity in burial settings. The bones are dated to the Early Palaeolithic up to the Bronze Age, thus covering periods from about 15,000 to 2000 BC and comprising wild and domestic horses (Table 1).

The aim of this study was to separate usable and non-exploitable samples for archaeometric analyses and not to assess the exact range of diagenetic alteration. For this reason, all data obtained were expressed in a binary classification system to reflect this different approach.

For the determination of their individual fluorescence, cross-sections of each bone were prepared by using a band saw or a multi tool equipped with a common 15 mm corundum cutting wheel. We chose a thickness of ca. 5 mm to have a common base for better comparison, but smaller and thinner bone pieces are also suitable for fluorescence analysis. In order to get a smooth, even surface, the cross-sections were polished by using a water cooled rotary sander and/or fine sandpaper, grit 800 or finer. After brief drying on paper towels, samples were then exposed to long-wave UV light, wavelength 366 nm, with the light source (Benda ultraviolet beamer, NU-4KL) located vertically above the bone sections. Each sample was inspected together with a positive control, a fresh bone with the typical, intensive light blue fluorescence, in a darkened room.

Fluorescence appearance of the compact portion of all bone samples was assessed by direct visual observation under UV light. Samples showing predominantly blue fluorescence (approx. 85% or more) across the whole section were considered as “type A” and those that did not match this criterion were assigned to “type B”. No difference was made between darker and lighter blue.

For histological investigation, bone pieces were embedded into Biodur E12 resin (Biodur™ Products) and cross-sections of 65–80 μm thickness were prepared using a lock saw microtome equipped with a diamond edged blade (Leica SP1600 and Leica 1600, Leitz). The thin sections were inspected at 50× magnification in bright field mode using an Axioskop 2 plus microscope (Zeiss) with an attached CCD camera (Zeiss AxioCam MRc colour). For classifying the preservation of bone microstructure in two categories, we followed the basic idea of the Oxford Histological Index (OHI, Hedges et al., 1995; Millard, 2001). Since we assume that an analysis of microstructural traits is most unproblematic for OHI stages 4 and 5, we merged these two categories into “well preserved microstructure” (which means that