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Make it clear: molds, transparent casts and lightning techniques for stereomicroscopic analysis of taphonomic modifications on bone surfaces

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Summary - *This paper provides detailed description of a non-destructive, low-cost, and low-time consuming technique for producing high-resolution casts for the observation of taphonomic modifications on bone surfaces. The aim of the whole process is to obtain molds that accurately replicate the original bone surface at both the macro- and microscopic levels. The high quality transparent epoxy casts produced are analyzed by light microscopy and used to produce detailed microphotographs of bone surfaces. After describing each step of the process, we present some examples of its application in the case of anthropic activity, carnivores, or other post-depositional modifications.*

Keywords - *Molding, Casting, Bones, Taphonomy.*

Introduction

Molds of archaeological remains are commonly used to produce high-resolution replicas for studying tooth microwear (Solounias & Semprebon, 2002; Rivals *et al.*, 2009, 2015; Sánchez-Hernández *et al.*, 2014), for lithic use-wear analysis (Ollé & Vergès, 2014), and on some occasions, for casting bone surfaces (Bello *et al.,* 2009, 2011). Molding of specimens has some advantages over the direct study of the original remains. It permits (1) the sampling of specific part(s) of larger remains, which sometimes cannot fit into the equipment employed for their analysis [a scanning electron microscope (SEM), for example]; (2) easy transport of samples from the collection to the analytical facilities; (3) the exportation of molds abroad for specific studies without requiring any permits; and 4) better observation, in some cases, of taphonomic damage present on the bone surfaces.

The technique of molding is relatively standardized, with the use of high-resolution silicone (principally Provil® novo by Heraeus or President® Jet by Coltène Whaledent) to produce the mold. The casting process, depending on the method of analysis, uses a wide diversity of resins (araldite, epoxy, polyurethane, etc.). These resins permit the analysis of the samples using a wide range of techniques, from low magnification using a stereomicroscope to high magnification at the SEM level. Previous studies demonstrated that molds and casts produce highly reliable replicas of surfaces, even for use at high magnification using scanning electron microcopy (Galbany *et al.*, 2004, 2006) or focus variation microscopy (Goodall *et al.*, 2015).

We provide guidelines for producing high-resolution molds and casts for the observation of taphonomic modifications on bone surfaces, including those made by anthropic activity, carnivores, or other post-depositional modifications. The high quality of the resulting transparent epoxy casts allows their analysis by light microscopy to produce detailed microphotographs of bone surfaces. The technique proposed here is based on the protocol of Solounias & Semprebon (2002) devised for the study of tooth microwear, which we have modified for its application to the analysis of bone surfaces.

Methods

We describe the technique of molding and casting of bone surfaces (Fig. 1). The full process, from cleaning to production of the cast, and then the observation at the light microscope level, is illustrated with pictures and with a video, available at https://vimeo.com/137151546.

The aim of the whole process is to obtain molds that accurately replicate the original bone surface at both the macro- and microscopic levels. We follow the protocol developed by Solounias & Semprebon (2002) for the study of tooth microwear.

The cleaning process

The first step is the cleaning of the bone surface to be analyzed with acetone and ethanol. This non-destructive process (Fernández-Jalvo & Marín, 2008; López-Polín, 2012) requires removal of any dirt or dust present, or any chemicals used during the excavation or conservation of the bones. If the bone surface is not properly cleaned, the resulting cast might not be suitable for observation. It is preferable to work in a stable environment where the temperature and humidity are not too extreme. The cleaning process is composed of three steps:

Step 1. Cleaning with acetone. The bone surface is cleaned by swiping with cotton swabs moistened with acetone (≥99.5%). The drying time of acetone is very fast; thus, in a few minutes, the surface will be dry and ready for the next step.

Step 2. Cleaning with ethanol. We eliminate any residue of acetone that could remain on the bone surface using ethanol (≥96%) and a cotton swab.

Step 3. Drying. The surface to be sampled must be totally dry (ca. 5 minutes) before applying the high resolution silicone. Specific temperature conditions will influence the drying (e.g., extreme cold and humid conditions will delay the process).

The molding process: producing high-resolution molds

As mentioned previously, the technique is a non-destructive and conservation-friendly method for the study of archaeological materials. Creating negative impressions requires a product with high plasticity and non-aggressive chemical composition. For this purpose, high-resolution dental vinylpolysiloxane silicone (e.g., Heraeus Provil® novo light regular set) is used. The kit used comes as a cartridge that is divided into two independents tubes: one contains the base (silicone) and other the catalyst. A mixing tip is attached to the cartridge to provide automatic mixing of the silicone and the catalyst. The cartridge is loaded into a dispensing gun.

Vinylpolysiloxane silicone is characterized by an impressive detail resolution of up to 1 µm (Goodall *et al.*, 2015), a high recovery after deformation (99.7%), a low linear dimensional change (shrinkage 0.2%), and a high temporal stability. The setting rate of the silicone is fast - 5 to 10 minutes, depending on the environmental conditions - which allows repetition of the process several times in a short period. Therefore, when a large number of samples need to be molded, we recommend cleaning them all first before starting the molding process. This will speed up the whole process and conserve silicone components (the mixing tip easily gets blocked due to progressive setting of the silicone). Temperature and humidity will influence the efficiency of the process. From our experience, optimal conditions are a temperature between 18°C and 22°C and a humidity of ca. 50%.

Step 4. The silicone is directly applied to the bone surface with the dispensing gun. Before

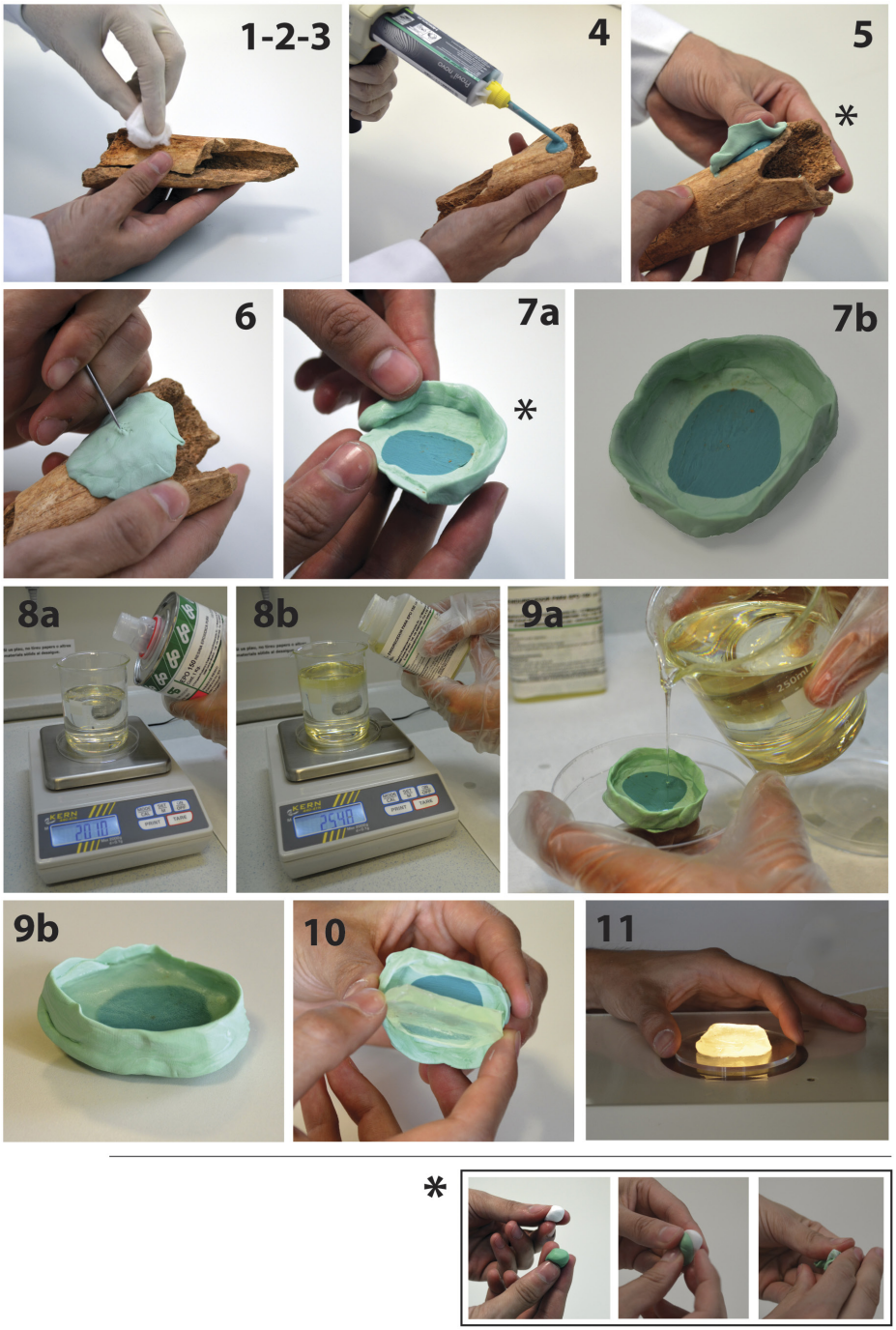

Fig. 1 - Molding and casting process described in the text. A detailed video of the process is available at https://vimeo.com/137151546. The colour version of this figure is available at the JASs website.

starting this process, we recommend discarding the first amount of mixed fluid, as the silicone and the catalyst are sometimes not well mixed. The mixing tip must be as close as possible to the bone surface to avoid the formation of air bubbles, and the spreading has to be constant—making circles provides a better distribution of the silicone.

Once the high resolution negative impression (silicone) has set, a new layer of silicone putty is added to cover it, with the aim of providing support to the mold. The product required is a low-resolution silicone (Heraeus Putty Regular Set), which has a high recovery after deformation (99.7%) and low linear dimensional change (shrinkage) (0.3%). This kit is also composed of a base and a catalyst, which are mixed in a dosing ratio of approximately 1:1 by volume. Specific temperature conditions will influence the timing of the process (e.g., cold and hot temperatures will delay or accelerate the process, respectively). A recommendation under high-temperature conditions is to place the silicone into a refrigerator before starting the process.

Step 5. The same volume of silicone and catalyst is taken (e.g., the size of a chickpea each for a surface of about 10 cm^2). The two components are quickly mixed manually until the mixture has a homogeneous color. The mixture is then flattened to form a surface, which is used to cover the negative impression and an area of about 1 cm around it.

Step 6. While the silicone is still soft, it is recommended to write, as soon as possible, the reference of the sample onto the mold surface (e.g., register, museum or field number). A stylus can be used to scratch the surface. This step allows the direct identification of the mold without using paper labels or individual bags, thereby avoiding any future error concerning the management of large samples. In addition, as in Step 4, the same advice regarding refrigeration related to high-temperature conditions applies here.

Step 7. When the silicone is totally set, it is carefully removed. The high-resolution impression in direct contact with the bone surface is now adhered to the overlying putty silicone. A wall needs to be built around the mold in order to form a recipient, which will be used to create the positive casts (Fig. 1, 7a). The low-resolution resolution silicone putty is used to build a wall about 1 cm high to hold the epoxy resin (Fig. 1, 7b). Before continuing with the process, we recommend to clean the bone as in steps 1, 2 and 3.

The casting process: producing high-resolution transparent casts

The molds are filled with epoxy resin to produce transparent casts. Transparent epoxy resin (e.g., C.T.S.® EPO 150) and a catalyst (e.g., K 151 for EPO 150) are required. EPO 150 is a transparent liquid epoxy resin with very low viscosity (500-800 mPas) and high resistance and stability. Both the resin and the catalyst are toxic; therefore, the use of plastic gloves, security mask, and safety glasses is required. The work should also be conducted in a fume hood. A plastic or glass container can be used for mixing the epoxy resin and the catalyst.

The epoxy and catalyst need to be mixed in precise proportions, as indicated by the manufacturer. In the case of EPO 150, the components are measured by weight. The proportion of the catalyst is 25% of the total epoxy utilized. For example, 200 g of epoxy requires 50 g of catalyst (Fig. 1, Step 8a and 8b).

Step 8. The weight of the components is measured with a digital scale. A wooden stick is used to mix the components, with constant changes in the direction of motion to ensure a homogeneous mixture. Special attention needs to be taken to avoid the formation of air bubbles. If excessive bubbles are present, they can be removed by centrifugation or use of a vacuum pump (we recommend 500 rpm during 30 seconds using a low-speed centrifugation device).

Step 9. The mixture is poured carefully into the mold (Fig. 1, 9a) and left to harden for 1–2 days at ambient temperature under the fume hood (Fig. 1, 9b). High temperatures should be avoided as they accelerate the catalysis and might reduce the resolution of the cast.

Step 10. Once the epoxy is set, the positive cast is carefully separated from the mold (Fig. 1,

10). The sample reference can be scratched on the back of the cast.

Observation of transparent casts

Step 11. The resulting epoxy cast is transparent and uncolored, permitting observation of the surface (Fig. 1, 11). The observation is typically conducted using a stereomicroscope with transmitted light (e.g., Zeiss Stemi 2000C). The correct use of the transmitted light is a very important aspect of the observation. In cases where the observation is not sufficiently clear, the light can be changed to a different intensity or angle of transmission (with an incorporated mirror in the base of the microscope) to improve resolution. The clarity of the visualization of different features present on the cast may be enhanced by manipulating lighting parameters.

Application of the technique: proof of concept

The technique described can be applied to both experimental and fossil bone surfaces containing taphonomic modifications commonly found in archaeological sites. The aim is to provide examples and a proof of concept regarding the utility and benefits of the method described. The molding and casting technique can be applied to almost all bone surfaces, excluding those with deep fissures and/or exposed spongy tissue. Surfaces displaying those characteristics should not be molded because silicone percolates through the gaps and holes of the bone, making extraction of the mold impossible without damaging the bone specimen. Nevertheless, all other surfaces can be successfully molded. For instance, our examples provided here are related to both human activities and natural agents, such as:

- Chemical damage (e.g., roots and soil acidity) (Fig. 2a)
- Weathering damage (e.g., exfoliation) (Fig. 2b)
- Carnivore damage (e.g., scores) (Fig. 2c)
- Anthropic modifications, such as scraping with lithic tools (Fig. 2d) and polished surfaces (Fig. 2e)
- Experimental superposition of anthropic lithic cutmarks over carnivore scores (Fig. 2f)

As the examples show, the application of the technique described represents an improved approach for the study of different types of taphonomic damage. In this sense, taphonomic damage that is defined by characteristic irregular surfaces, such as vermiculations or exfoliation (Figs. 2a,b), can be easily observed. Anthropic modifications are also easily observed as they display complex surfaces composed of regular, flat, and angulated modifications. Scraping traces and cut-marks, for instance, are perfectly visualized as they contrast with the bone surface (Figs. 2c-d, f). Furthermore, polished surfaces, which present no difference in relief, also reflect light in a way that allows easy identification (Fig. 2e). All these characteristics are related to the fact that the light is located beneath the transparent cast. The angle of transmitted light can be changed to improving the resolution of the observation. As Figure 3 shows, moving the mirror in different directions highlights different features (Figs. 3a-d). This allows the researcher to focus on different variables present in a single case study. This type of observation is better than the traditional observation of the original bone surface under direct incident light. An advantage of using transparent casts is that the surface do not need to be metalized (with gold or graphite), unless observation of the cast using SEM is planned. This type of observation has been described previously by other authors (Fiorenza *et al.*, 2009).

The benefits of silicone casts for analysis of bone surfaces are also related to their high resolution. In this sense, the sampled bone surfaces are perfectly replicated, allowing macro- and microscopic observations. Therefore, the analysis of the casts with devices such as ESEM/SEM is possible without losing information present on the original bone surface (Galbany *et al.*, 2006).

The present technique has other benefits for analyzing taphonomic modifications on bone surfaces. It is a low-cost (e.g., ca. 150€ for 100 casts of 2 x 2 cm in size), low time-consuming (e.g., for 100 casts: ca. 6 hours of work on the material for steps 1 to 7 and 48 hours to obtain the final epoxy

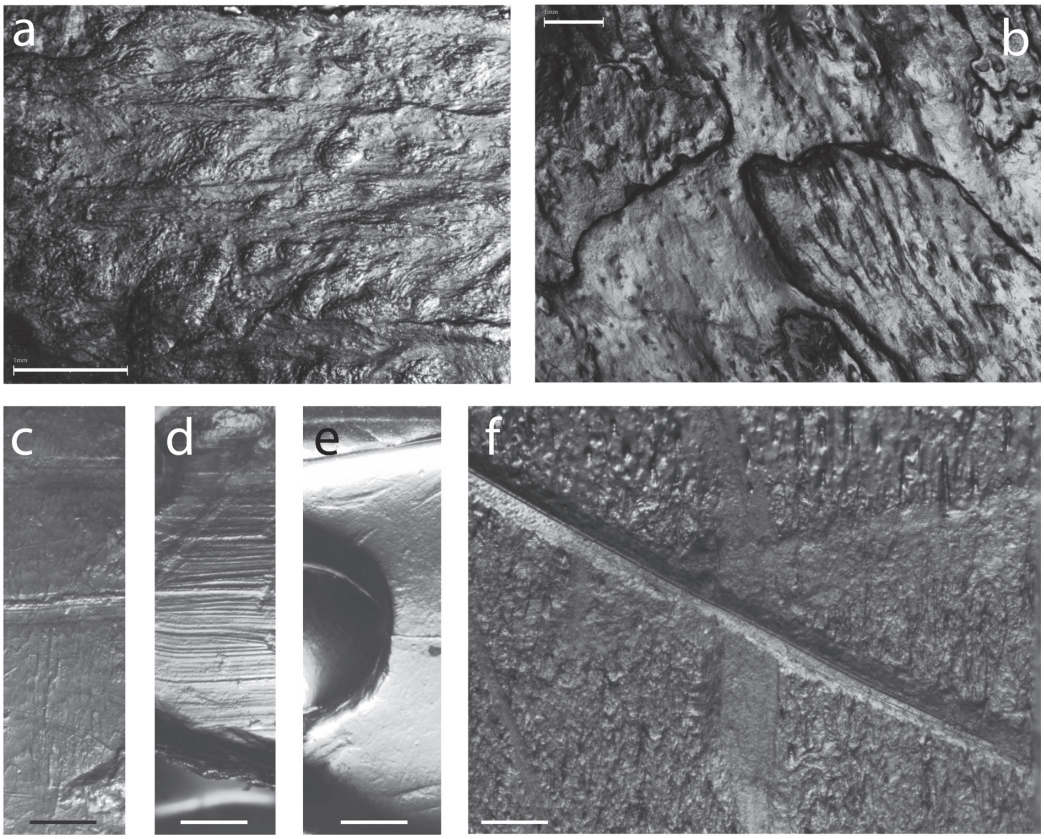

Fig. 2 - Casts displaying different taphonomical modifications on bone surfaces: a) Chemical damage (roots and soil acidity); b) Weathering damage (exfoliation); c) Carnivore damage (scores); d) Anthropic scraping; e) Polished surface; f) Superposition of cutmark over carnivore score. Scale bar is 1 mm.

casts in step 10), and non-destructive method that provides a cast with the dimensions of a specific area of the bone rather than the dimensions of the entire bone (case of Fig. 1). In this sense, the casts can be easily stored as reference, teaching, and scientific collections, and can also be easily transported and introduced into specific devices, such as ESEMs/SEMs and others.

The technique also facilitates experimental research protocols aimed at observing before and after scenarios, such as have been applied for the study of lithic tool marks (Ollé & Vergès, 2014; Camarós *et al.*, 2016). More benefits can be listed, such as the possibility of rapidly sampling large collections and studying the casts at greater leisure later. This provides the future option of analyzing the bone surfaces from different points of view, which is generally not possible in museum collections due to time and permit limits (e.g., SEM/ESEM analysis, direct measurements, high-quality microphotography, etc.). Furthermore, molds and casts can be stored for many years, allowing their study in the future; for example, when an advance occurs in an existing techniques or new scientific agenda develops. The molding technique is a useful method when working in museums and conducting fieldwork, where materials cannot be easily exported overseas. In addition, molds can be used to produce up to four consecutive casts for optimal observations at the SEM (Galbany *et al.,*

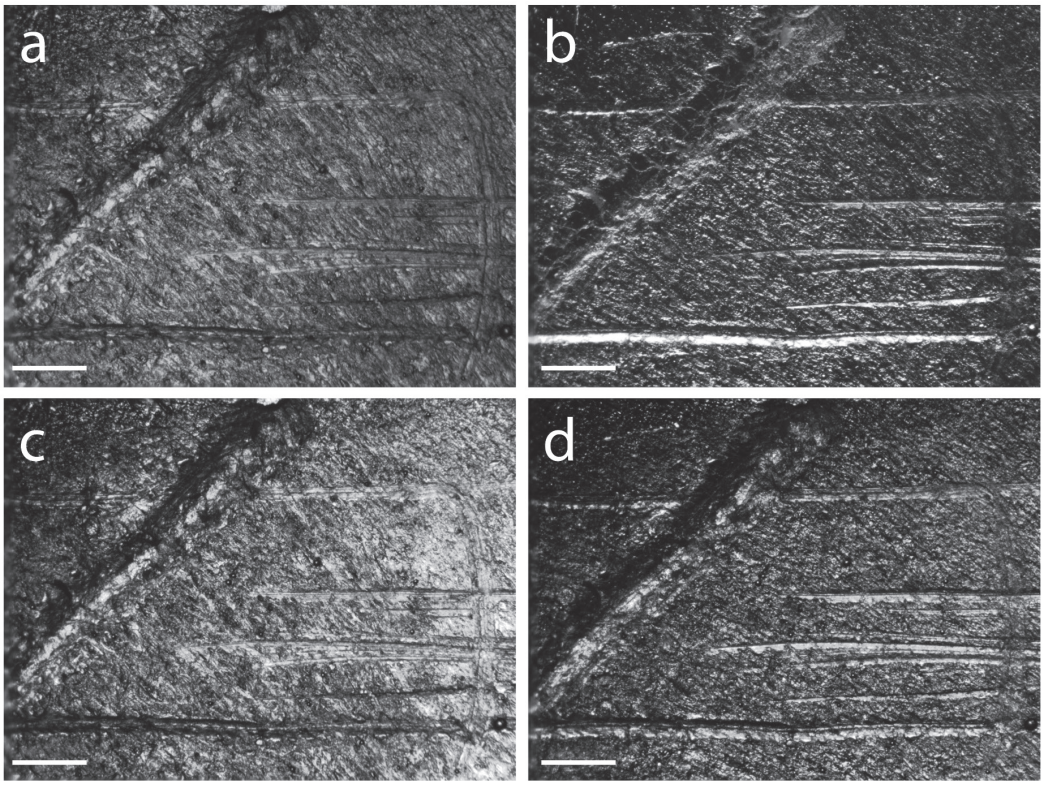

Fig. 3 - Epoxy cast displaying carnivore scores over anthropic cutmarks observed with light directed from different angles at same intensity (using a stereomicroscope Zeiss Stemi 2000C with transmitted light). Scale bar is 1 mm.

2006). Casts permit observations with different devices that require specific treatment of the surface (e.g., SEM) or for teaching purposes (thereby reducing the risks of handling original specimens).

Conclusions

The preparation of molds and casts of bone surfaces improves the stereomicroscopic analysis of taphonomic modifications. It is a non-destructive, low-cost, and low-time consuming method that is easy to replicate and provides important benefits related to different scientific agendas and objectives. In this sense, the high-resolution quality of the surface replicated, the size of the casts, and the intrinsic properties (e.g., the transparency and durability, among others cited here) are positive aspects to take into account when facing a taphonomic study of a bone specimen or collection. The case studies presented here are proofs of concept of this improvement when approaching different taphonomic studies.

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