An assessment of bone tool cleaning procedures in preparation for traceological analysis

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Abstract
The preparation of samples for traceological analysis is a key methodological aspect in the correct interpretation of use-wear; however, it is often poorly reflected in the archaeological literature. The treatment of osseous tissues is particularly overlooked, and receives even less attention than lithic raw materials. The presence of residues and contaminants on the surface of artefacts can conceal or even be mistaken for use-wear features, thereby affecting their interpretation. Therefore, the objective of this work is to contribute to the systematization of cleaning protocols and the preparation of experimental bone tools for traceological analysis. Through a sequential experiment, we tested the effects of different cleaning agents on experimental samples. Microscopic observation of the samples was complemented with microhardness testing. Our results made it possible to evaluate the cleaning effectiveness of the tested products, to determine how each product affects the bone surface at a microscopic level, and to assess the effects of these products on the treated bone tools in terms of cutting performance.

Keywords Bone tool · Cleaning procedure · Use-wear · Experimental archaeology

Introduction
Functional analyses aim to identify the functionality of archaeological artefacts through two well-established approaches: use-wear and residue analyses. The former arises from the studies of Semenov (1964), which were methodologically consolidated with Keeley’s contributions (1980). As for the latter, the earliest studies on residues adhering to stone tool surfaces appeared more or less contemporaneously (e.g. Briuer 1976; Shafer and Holloway 1979). Residue analysis presents a range of methodological issues, such as the nature of the residue itself, its synchronicity with the piece it is found on, and the activity performed using the piece (Pedergnana 2020). Numerous works have therefore attempted to define how best to recognize functional, incidental, or post-depositional residues (e.g. Fullagar et al. 1996; Rots et al. 2016; Croft et al. 2016; Pedergnana and Ollé, 2018). Functional residues are left during the use of the tools, while incidental and post-depositional residues can be produced by various agents and are sometimes mistaken for functional residues (Evans and Donahue 2005; van Gijn 2014; Pedergnana et al. 2016; Bordes et al. 2017; Fernández-Marchena et al. 2018, 2020; Martín-Viveros and Ollé, 2020a, 2020b).

In the field of lithic traceology, some studies have stressed the importance of appropriately preparing and cleaning tools, once the residues have been documented, for the subsequent study of use-wear traces (e.g. Keeley 1980; Ollé and Vergès 2008; Rots et al. 2016; Macdonald et al. 2018). Ancient or modern residues can conceal the object’s surface or give rise to false use-wear traces—even after washing—which can lead to the misinterpretation of the function of the tools analysed (Knutsson 1988; Pedergnana et al. 2016;
Hayes and Rots 2019; Martín-Viveros and Ollé, 2020b; Fernández Marchena 2021). In addition, the presence of residues is incompatible with the attainment of replicas of the working edge through mould and cast techniques because surface contaminants will be equally reproduced, possibly leading to misinterpretations in the analysis of the replica. Other authors have emphasised the lack of consensus regarding cleaning protocols (Evans and Donahue 2005; Macdonald and Evans 2014; van Gijn 2014).

Unlike in functional studies focusing on lithic materials, in bone traceology, methodological discussion regarding sample preparation and cleaning has not yet taken place. Although it is mentioned in some of the earliest functional works (e.g. d’Errico et al. 1984; Olsen 1984; Peltier 1986; Campana 1989), many publications fail to indicate the cleaning protocol followed prior to the analysis of the material or whether the material has indeed been cleaned in any way.

Specifically, in the case of functional analyses of experimental material, the presence of residues and contaminants makes it difficult to observe and characterise the use-wear traces created for that very purpose. For sequential experiments in particular, that is, those devoted to recording multiple stages of wear development through progressive monitoring of the active edges (Ollé and Vergès 2014), cleaning is especially important to study the formation process of use-wear patterns. To this end, some sequential studies on hard animal materials describe a cleaning protocol applied prior to each microscopic observation between the intervals of the sequence (Tumung et al. 2015; Martisius et al. 2018; Mateo-Lomba et al. 2020; Gilson et al. 2021).

Another decisive aspect in sample preparation are the properties of the materials to be treated. Fresh bone is made up of living cells in a mineralized organic matrix. This matrix is primarily made up of inorganic material—hydroxyapatite and other calcium and phosphate salts—as well as organic materials, mainly collagen fibres. These components are what determine the physical properties of the material (Currey 2012; Kendall et al. 2018). Osseous remains recovered in archaeological contexts have rarely been addressed in functional studies (Graziano 2014). However, specific works on the preparation of osseous elements in other disciplines, such as taphonomy, microscopy, and conservation and restoration, have also documented the effects of certain sample cleaning procedures on these materials (Shipman and Rose 1983; Bromage 1984; Fernández-Jalvo and Marín Monfort 2008; Martínez-Maza et al. 2010; López-Polín, 2012; Graham and Allington-Jones 2018; Marin-Monfort et al. 2018; Wiest et al. 2018; Cazalla Manceras 2019; Valtierra Pereiro 2019; Valtierra et al. 2020).

Therefore, the aim of this work is to propose an effective cleaning protocol that does not visually or physically modify the state of experimental specimens used in traceological studies of bone industry. In sequential use-wear experiments, it is essential to record the microtopography of tool surfaces in order to understand the process of wear formation. For that reason, this paper focuses on residues present on a fresh bone sample: blood, internal grease, and superficial flesh remains. This work therefore continues the series of previous experiments designed to study minimally elaborated bone tools (Mateo-Lomba et al. 2020), as the identification of use-wear traces is a key factor in recognizing bones used as tools (Shipman 1988).

The analysis of adhering residues from worked materials, being experimental or archaeological, as well as sedimentary deposits on archaeological specimens is beyond the scope of this work.

**Materials and methods**

We performed a multi-stage experiment to address our objective using a trial-and-error approach. The materials used in this study were diaphyseal bone fragments (n = 10) obtained from the intentional fracturing of a fresh femur (Bo taurus) using a direct percussion technique. Those that could potentially be used as tools in the performance of tasks were selected based on their shape and size and the presence of usable edges for different actions (i.e. simple bone tools sensu Mateo-Lomba et al. 2020). First, these samples were cleaned with different products to evaluate the efficacy of those products and any alterations that might occur as the result of the cleaning protocol. Second, a selection of the fragments (n = 3), cleaned with the most effective products, were subjected to a cutting test to ascertain whether they were still effective for use. Complementary microhardness tests were performed on this smaller sample.
Cleaning experiment

Through a systematic review of functional studies of osseous tools, we catalogued the various cleaning protocols used to date. Only a low percentage (21.6%) of the publications consulted ($n=204$; see all references consulted in Online Resource 1) indicate that the analysed tools had undergone a cleaning process prior to microscopic observation.

The products most commonly reported in the reviewed literature were selected (Table 1). Our objective was to test these products to assess their effectiveness in cleaning bone surfaces, the degree of alteration induced on the surfaces, and any changes in the physical properties of the tools, especially possible effects on their effectiveness for cutting activities (Mateo-Lomba et al. 2020).

The products selected were (1) distilled water with Fairy® (11 2%), (2) distilled water with Derquim® (2%), (3) distilled water at 50°C, (4) pure acetone (CH$_3$(CO)CH$_3$), (5) ethanol (or 96° alcohol), (6) dilute solution of sodium hypochlorite (NaClO <5%; i.e. household bleach), and (7) hydrogen peroxide (H$_2$O$_2$) to 35% (130 vol.). The two detergents used are products composed of anionic and non-ionic surfactants. Derquim® is a phosphate-free detergent (pH in a 2% solution: 8–9), used for sensitive materials in lab tasks. Fairy® is a common dishwashing soap.

The bone samples ($n=10$) were analysed when they were fresh, immediately after intentional bone breakage. Blood, soft tissues, and bone grease were naturally present on their surfaces, as described below.

At least one control point was documented on each piece with a digital microscope (Hirox KH-8700, MXG-5000REZ Triple Objective) and additionally with an optical microscope (Zeiss Axio Scope A.1) and/or a scanning electron microscope (ESEM, FEI QUANTA 600, used on low vacuum mode). The technical specifications for this equipment can be found in Ollé et al. (2016), in Courtenay et al. (2019), and in Martín-Viveros and Ollé (2020a). All control points were selected for being easily recognizable at the margins of the active cutting edges by means of low- to high-magnification images, and all were documented before cleaning (stage 0) and after each of the subsequent stages of cleaning.

The experiment consisted of the sequential cleaning of each fragment by means of immersion in an ultrasonic cleaner (J.P. Selecta 3,000,513, 50 kHz or Branson 2510, 40 kHz). Other types of mechanical action on the surfaces were avoided to prevent any microscopic alterations that might be generated in this process, as mentioned above. The cleaning procedure consisted of individually placing each sample, along with the cleaning product, in low-density polyethylene zip-lock bags. The samples were then rinsed with tap water to stop the action of the products.

First, we performed a sequential cleaning experiment at all stages. We selected seven bone fragments whose control points were documented between each cleaning. Our aim was to document the evolution of the changes on the sample surfaces due to the action of each cleaning protocol as well as to evaluate the cleaning efficacy of each procedure. The first stage was longer to ensure that any possible changes caused by cleaning were evident. In the following stages, shorter cleaning times were chosen because the surfaces were not obscured by flesh and blood remains and the changes were readily noticeable. These times were exaggerated so that the action of the products would be much more evident on the experimental samples. In other ongoing use-related experiments, shorter cleaning times have proved sufficient to clean fat and soft tissues as well as the worked materials (i.e. wood) from the bone surface. Second, based on the results obtained from the first set (in which the cleaning efficacy of the products was tested; see below for details), three additional fragments were cleaned once for 10 min for further documentation with a digital microscope and ESEM (10–20.8; 10–20.9; 10–20.10). In this case, the control points were documented before and after cleaning (Table 2). The aim of this additional step was to document possible alterations caused by the most effective cleaning protocols with a higher resolution equipment.

| Product | References |
|---------|------------|
| Water   | Bromage 1984; Campana 1989; d’Errico 1993; d’Errico et al. 1995; Buc and Silvestre 2006; van Gijn 2006 |
| Water+detergent | Olsen 1984; Peltier and Plisson 1986; LeMoine 1991; Gates St-Pierre 2007; Terradas et al. 2011; Mallye et al. 2012; Boñill and Buchra 2013; Guzzo Falci 2015; Orłowska 2016; Hutson et al. 2017; Xie et al. 2017; Osipowicz et al. 2019 |
| Ethanol | Shipman and Rose: 1983; Campana 1989; Fernández-Jalvo and Marín Monfort 2008; Martinez-Maza et al. 2010; Boñill and Buchra 2013; Lisowski et al. 2017; Xie et al. 2017; Guzzo Falci et al. 2019 |
| Acetone | Shipman and Rose: 1983; Peltier and Plisson 1986; Campana 1989; LeMoine 1991; d’Errico 1993; d’Errico et al. 1995; Fernández-Jalvo and Marín Monfort 2008 |
| Solution of sodium hypochlorite | Bromage 1984 |
| Hydrogen peroxide | Fernández-Jalvo and Marín Monfort 2008; Santiago et al. 2019; Mateo-Lomba et al. 2020 |
In addition, the cutting efficacy of the second set of fragments—those cleaned with an effective cleaning protocol—was tested after the cleaning protocol by cutting fresh meat with the sharp edges of the selected experimental samples (Online Resource 2). The cutting test was performed as described in previously published use-related experiments (Mateo-Lomba et al. 2020, pp. 53–54).

**Microhardness test**

The mechanical properties of bone, such as hardness and resistance to stress, can contribute relevant information to functional experiments with this type of material. We therefore sought to determine whether the microhardness of fresh bone changes when exposed to the products applied in the cleaning protocols, and if these products might consequently affect the experimental results. To explore this possibility, we tested the products that were considered effective for cleaning in the first qualitative assessment, excluding those too aggressive for the material or lacking sufficient cleaning action.

We used the Knoop test (Knoop et al. 1939; Riches et al. 1997) to evaluate the microhardness of the samples. It is based on the application of a rhombohedral indenter on the surface of the material. The microhardness value is obtained from the measurement of the length of the longest diagonal of the resulting indentation. The Knoop test was performed with a microindentation tester (Wilson-Wolpert 401MVA). Given the variability of the material, all indentations \((n = 50\) per sample) were made with the same orientation (Ziv et al. 1996), always perpendicular to the direction of the lamellae. Ten of the measurements with variability of less than 20% were selected. These provided an average microhardness value for the sample and the standard deviation of that value. These exclusion criteria ensured that erroneous measurements were not included. A digital microscope (Hirox KH-8700) was used to examine and measure the indentations.

Thus, three additional samples extracted from fragments of a *Bos taurus* femur were selected. The samples required preparation prior to testing because it is important that the indentations are made on a flat plane. The samples were next fixed to a methacrylate plate with Araldite® bicomponent epoxy resin and their surfaces were levelled with a drilling-milling machine (Travis-4VS) until the surface was as flat and regular as possible. The indentation surface was planed to a flat surface in 0.02-cm intervals. Once a flat surface was obtained, it was stained with red and black ink to improve the visibility of the indentations.

Two of the samples were subjected to the cleaning protocol described above with the Derquim® water solution and hydrogen peroxide. Then, they were left to dry for 5 days. Lab detergent was chosen because it was the most effective product for cleaning the surfaces (see below), and hydrogen peroxide was selected because after cleaning with this product simple bone tools were rendered ineffective for cutting actions (Mateo-Lomba et al. 2020, p. 56). The remaining fragment was a fresh fragment of the same anatomical element and taxon; it was not subjected to any cleaning protocol and was used as a control sample.

**Results**

**Cleaning experiments**

The products used for cleaning had exerted effects on the experimental samples, as described below.

**Water + Fairy®**

The samples subjected to cleaning (08–20.1 and 10–20.9) with a solution of water and household detergent exhibited

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**Table 2 List of the experimental samples and summary of the cleaning protocol**

| ID     | Product                                | Documentation | Ultrasonic cleaner | Cleaning protocol |
|--------|----------------------------------------|---------------|--------------------|-------------------|
| 08–20.1| Water + Fairy® (2%)                     | Hirox + Zeiss | J.P. Selecta       | 15 min 5 min 5 min |
| 08–20.2| Water + Derquim® (2%)                   | Hirox + Zeiss | J.P. Selecta       | 15 min 5 min 5 min |
| 08–20.3| Distilled water (50 °C)                 | Hirox + Zeiss | J.P. Selecta       | 15 min 5 min 5 min |
| 08–20.4| Pure acetone                           | Hirox + Zeiss | J.P. Selecta       | 15 min 5 min 5 min |
| 08–20.5| Ethanol                                | Hirox + Zeiss | J.P. Selecta       | 15 min 5 min 5 min |
| 08–20.6| Solution of sodium hypochlorite         | Hirox + Zeiss | J.P. Selecta       | 15 min 5 min 5 min |
| 08–20.7| Hydrogen peroxide (130 vol.)           | Hirox + Zeiss | J.P. Selecta       | 15 min 5 min 5 min |
| 10–20.8| Water + Derquim® (2%)                   | ESEM + Hirox  | Branson            | 10 min - -         |
| 10–20.9| Water + Fairy® (2%)                     | ESEM + Hirox  | Branson            | 10 min - -         |
| 10–20.10| Hydrogen peroxide (130 vol.)           | ESEM + Hirox  | Branson            | 10 min - -         |
black residues (when viewed under optical microscopes) of unknown origin (100–120 μm in diameter) (Online Resource 2, Figs. S1, S3, S5, S6, and S7) on their cortical surfaces, as well as fat and the remains of tissues that covered the bone, clearly organic in composition (C) as shown in the SEM-BSED image (Fig. 1A).

The cleaning process exposed the entire microstructure of the surface (Fig. 1B). To a great extent, the blood and superficial fat disappeared; however, within the osteons and channels it was still possible to see brighter elements most likely corresponding to fat remains. The deposited black residue particles also disappeared over the course of the cleaning sequence.

The cleaning process did not appear to have altered or damaged the surface of the bone. There were scarcely any changes documented between the intervals in the sequence. The edge of the fragment did not exhibit any significant changes either.

Water + Derquim®

The lab detergent water solution was used to clean two samples (08–20.2 and 10–20.8). The surfaces of the samples before cleaning presented different accumulations of fat, tissues, and blood (Fig. 2). After cleaning, the bone surface was free of natural bone grease, blood, and soft tissues at all of the different intervals of the sequence. Some of the materials deposited on the surface moved slightly across the surface. In addition, some of these residues were trapped between lamellae and channels. The structure of the bone was also not modified (Online Resource 2, Fig. S2) on the surface or at the edges.

After cleaning, we found that if the samples were not well rinsed with water and left to dry before microscopic analysis, residues of the detergent itself could be observed on the surface (Fig. 3).

Fig. 1 A Experimental sample (10–20.9) before cleaning. Abundant grease is documented on the medullar surface. A.1 Detail of the same point. B Same point after cleaning with water and Fairy® for 10 min. Most of the residues have been removed. B.1 Detail of the micro-topography of the bone. Images obtained with SEM (low. vac.). Original magnification: 300×(A, B), 600×(A.1, B.1). Scale bar: 500 μm (A, B), 300 μm (A.1, B.1)
Water

A single sample (08–20.3) (Online Resource 2, Fig. S3) was cleaned with distilled water. Before cleaning, the cortical surface of the sample bore traces of fat, tissues, blood, and black residue particles of unknown origin. After cleaning, the water had dissolved and removed some of these elements from the surface. However, the bone still had a greasy appearance that created surface shine. In the fracture plane, soft tissues were effectively cleaned as well (Fig. 4). Cleaning did not alter the appearance of the bone structure.

Pure acetone

The sample subjected to cleaning with pure acetone (08–20.4) obtained similar results to those obtained with water. Blood, fat, and fresh tissues present prior to cleaning were partly removed, exposing the surface of the bone (Fig. 5). However, much of the fat was still present on the surface, leaving it shiny. There were no changes to the bone edges (Online Resource 2, Fig. S4).

Ethanol

Ethanol was used to clean one sample (08–20.4), the surface of which exhibited grease, particles, and tissue remains. After cleaning at different intervals, part of the superficial fat and blood was removed. However, other black residue particles of unknown origin deposited on the surface were still present after the experiment (Fig. 6). Like water and acetone, treatment with ethanol did not change the greasy appearance of the surface or damage the bone structure. It did not dry out the bone or alter it (Online Resource 2, Fig. S5).
Solution of sodium hypochlorite

One sample (08–20.6) was cleaned with dilute sodium hypochlorite. As described for the other samples, before cleaning, the fresh sample exhibited particles on the surface as well as traces of tissue, blood, and fat. After the experiment, the structure of the bone was exposed without any trace of organic residues. In the successive intervals of the sequence, sodium hypochlorite modified the cortical surface, which exhibited a smoother, rounded surface with a dull appearance. The holes and channels that constitute the microstructure increased in size because the bleach had removed part of the microstructure. Similarly, the general shape of the sample was also modified (Online Resource 2, Fig. S6). However, changes were not as perceptible on the fracture plane (Fig. 7).

Hydrogen peroxide

Two samples (08–20.7 and 10–20.10) were cleaned with hydrogen peroxide. Before cleaning, the surface of the samples had a greasy appearance and other tissues and blood were visible. The action of peroxide exposed the microstructure of the bone. Observation at low magnification seemed to indicate that the microstructure was not affected (Online Resource 2, Fig. S7). However, the higher resolution analysis performed with ESEM showed that hydrogen peroxide did not leave the surface as clean as it appeared. Higher magnifications revealed that the irregularity of the bone surface had been smoothed and part of the matter that constitutes the bone structure had disappeared (Fig. 8).

Cutting test

After the cleaning tests, we performed a cutting test on a piece of meat using the tools that were cleaned with lab (Derquim®) and household (Fairy®) detergents and with hydrogen peroxide. The cutting test was not performed with all the samples because some of them had yielded negative results in the cleaning test. No differences were found in the cutting capacities of the samples treated with surfactants. However, the sample subjected to cleaning with peroxide was not as effective at cutting the meat as it was when the bone was fresh (Mateo-Lomba et al. 2020, p. 56). Although the other untested samples are likely to be effective for cutting as well, since the goal of this work was to achieve an effective cleaning treatment that is innocuous to the material surface, testing the remaining samples was not considered.

Cleaning protocols vs. cutting efficiency

The different cleaning protocols used can be classified according to the degree to which they clean the specimens and the degree to which they alter the bone surfaces (Table 3). Solutions made up of water and surfactants are effective in removing superficial fat while not altering the surface of the bones or affecting their cutting capabilities. Therefore, the cleaning process was repeated with these
products on a second set of samples (10–20.8 and 10–20.9; see Table 2) to perform a complementary analysis with a higher resolution technical device (ESEM). No additional modifications to those documented by digital and optical microscopes were observed. Another group consists of products that do not adequately clean the surface but do not alter it either. Finally, sodium hypochlorite and hydrogen peroxide effectively clean surfaces but also affect bone microstructure to different degrees. An analysis was also performed on a sample cleaned with hydrogen peroxide (10–20.10; see Table 2), and it was found to cause loss of material and smoothing of the surfaces, which is only evident with high-resolution equipment such as an ESEM (Fig. 8).

Microhardness test

The control sample obtained a mean hardness value on the Knoop scale of 40.23 Hk (SD = 4.70). The sample exposed to water solution with lab detergent was not as hard (20.74 Hk, SD = 1.93, the lowest value of the three samples). Hydrogen peroxide-cleaned sample showed an intermediate hardness value of 30.17 Hk (SD = 2.00) (Fig. 9). The ten measurements per sample taken to obtain these values can be found in Online Resource 3.

Discussion

Functional analyses require the consideration of a range of variables that go beyond the type of raw material. One of these key aspects is a proper cleaning protocol. However, not all types of cleaning are valid when preparing samples for traceological study. Previous works have pointed out that mechanical cleaning should generally be avoided, since interaction with cleaning tools can alter the surfaces to be studied (Peltier and Plisson 1986; Bromage 1984;
Martinez-Maza et al. 2010) and give rise to additional modifications (Shipman and Rose 1983; Bromage 1984; Fernández-Jalvo and Marín Monfort 2008; Pedergnana et al. 2020; Valtierra et al. 2020). For that reason, mechanical methods were not tested in this work.

In addition, other types of non-mechanical cleaning, such as cleaning with water, do not effectively clean fresh bone samples, as they alone cannot remove the blood, soft tissues, and natural bone fat present on the surface of the samples. These types of solvents must be used with an ultrasonic cleaner, as the waves emitted by these devices vibrate the aqueous medium in which the pieces are immersed, which helps to detach the surface particles (Caldararo 1993).

In the case of experimental bone materials, other authors have reported that water alone does not clean away meat or fat (e.g. Fernández-Jalvo and Marín Monfort 2008). This is due to the incompatibility of water with fat. Water is a polar substance and fat is nonpolar; in other words, they are immiscible materials. Therefore, it is necessary to use some other products that act in conjunction with water.

However, the use of different products for cleaning without a tested protocol can cause qualitative and/or quantitative modifications in the sample to be studied. The effects that may occur as a consequence of these products must be known before initiating cleaning. Uncontrolled exposure to acidic aqueous solutions used to remove calcareous matrices can damage some materials, including fossil bones (López-Polín 2012). Furthermore, other agents such as enzymes can give rise to holes, cracking, and light rounding on bone surfaces when temperature and exposure are unrestrained (Fernández-Jalvo and Marín Monfort 2008).

The surfactants used in this work, both the household and the lab detergent, were effective in removing most organic remains from the bone surface. Neither of them caused qualitative alterations to the bone surface. The molecular structure of surfactants, made up of a polar head and a nonpolar chain, lowers the surface tension of water, which improves its wetting capacity. In this way, two immiscible substances become compatible, allowing the extraction of solid particles—in this case the organic remains—and their dispersion in the liquid medium through the formation of micelles (Doménech Carbó 2013; Pérez 2019).

Lab detergent was chosen over household detergent because it was the most suitable product tested, its composition is duly indicated by the manufacturer, and it is used by other analysts (e.g. Ollé and Vergès 2008).

In contrast, we found that neither acetone nor ethanol modified the fat or organic matter adhering to the surface of the experimental bone, so they alone cannot affect these types of residues. The action of the vibrations to which the sample was subjected in the ultrasonic cleaner caused the soft tissue remains to move slightly out of place, but did not eliminate them. We did not observe any of the alterations reported by other authors for either acetone or ethanol (e.g. Buc and Silvestre 2006; Fernández-Jalvo and Marín Monfort 2008; MacDonald and Evans 2014), because immersion in these solvents for the amounts of time used in this experiment did not in itself cause any alteration (Matienzo and Snow 1986; Valtierra Pereiro 2019). The striations documented in some works (e.g. Fernández-Jalvo and Marín Monfort 2008) may have been caused by applying the products using mechanical means, such as cotton swabs or brushes (Bromage 1984; López-Polín 2012; Pedergnana et al. 2020).

Other products used in cleaning have more visible effects. Hydrogen peroxide is an oxidizing agent that reacts with lipids and proteins. In the field of dentistry, hydrogen peroxide has been found to react both with the organic fraction of dentin (oxidizing it) and with the inorganic fraction. In the
latter, it triggers a demineralization process, increasing the presence of oxygen and decreasing that of calcium and gives rise to surface morphological alterations (Rotstein et al. 1996; Hegedüs et al. 1999; Chen et al. 2002; Baldión et al. 2011). These processes have been related to those found on bone material (Chen et al. 2002). According to some authors (e.g. Fernández-Jalvo and Marín Monfort 2008), hydrogen peroxide would round the surfaces of bone specimens. Our results confirm that hydrogen peroxide causes loss of material and smooths the bone surfaces, which is clearly observable with high-resolution tools such as ESEM (Fig. 8).

The abovementioned striations and rounding caused by an inadequate cleaning protocol could be mistaken for use-traces. Other types of traces (linear marks, polishing, or edge damage, sensu Mateo-Lomba et al. 2020) may be concealed by fat and soft tissue remains. In addition, natural bone grease creates shiny surfaces that could be confused with polishing generated during functional tool use, leading to inaccurate descriptions of the microwear produced on active edges during experiments.

Another highly modifying agent is sodium hypochlorite. A chemical reaction takes place when it comes into contact with the components of the bone, altering its physical and histological properties, weakening it, and giving rise to substantial alterations on its cortical surface (Kerbl et al. 2012). It is an oxidizing chemical compound which reacts with organic matter, in this case, with the fat remains deposited on the bone and the organic components of the fresh bone itself (Bromage 1984).

The complementary microhardness analysis, using the precise and quantitative Knoop test, revealed changes in the materials after the application of cleaning products. We found that microhardness decreases (to differing extents depending on the product applied) once the specimens are subjected to cleaning. These microhardness values may be affected by a certain degree of water content in the cleaned

Fig. 6 A Experimental sample (08–20.5) before cleaning with ethanol. B Same control point of the sample after cleaning (stage 3). There are not significant changes in the appearance of the surface. Images obtained with a 3D digital microscope with lateral (A, B) and coaxial light (A.1, B.1). Original magnification: 140×. Scale bar: 500 μm
samples (Rho and Pharr 1999). However, when relating the results of the Knoop test to the changes observed at the microscopic level, we found that a lower degree of hardness does not translate to decreased cutting capacity. In the cutting test, the peroxide-cleaned sample had clearly decreased cutting capacity, even though its microhardness was not affected as much as that exposed to detergent. The Derquim®-cleaned sample exhibited better results despite the fact that its microhardness value was lower than that of fresh bone. This seems to indicate that microhardness is not the only property that influences cutting capacity. Derquim® has a slightly basic pH, which may affect the organic phase of the bone, and consequently, mechanical bone properties such as elasticity and microhardness (Weiner and Wagner 1998; Natali et al. 2014). But, in the case of the hydrogen peroxide–cleaned sample, not only was the organic fraction attacked, but it was also clear that the microstructure of the bone was affected (Fig. 8) (Chen et al. 2002). The state of the bone microstructure and/or other untested mechanical properties, such as flexibility and elasticity, might have a greater influence on cutting capacity since the microhardness value of the material is not related to this variable.

**Conclusions**

Traceology on bone industry has been the focus of considerably less methodological discussion than lithic traceology. However, despite the clear differences between these two types of raw materials, some steps in the methodology, such as sample preparation, have scarcely been developed. The lack of systematicity and standardization in the criteria for the use and effectiveness of the different cleaning protocols is clear.
The results presented in this work show that, after testing different products for cleaning experimental fresh bone tools with an ultrasonic bath, the use of laboratory detergent offers the best results in terms of both cleaning efficacy and conservation, as it does not alter the sample. This protocol may therefore be suitable for specimen cleaning in sequential experiments. Exposure time to cleaning must be adapted

Fig. 8  A Experimental sample (10–20.10) before cleaning. Some grease appears on the fracture plane. A.1, A.2 Details of the same point. Rough surface is visible. B Same point after cleaning with H$_2$O$_2$. Most of the grease has been removed except that trapped between lamellae. B.1, B.2 Bone surface has been extensively smoothed and some material disappeared. Images obtained with SEM (low. vac.). Original magnification: 300×(A, B), 600×(A.1, B.1), 1200×(A.2, B.2). Scale bar: 500 μm (A, B), 400 μm (A.1, B.1), 200 μm (A.2, B.2)
to each experimental sample, in keeping with its state and considering the worked material.

The effect of cleaning protocols on the materials requires further research, as the adaptation and use of procedures used on other materials such as lithics (e.g. hydrogen peroxide) may have modifying effects on the physical properties of bone material (Mateo-Lomba et al. 2020). We were able to verify that microhardness is not related to cutting capacity, since both lab detergent and hydrogen peroxide yielded similar values, both of which were lower than those of the fresh bone sample. The proposed cleaning protocol presented here is intended to be a first step towards the systematization of a traceological methodology for the study of both experimental and archaeological bone tools. Traceological publications should explain in detail the cleaning protocols performed, if applicable, prior to analysing the material.

Further studies could explore the issue of cleaning procedures in traceology using different variables or modifying those used here, such as the percentage of the reagent used, the application time, and the cleaning method (immersion, ultrasonic bath, application by pipette, etc.), amongst others. Likewise, chemical analyses should also be performed to characterise the contaminating substances present on bone materials, or blind tests might be conducted into the recognition of the characteristics of these substances and their subsequent elimination.

Any possible application of our results in the context of archaeological bone tools should be done with attention to several different aspects. First, studies of the functionality of these artefacts should document the possible presence of residues. Once they have been analysed and their origin established, a decision must be made as to whether they should be eliminated or preserved for the observation of use-wear. At the same time, situational reasoning and diagnosis of each element must be undertaken in order to determine the state of the material and the possible application of cleaning protocols that will not damage the integrity of the sample. To this end, the dialogue between microwear analysts, curators, and taphonomists is fundamental, so that the cleaning treatment must be performed having tested the effects of the protocols applied.

In addition, it is important to emphasise the need for appropriate sample preparation for the correct identification of use-wear. Such preparation removes elements that can be mistaken for use-wear traces and that can lead to erroneous interpretations about the functionality of these materials. Knowing how to recognize other elements that can be misinterpreted as use-wear should be a priority for any analyst. Furthermore, including detailed information about the protocols applied to specimens described in scientific publications is also recommended. Despite cleaning protocols, the contamination of the samples is difficult to prevent even if strict protocols are followed (Hayes and Rots 2019), so artefacts must be handled very carefully from the moment they are recovered.

This last point is especially important in the correct identification of minimally elaborated bone tools. These artefacts tend to present questionable technological modifications and the determination of use-wear can be decisive in the recognition of an item as an artefact or pseudo-arte-fact. Experimental archaeology and specifically sequential functional experiments provide reliable data collections with which to compare and identify use-wear. The correct interpretation of these tools could have technological and cognitive implications in the study of the behaviour of early human groups.

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Data availability Not applicable.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare no competing interests.

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