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Anthropology and Ethnology Open Access Journal
ISSN: 2639-2119

How to Perform SEM/EDX Analysis on Bone? Procedural Aspects and Main Anthropological Applications

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Research Article

Volume 5 Issue 2

Received Date: July 15, 2022

Published Date: August 16, 2022

DOI: 10.23880/aeoaj-16000178

Abstract

The application of scanning electron microscopy with energy-dispersive X-ray analysis (SEM/EDX) to the anthropological field has begun to open up wide-ranging perspectives of evaluation and investigation of great interest from various points of view, including the forensic one. Indeed, when bones are directly affected by penetrating detrimental means, they become valuable substrates on which to perform SEM/EDX analysis for forensic purposes. Moreover, on partially or completely skeletonized corpses, anthropologists take over or work alongside the medical examiner and their contribution plays a major role. In addition, SEM/EDX analysis may also prove useful in pursuing the identification of a victim. For these reasons, anthropologists need to be increasingly aware of the huge potential of this technique. However, it requires special precautions in order to provide appropriate and reliable results. Therefore, we present the proper approach to bone tissue for SEM/EDX analysis, reporting some of its main applications in the anthropological field.

Keywords: Anthropology; Bone Tissue; Forensic Pathology; Sampling Procedure; SEM/EDX Analysis

Introduction

In the forensic field, clarifying the nature of a detrimental mean involved in a homicide is of extreme importance, both in cases in which the weapon is found at the crime scene and especially if it is missing. Indeed, according to the Locard's principle, a transfer of micro-particles takes place from the wounding mean to the biological substrate with which it comes into contact. Such micro-particles may deposit in the wounded tissues by direct contact, penetration, or dislocation [1]. If there is consistency between the micro-traces found in such substrates and the detrimental mean found during the crime on-site inspection, the latter can be considered as the murder weapon. Conversely, if no weapon is available with which to make comparisons, the analysis of the micro-traces in the wounded tissues is crucial for guiding the subsequent investigations on the nature of the weapon involved [2]. The interaction between biological matrices and detrimental means can be effectively studied through the scanning electron microscopy with energy dispersive X-ray analysis (SEM/EDX) [3]. This technique, using electron beams, provides a three-dimensional view of the examined samples (different types of particles, such as fibers, organic or inorganic residues, minerals, dust, etc.), as well as an analysis of the chemical composition of all the identified elements [4]. As this technique have a high sensitivity, specificity, resolution power and depth of field greater than that of an optical microscope, it has assumed over time a role of primary importance in forensic investigations [5].

To date, in the literature, SEM/EDX has been almost exclusively applied to soft tissues, but when the used detrimental mean has penetrated so deep as to have injured bone structures, these latter become valuable substrates on which to perform SEM/EDX analysis [2]. For this reason, it is necessary for anthropologists to be aware of the great potential that injured bone can have for also forensic purposes. In fact, on partially or completely skeletonized corpses or even on more or less disarticulated bone remains, anthropologists take over or work alongside the medical examiner and their contribution plays a major role [6]. We therefore present the most appropriate approach to the bone tissue for SEM/EDX analysis. As a further demonstration of the great versatility of SEM/EDX investigation and the need for anthropologists to increase their awareness of the importance of this investigation, we report some of the other main applications of SEM/EDX for anthropological purposes.

Sampling Procedure

In order to preserve any residual exogenous material on exposed bone tissue and to obtain a reliable SEM/EDX analysis result, it is essential to avoid the preliminary washing of the body [2]. Next, it is necessary to identify the injured bone (or dental) structures of potential forensic interest as the possible localities of exogenous micro-traces. If there are completely detached bone fragments, they can be directly picked up using plastic forceps. On the contrary, if the area of interest is in continuity with the main bone structure (e.g. an area of depression or chipping of the skull), then it is necessary to cut out a bone piece (approx. 1 x 1 x 0.5 cm) using an oscillating saw with a ceramic blade. The need not to use metal instruments is to preserve the specimen from possible contamination by metallic micro-traces deriving from the autopsy instrumentation. This specific aspect is relevant in the search and potential identification of any exogenous metal traces; those of iatrogenic origin could be confused during SEM/EDX analysis. In addition, a negative control is required and, for this purpose, it is essential to take a fragment of intact bone, from a distant and unscathed area. The collected bone fragments must be immediately placed in sterile and hermetically sealed containers to avoid environmental contamination. They do not require any further pre-treatment and can be sent directly for SEM/EDX examination. However, if this is not possible for logistical reasons, samples can be stored in the freezer at -20°C until analysis. Dental elements can be approached in the same way as bone fragments.

If the specific ceramic instrumentation is not available and it is not possible to sample a bone piece, a double-sided graphite tape can be used as an alternative. Approximately 5 cm pieces of such a tape can be placed and pressed directly onto the portion of the damaged bone tissue that is to be analyzed. The tape should be positioned in a way that it also cover at least part of the adjacent free-of-injuries bone tissue that stands as a negative control. Subsequently, the scotch is fixed on a microscope slide and placed in a sterile, hermetically sealed tube, ready to be directly analyzed. Unlike bone fragments, graphite scotch can be stored at room temperature without any deterioration of the substrate.

Anthropological-Forensic Applications of SEM/EDX to Different Injury Modalities

In blunt force trauma associated with skin laceration, the direct impact of the detrimental mean on the underlying bone tissue may occur, causing its exposure frequently. This typically happens in the case of blunt head trauma, in which the impact may also cause either areas of depression/indentation or the formation of multiple and completely detached fragments [7]. The impact point represents the best site on which to investigate the presence of any exogenous micro-traces resulting from the contact of the detrimental mean on the victim [2]. In this context, the great versatility of SEM/EDX makes it possible to successfully identify on bone [8] particles from various types of tools: glass [9], minerals [10,11], metal [12,13], wood and hard plastic, as well as various impact surfaces, such as bricks, tiles, asphalt, soil [14], wall or floor paintings [15], and painted car surfaces [16-18].

With regard to sharp force injuries, penetrating wounds are typically able to affect the underlying bone tissue and result in lesions whose morphology can reproduce the cutting edge of the detrimental mean [19]. In these cases, the bone piece to be taken must necessarily contain the cutting margins as these constitute the most significant points where the micro-metallic traces are deposited during the friction of the blade on the bone tissue [20]. Furthermore, these same margins are also relevant from another point of view. In fact, they can look different depending on whether the cutting edge of the weapon is smooth or serrated, presenting in the latter case microgrooves caused by the teeth of the blade [21]. All of these aspects can be effectively investigated by SEM/EDX analysis, and a few have been reported in the literature [8,22-24]. In general, the metallic particles typically associated with sharp weapon injuries include manganese (Mn), nickel (Ni), iron (Fe) and chromium (Cr) [25]. The latter two result from the plating of steel and give the blade greater hardness and corrosion resistance [26].

In the case of gunshot injuries, gunshot residues (GSR) are of particular importance, among which lead (Pb), barium (Ba) and antimony (Sb) are considered as pathognomonic particles [27]. They can be characterized both morphologically and compositionally using SEM/EDX [28]. The penetrating force of a projectile is such that bone tissue is very often involved and perforated. This typically occurs in the case of gunshots to the head [29]. The resulting fragments are an excellent substrate for the search for GSR, the detection of which makes it possible to diagnose a gunshot wound. Furthermore, in the case of penetrating head injuries, the analysis of the bone margins of the two holes aids the differential diagnosis between the entry and exit bullet hole [2,30]. Using SEM/EDX analysis, GSRs have also been successfully detected on decomposed and cremated human bodies [31] as well as skeletal remains [32]. In the context of all the above injury modalities, in addition to the described specific elements of each of them, SEM/EDX analysis typically reveals also calcium (Ca) and phosphorus (P) particles due to the involvement of bone tissue as a result of bone fractures [26].

Other SEM/EDX Applications of Anthropological Interest

The usefulness of SEM/EDX in anthropology does not end with the applications to the above mentioned injury modalities. Indeed, through this analysis it is possible, for example, to distinguish fresh bone from archaeological bone after a prolonged period of immersion in water [33]. Furthermore, this technique also allows for rapid diagnostic classification as a bone or dental element in the context of fragmented or taphonomically altered evidence that cannot be interpreted macroscopically [34,35]. In this context, quickly confirming the biological nature can also allow for genetic analysis [36].

Even in attributing an identity to an unidentified or severely damaged corpse or to bony remains without any particular identifying marks, SEM/EDX can prove decisive. In this regard, it was applied to a crown of a tooth and root fragments found at the crime scene where the victim was charred. A suspected identity was confirmed by parallel striae of the tooth enamel and dentine, which demonstrated a previous dental restoration that the suspected victim had undergone prior to death [37]. Furthermore, dental materials and restorative teeth were analyzed with SEM-EDX before and after carbonization, demonstrating that the original elemental composition was preserved. The ability to distinguish dental materials has an important impact on the identification process [38,39]. Even in cases of bodies in which metal osteosynthesis devices have been implanted, SEM/EDX could be considered for identification purposes. In fact, the interaction between bone and metal matter creates a unique connection between the subject and the prosthesis itself, with the release of metallic micro-traces (iron (Fe), titanium (Ti), nickel (Ni), cobalt (Co) and chromium (Cr)) into the surrounding bone that reflect those of the prosthesis in both quantity and quality [40].

A further application of SEM/EDX can be the assessment of the vital reaction of fractured bone fragments, even months after death. Indeed, in the context of fractures, red blood cells may remain and can be detected and recognized by SEM/EDX investigation [41]. Innovative research frontier is the evaluation of the vitality and healing process of fractures by analyzing the Howship's lacunae along the fracture lines [42]. Finally, on ancient human remains (an archeological setting), SEM/EDX analysis has proved to be very useful, as it allowed the identification of the cause of death on the remains of King Louis IX of France. Indeed, it showed that the king died due to a schistosomiasis infection, a parasite that was found in its adult form in the mummified viscera and bone tissues of the corpse [43].

Conclusion

The anthropological field is characterized by areas that are still little explored and for which no particularly sensitive analytical methods exist to date. In this context, the application of the SEM/EDX technique has begun to open up broad perspectives of evaluation and investigation of great interest, mainly in order to better assess the victim's death event. The injury modalities that cause bone trauma certainly represent one of the main applications of SEM/EDX analysis in the forensic anthropology and pathology setting. Indeed, identifying and diagnosing the real nature of some bone injuries (especially in the case of completely or partially skeletonized human remains, as well as those in an advanced state of decomposition) can be very challenging. It is true that the pattern of fractures can often be suggestive of the injury modality involved, but often the interpretation is not straightforward at all. Just consider the differential diagnosis between blunt force trauma and gunshot injury if only a few fractured and dissociated cranial bone fragments are available [6]. Concerning sharp force trauma, as the morphology of point-and-edge wounds can be multiple and insidious, the morphological assessments are often not sufficient to link the wounds on a corpse to the weapon involved even when the weapon is at the crime scene [26]. In all these situations SEM/EDX analysis can be used to guide the subsequent investigations and allow to spread some light, since it proved to be a sensitive technique in detecting exogenous micro-traces deriving from the weapon and means of wounding and killing [25]. This technique could also help to study in-depth more recent injury modalities, such as lesions caused by non-powder guns on bones [44]. In addition to all this, SEM/EDX analysis may also prove unexpectedly useful in pursuing the identification of a victim. This is, therefore, clear why anthropologists must be aware of the potential of this investigation, which while on one hand represents a great opportunity, on the other one requires extreme procedural accuracy. Indeed, the greatest criticality lies in the fact that the target of this technique mostly consists of particles that are invisible at the naked eye, and as such at high risk of being overlooked. For this reason, caution and foresight are required in selecting the areas of interest to be sampled, before washing the body. A superficial procedure would lead to the careless and unconscious elimination of possible evidence resulting in the inevitable loss of relevant and otherwise unobtainable information.

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