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ADVANCED MORPHOLOGICAL AND ANALYTICAL INVESTIGATIONS OF MUMMIES. THE PATHOLOGIST'S APPROACH

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SUMMARY

ADVANCED MORPHOLOGY AND ANALYTICAL CHEMISTRY IN MUMMY PALEOPATHOLOGY

Advanced morphological and analytical methods play a very important role in paleopathological investigation. Depending on the availability of the instruments, a modern pathologist should be prepared to choose the most suitable technique to the specific question and be able to interpret the results obtained.
The aim of this paper is to illustrate the application of such methods through a series of practical examples dating back from Egyptian dynastic to contemporary mummified tissues, in order to outline a basic guideline for paleopathologists in different settings.

Introduction

Human remains can give us a huge amount of information about disease in past times, helping us to better understand contemporary pathological conditions. As a multidisciplinary science, paleopathology is widely based on modern investigation techniques and the pathologists involved in the study of ancient diseases should be aware

Key words: Paleopathology - Mummies - Morphology - Analytical chemistry

of the countless options available. Beside the familiar morphology-based methods (macroscopic examination, radiology, endoscopy, autopsy, and histology), there is a growing number of procedures that may be used to obtain information from mummies[1,2]. The application of non-invasive/minimally destructive approaches is to be preferred, following a careful planning of the possible examinations in connection with the specific case, before any conservation procedure, and ahead of extensive handling[3]. Among the available techniques, advanced morphological and analytical methods play a very important role in paleopathological investigation.

The purpose of this paper is to illustrate my personal experience in the application of such methods through a series of practical examples, in order to outline a basic guideline for paleopathologists in different settings[4,5].

Material and methods

Different ancient materials obtained from natural or artificial mummies were studied in order to complete the paleopathological investigation.

The renal stones found in the natural mummies of Pandolfo III Malatesta, Lord of Fano (1370-1427)[6] and an anonymous nobleman from Popoli (18th century)[7] were investigated to establish their structure and composition. The material from four canopic jars in the Egyptian Museum of Florence, found in Thebes and belonged to an individual of the New Kingdom (1550-1069 BC)[8] was studied in order to identify organs and substances. A white-coloured nail, belonged to a partially mummified adult female from Sermoneta (20th century)[9] was studied to evaluate its features and establish the chemical composition of the white substance.

One of the calcified nodules found near the lumbar vertebrae in the partially skeletonized body of the Blessed Sante Brancorsini (1343-1394)[10] underwent thorough examination to solve the differential di-

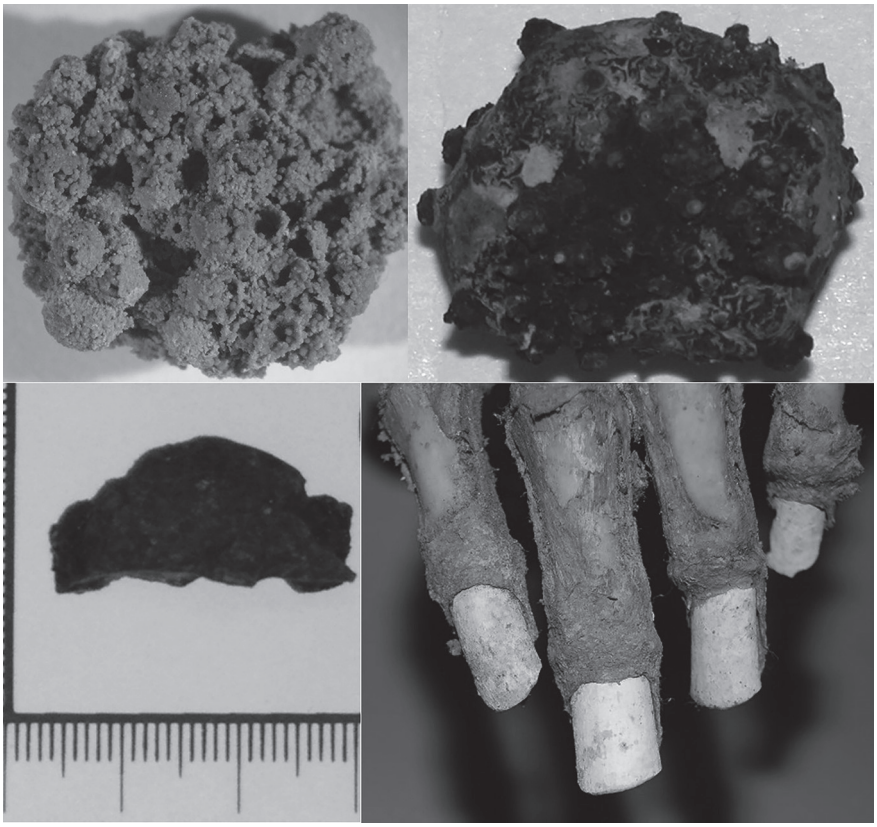

Fig. 1. From left to right: Pandolfo and Popoli renal stones; material from canopic jars; mummy fingernails from Sermoneta.

agnosis between urinary stones, calcified lymph nodes, phleboliths, and restoration material. The anterior thoracic and abdominal skin from the artificial mummy of Saint Giacomo della Marca (1393-1476)[11] underwent analysis in order to establish the presence and chemical composition of surface materials. The brown, chalky material from a foot compress found in the natural mummy of a Sicilian nobleman from Modica (18th century)[12] with signs of osteoarthritis, was investigated to establish its chemical composition.

Fig. 2. From left to right: cut surface of the nodule from Blessed Sante; the artificial mummy of Saint Giacomo; foot compress of the Modica nobleman (CT scan).

The following techniques were employed. Binocular stereomicroscopy (BSM) was performed using a LEICA S8APO stereomicroscope, equipped with a LEICA EC3 camera. Scanning electron microscopy (SEM) with energy dispersive X-ray analysis (EDX) used a Philips XL30/CP scanning electron microscope, equipped with OXFORD-Inca x-act Energy microanalysis. X-ray diffraction (XRD) analysis was carried out using a Philips X'Pert PW 1830 X-ray diffractometer. Fourier transformed infrared (FT-IR) spectroscopy employed a Thermo Nicolet Nexus and a Perkin Elmer Spectrum Two instrument. Inductively coupled plasma optical emission spectrometry (ICP-OES) was made through a Perkin Elmer Optima 7000 DV system.
The features of the samples and the needed information addressed the choice of the most suitable options case by case. The kidney stones were investigated by BSM, SEM/EDX, and XRD. The canopic jars content underwent BSM and SEM/EDX, along with histology on paraffin- and methacrylate-embedded material. The nail was carefully extracted from its bed and submitted to BSM, and SEM/EDX with a fully conservative approach. The abdominal nodules of the Blessed Sante Brancorsini underwent BSM, SEM-EDX, XRD, and FT-IR, along with histology on paraffin-embedded material. The skin of Saint Giacomo della Marca underwent BSM, SEM/EDX and surface XRD analysis. The material from the foot compress of the Sicilian nobleman was investigated by FT-IR and ICP-OES.

Results

BSM and SEM investigation of the renal stones enabled us to observe the morphological details of their external surface and inner portions, whereas SEM/EDX and XRD analysis allowed to understand their elemental and chemical compositions. The stone of Pandolfo had a mulberry-like surface with honey brown colour and measured 12 mm in largest diameter. Along with the organic constituents (C, O, N), the following chemical elements were detected: K, S, Si, Cl, Ca, P, Na, and Ba. The calculus was composed of ammonium acid urate (95%) and calcium oxalate dihydrate (weddellite) (5%). In the case from the Popoli nobleman, the ovoidal mass with small superficial spherical buds measured 22 x 16 x 15 mm. The cut surface showed a central nucleus of sharp-edged crystals and concentric laminations. Detected chemical elements were: C, O, N,Ca, P, K, S, Cl, Na. The

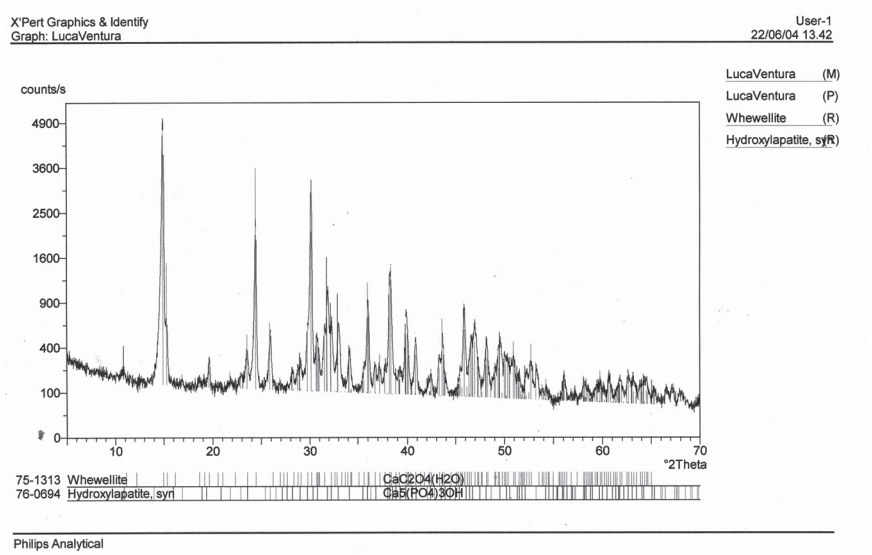

Fig. 3. X-ray diffractogram of the Popoli stone revealing 90% calcium oxalate monohydrate (whewellite) and 10% calcium phosphate (hydroxylapatite).

stone composition was calcium oxalate monohydrate (whewellite) (90%) and calcium phosphate (hydroxylapatite) (10%).
In the material from canopic jars, histology allowed to identify pulmonary tissue with silico-anthracosis in two of them, and intestinal content with starch particles in another one. The fourth sample held amorphous material enclosing wide birefringent fibres in slides from paraffin-embedded material. It melted away after processing for methacrylate embedding, leaving only entwined fibers related to the linen fabrics used to wrap the organs. Furthermore, chemical constituents of natron salts (sodium chloride, sulphate and carbonates) used during embalming were identified using SEM/EDX.
BSM of the nail allowed to appreciate differences between dorsal (polished) and ventral (unstained) surfaces, as well as to closely inspect the nail root and the free edge contours. SEM evidenced further details of these structures, enabling to select areas for EDX

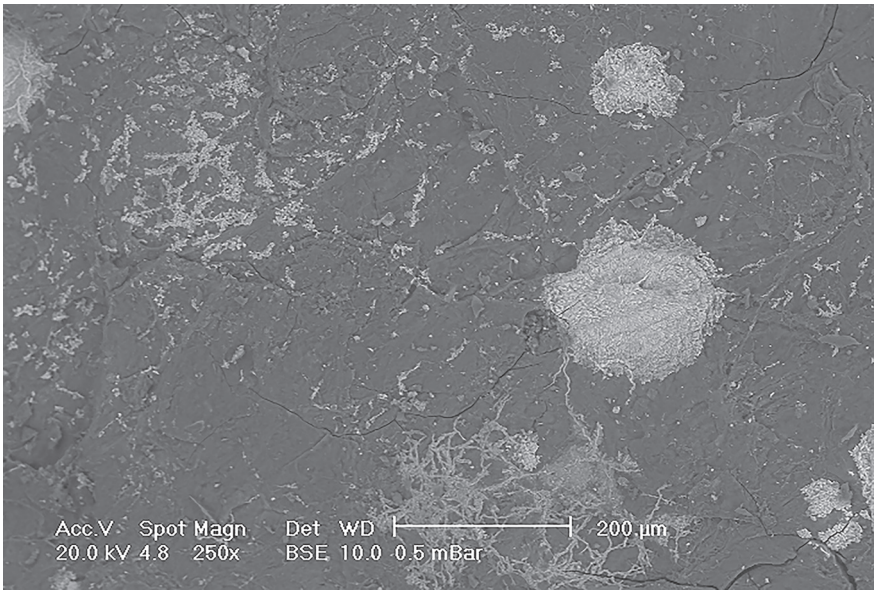

Fig. 4. Crystals of natron salts identified by SEM in canopic jar material.

measurements. C, O, S, Mg, Cl, K, P and Ca were detected in unstained areas of the dorsal surface and in the ventral one, indicating the organic structures of nail. The pigmented areas of the dorsal surface displayed O, S and C only, suggesting the presence of calcium sulphate (CaSO4) used as a nail polish. Al, Fe and Si in the free edge of the nail were referred to remnants from manicure devices.
Paraffin-embedded histology of the nodule from the body of the Blessed Sante Brancorsini showed evidence of a fibrous capsule with an amorphous acidophil content. The composition in apatite revealed by EDX and XRD allowed to rule out urinary stones and restoring material. The presence of DNA and serum proteins between the organic components displayed by FT-IR confirmed the biological nature of the nodules, more likely related to phleboliths than to reactive lymph nodes.

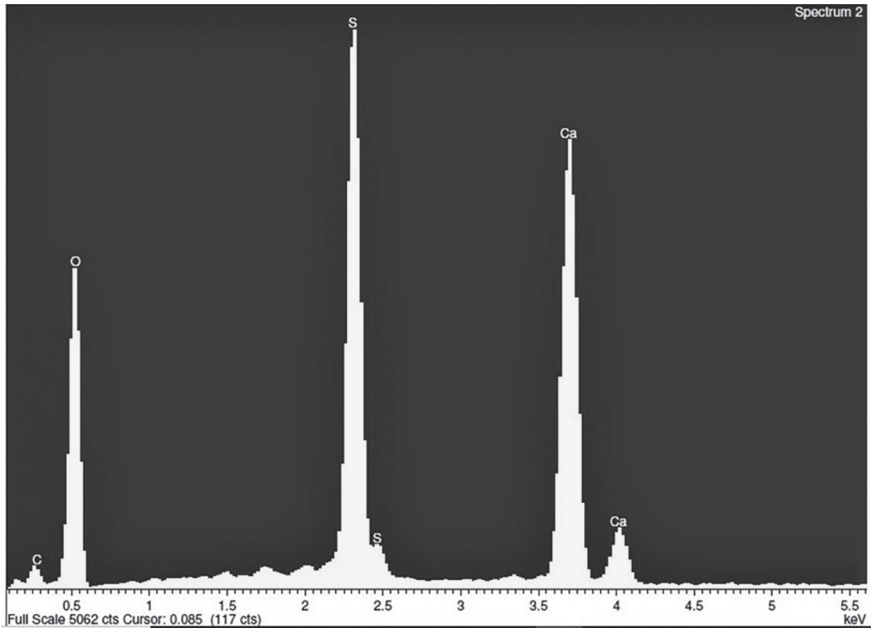

Fig. 5. Elemental analysis of the nail polish with SEM/EDX.

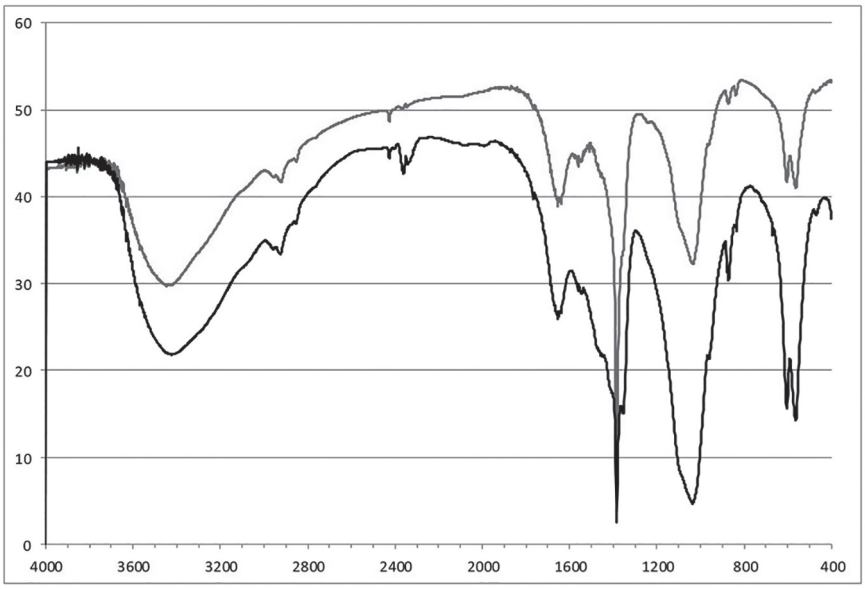

Fig. 6. FT-IR spectrum of the nodule from the Blessed Sante.

The preliminary SEM/EDX of the skin from Saint Giacomo della Marca showed significant traces of mercury. XRD analysis of the external surface did not show crystalline components.
ICP-OES analysis of the powder from the foot compress of the Sicilian nobleman allowed to identify and quantify the chemical elements. FT-IR spectroscopy revealed the presence of clay, plaster and calcium oxalate. The latter may be related to bacterial activity. Additional unidentifiable organic components may be derived from tissue or bandage decomposition, as well as from components added to the device. Salicylic acid was ruled out.

Discussion and Conclusions

Advanced morphological and analytical investigation methods may be of great help in understanding ancient diseases. Although one may

be discouraged by the great number of methods, the paleopathologist should be prepared to choose the best process to be employed in every instance. The first and most important distinction is between conservative and invasive/destructive methods. This distinction applies to both morphological and analytical techniques and is based on the capability of maintaining the existing conditions of the body or the object under examination. Analytical chemistry methods may also be classified as qualitative if they are designed to identify chemical elements, inorganic or organic molecules, and quantitative when they are able to measure the amount of the substances. The application of carefully selected analytical-chemical techniques to archaeological research provides data otherwise not available to the investigators[13].

The protocol for the examination of renal stones employed primarily conservative morphological instruments. After BSM observation, the specimen underwent SEM-EDX for elemental analysis[14]. The combination of optical microscopes and SEM-EDX is widely used in different circumstances, as the study of putrefaction fluid deposits[15], to establish differential diagnosis between taphonomic effects and pathological conditions[16]. XRD analysis allowed to obtain an affordable evaluation of the chemical composition of the stones with minimally destructive approach in the case of Pandolfo and after section in the case from Popoli. A similar approach could be used also in the case of the Blessed Sante, but in that particular setting, the application of minimally destructive methods was preferred. This option allowed to compare morphology by different optical systems, also obtaining data about inorganic and organic components by submitting small amounts of powder to XRD and FT-IR[17]. FT-IR spectroscopy represent a powerful technique to study organic molecules, as it is particularly sensitive to the secondary structure of the proteins[18, 19].

The evaluation of canopic jars contents by a multidisciplinary approach favoured histological techniques. Despite the lack of organic

components evaluation, we were able to perform identification of human organs and non-human materials, providing useful information about the diseases of ancient Egyptians[20].

The studies of the white-coloured nail and the skin from Saint Giacomo were based mainly on SEM with elemental analysis. The presence itself of calcium sulphate (plaster) may explain the preservation of the fingernails, often disappearing after death. According to the history of nail care, the chemical composition of the polish and the nail shape helped to date back the death of the subject to the very first decades of 20th century. At that time women used to stain their nails with dyes, buffing them shiny and naturally coloured, even though in this case the procedure could also be performed after death[21].

The body of Saint Giacomo revealed indisputable evidence of artificial mummification, by a xipho-pubic incision and posterior craniotomy, both sutured with stitches. This makes more likely the conservative use of mercury than its employment as a therapeutic ointment. Further analyses are planned in order to detect the organic substances used for embalming[22, 23].

The presence of natural remedies in an ancient body is a point of great interest. The use of such remedies in past times is well known but, to the best of my knowledge, no traditional remedy for osteoarthritis has been described in a mummy to date. The presence of a clay pack on a foot as a natural remedy in folk medicine dates back to prehistoric times, and a hot clay cataplasm (poultice), with or without other organic substances can soothe aching joints and relieve swelling and inflammation[24]. The use of ICP spectrometry yields quantitative results on the inorganic component[25].

Among the techniques not available X-ray fluorescence (XRF) analysis deserves a special mention, as it is being increasingly used[26]. Portable XRF instruments allows compositional measurements to be undertaken directly on the mummy. Also, Raman spectroscopy represent an extremely valid tool in organic substance characterization[27].

In conclusion, when facing off these particular procedures we must be aware of these basal rules: there are many different techniques, the researcher should know how to choose the most suitable to the specific question and be able to interpret the results. This means that a skilled pathologist has to know the main features of each method, in order to select the best available options.

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Acknowledgements
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