

Beyond Death: Forensic Investigations of pre-Columbian Mummies from the Tarapacá Valley, Chile, Using Variable Pressure SEM and Raman Spectroscopy

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Introduction

Variable pressure scanning electron microscopy (VPSEM) coupled with other non-destructive analytical methods, such as energy dispersive (EDS) and Raman spectroscopy (RS) offers new capabilities for non-invasive imaging and chemical characterization of archaeological materials. This article underlines the application of VPSEM-EDS-RS on bioarchaeological specimens of pre-Columbian mummies from the Tarapacá Valley in northern Chile. The aim of the scientific investigations is to identify nonanatomical features and to provide qualitative and quantitative information at molecular levels, complementing the morphological record from studies in physical anthropology [1], in an effort to understand mortuary practices in the Tarapacá Valley and the effects of the burial environment in the preservation of mummified human remains.



Figure 1. Mummy bundle from the Tarapacá 40 cemetery (TR40) in a fetal position and with a strap secured around the neck.

Since 2005 an international multidisciplinary team from UCLA and the University of Chicago, in collaboration with faculty and students from the Universidad de Chile, carried out archaeological investigations in the Tarapacá valley in northern Chile to explore the relationship between Tiwanaku and mid-valley polities during the Middle Horizon and Late Intermediate Period (ca AD 400-1535). Systematic surveys recorded hundreds of sites in the valley and in the pampas immediately above. Excavations at one large habitation and cemetery site, Tarapacá 40 (TR40), exposed naturally mummified human remains. Six bodies were retrieved, two of which were well-preserved while the others were in poor condition of preservation. The mummies were wrapped in bundles of textiles, feathers, and basketry. There were in a fetal position, typical for the region and time period. One mummy bundle had a strap at either end possibly intended for transportation

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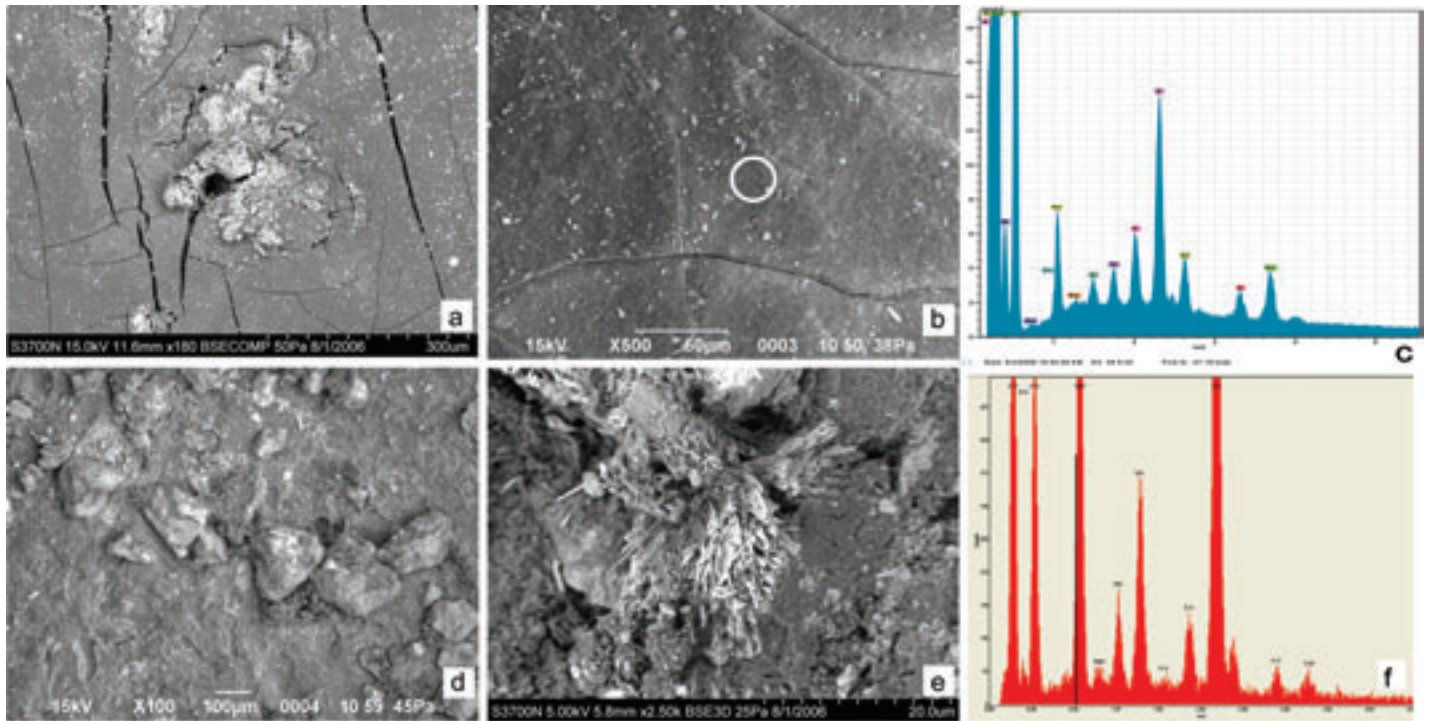


Figure 2. Secondary electron images (a, b, d, e) and EDS spectra (c, f) of skin samples from Pre-Columbian mummies. The spectrum shown on image (c) corresponds to the area highlighted on image (b); spectrum shown in figure (f) corresponds to the entire area shown in figure (e). Images of the skin revealed extensive cracking due to the processes of spontaneous desiccation and mummification (a, b). Debris representative to burial depositional layers cemented into the skin (d). Elemental analysis indicate presence of Al, Si, Mg, Fe (several weight %) and some other elements. Salt crystallizations on the skin were also observed in several locations (e). These were identified as halite - NaCl (f).



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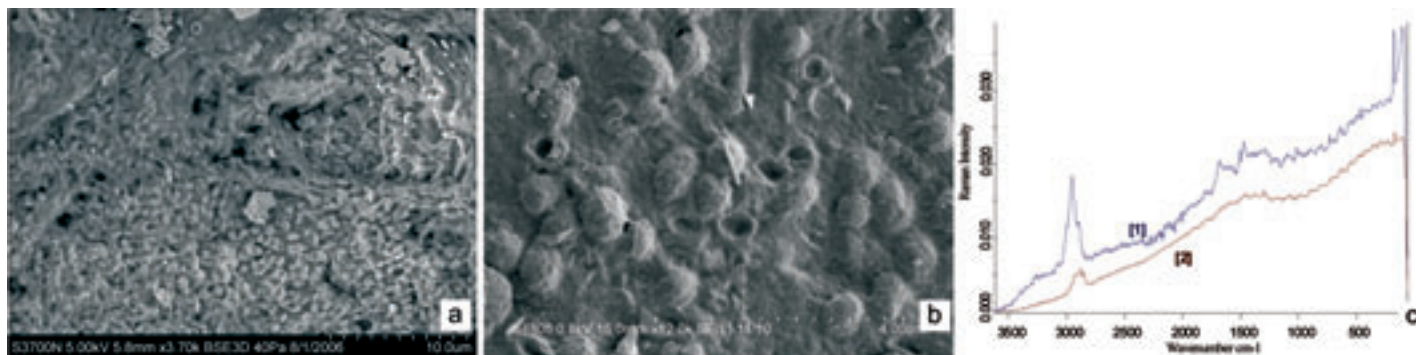


Figure 3. High magnification secondary electron images (a, b) and Raman spectra at 1,064 nm excitation of well-preserved and degraded mummified skin of pre-Columbian mummies in Chile (spectrum 1-blue and 2-red respectively). Spectrum 2 shows a marked loss of protein amide I (1680-1645 cm^{-1}) and amide III (1305-1230 cm^{-1}) band intensities (c).

(Figure 1). These preliminary observations raised a series of questions: *Does the presence of the strap imply that this mummy was carried to the final burial destination from another location? What were the mortuary practices of these people? Can the level of decomposition of organic material, namely the proteins still preserved in the hair and skin be used as a biomarker of taphonomic processes?*

The area of TR40 is of particular interest since it is located very close to the important residential site of Caserones, a large habitation site in the mid-valley. Ceramic and textile analysis together with studies in physical anthropology and paleopathology will provide data to test the models in the research design for the larger project.

Experimental Methodology

Our approach is based on the Locard Exchange Principle of fractured patterns as factual physical evidence [2] and follow the non-invasive \rightarrow invasive \rightarrow non-destructive \rightarrow destructive protocol of examination. Where *invasive* refers to procedures that require micro-sampling. Non-destructive, refers to the application of techniques that do not consume or alter physically and chemically the sample and, therefore, the sample can be re-used for more analyses. Finally, destructive, refers to analytical procedures that in order to yield results the samples are destroyed or changed chemically and therefore become unusable for further analysis. Examples of the latter are DNA/PCR (polymerase chain reaction), and gas chromatography—mass spectrometry (GC-MS). This approach allows the collection of

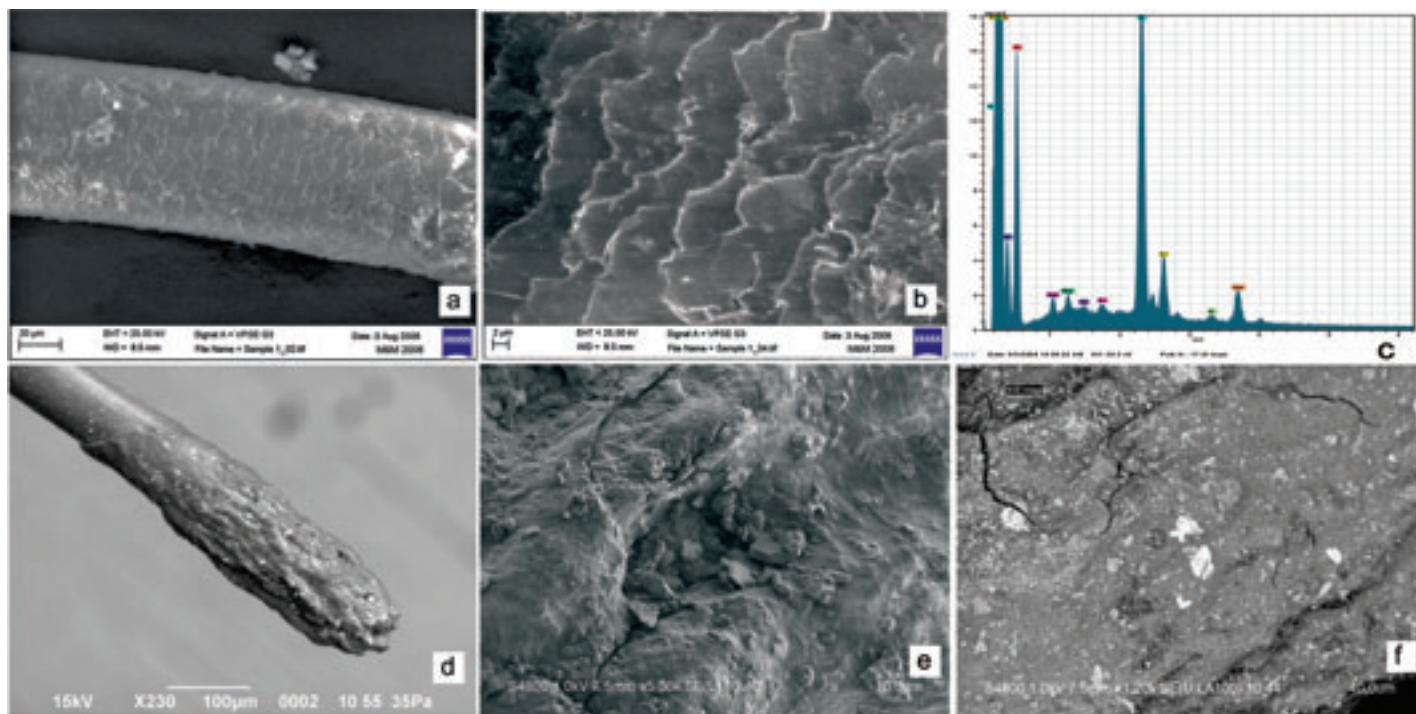


Figure 4. Secondary electron (a, b, d and e) and backscattered electron (f) images, and EDS spectra (c) of hair samples from mummies. The images show the degree of preservation of the entire hair and the natural shrinkage of its clock-bag. Debris and mineral inclusions attached to the surface of the hair are also visible, especially with the use of backscattered electrons signals. EDS elemental analysis shows measurable amounts (several wt. %) of Al, Si and Mg. Analysis of hair samples can be used for the sensitive biomonitoring of environmental exposure or the ingestion of an individual during his or her life time via food and drinking water. This is because hair is stable and robust and its composition does not change significantly after death compared to other tissues. Hair also has the unique ability to reflect the total body intake over an extended period and it is possible to trace changes over time depending on the length of the hair [4]. These preliminary results collected on hair using VPSEM coupled with EDS and RS can be used to select the best “candidates” for other types of analysis including inductively coupled plasma mass spectrometry (ICP-MS).

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Figure 5. Secondary electron images of hair from the mummy illustrated in Figure 1, with a head lice egg (nit). Examination of the lice egg's size, dimension, and other morphological characteristics can be performed and the distance from the place of attachment of the nit to the hair shaft to the clock-bag can be measured. Images show that the egg had hatched. Parasite remains from archaeological sites can address a remarkably broad range of questions regarding past behavior of human and environmental factors [5].

important data without having to take unnecessary samples. It is also an excellent procedure for the design and development of the subsequent steps of the examination since some questions may be answered and at the same time others may arise from the findings.

Having completed x-ray radiography and CT scanning of the mummy bundles for the examination and visualization of the bones and internal organs, the mummies were further subjected to an exhaustive visual examination and documentation using photographic-based glossaries and descriptive recounting. Subsequently, twelve samples were taken from exposed areas of the skin and hair from four different individuals and analyzed using VPSEM-EDS-RS.

Results and Discussion

Examination and analysis of the skin: High resolution and high magnification secondary electron (SE) and backscattered electron (BSE) imaging using VPSEM in areas where we had previously observed whitish extrusions protruding from the skin, revealed the presence of a biofilm probably formed by bacterial infection (Figure 3). Although it is still uncertain whether this biofilm is due to antemortem pathological lesions caused by parasitic infections or a postmortem artifact forming this pseudopathological effect, these observations are significant and require further diagnostic investigations. Analysis using RS of skin specimens from different cemeteries indicated that some skin specimens are better preserved than others (Figure 3 c).

Examination and analysis of hair: Close examination indicated that one of the mummies was laden with head lice eggs. SE imaging shows clearly the hatched nit attached to hair closer to the scalp (Figure 5). However, it is still unclear whether the individual was infested by louse-transmitted diseases such as typhus or other epidemic diseases. Head lice infestation is common among pre-Columbian populations from Peru and the Atacama desert [3]. Since louse infestation is transmitted between people that come in contact with each other, this study can contribute to the larger research goals of tracing patterns of transhumance and trade.

These preliminary results underline the potentials of VPSEM coupled with EDS and RS for the examination and

analysis of bioarchaeological materials. Of great importance is to be able to understand the degree of organic preservation and the detrimental role of the depositional burial environment, and to explore the environmental factors associated with pathogenic processes [6, 7]. Analysis of mummified skin and hair has the potential to address directly many of the anthropological questions in the Tarapacá Valley research. *Are there skin diseases more common to people living above 3000 meters than to people on the coast? How prevalent are tattoos in the populations, and can these be related to ethnic identities?* These and other questions can be addressed by harnessing the capabilities of a VPSEM-EDS-RS system.

Summary

The coupling of VPSEM with EDS and RS is a novel technology that has increased the versatility of the individual methods by allowing spatially resolved analysis and compositional characterization of single components in complex and heterogeneous samples at the micron length scale. This permits non-conductive samples, such as bioarchaeological specimens, to be analyzed without the need for the deposition of a conductive coating. Scanning electron microscopy combines large depth of focus, high resolution and compatibility with EDS, providing elemental compositional measurements for a broad range of applications in the archaeometric field. The addition of RS to the VPSEM-EDS configuration can significantly strengthen the analytical capabilities of the system as it can also provide molecular characterization of materials. ■

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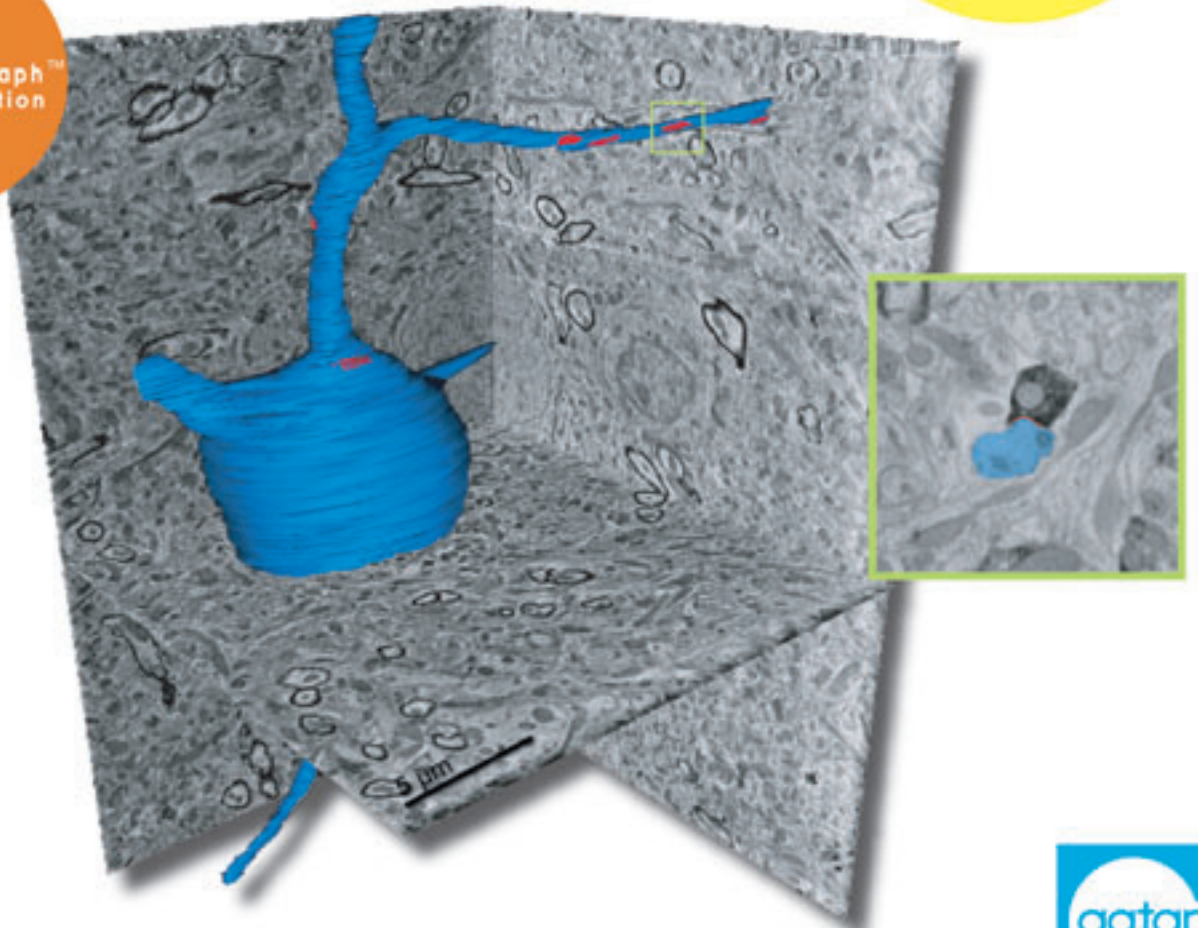


Image showing a neuron (axons through a mouse barrel cortex (neuron shown in blue and synapses shown in red) processed for EM and generated using Gatan's 3View™ SRPSEM microscopy system. Eight hundred perfectly aligned image slices of 30 nm were acquired. Gatan's DigitalMicrograph™ 3D Visualization tool was used to show the Z projection of the 800 slices. Manual segmentation using Reconstruct (GNU General Public License version 2) software generated a 3D reconstruction of the neuronal cell including the dendrites and axon. The inset image shows an identified synapse between a dendrite of the reconstructed neuron and the axon labeled for parvalbumin (pre-embedding immunocytochemistry with biotinylated antibody). Sample courtesy of Dr. Graham Knott, DBCM, University of Lausanne.

