

Herculaneum 79 AD: Neuronal Tissue Preservation from a Vitrified Human Brain

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Abstract

In AD 79 the town of Herculaneum was suddenly hit and overwhelmed by successive volcanic ash-avalanches, fast moving clouds of hot volcanic ash and gases, capable of killing all residents who were not yet evacuated. The scientific studies on the Herculaneum victims are now standing from the first discovery in the early 1980s of hundreds of skeletons of people crowding the beach and a series of waterfront chambers, fixated into a final vital stance by the first of the deadly incoming pyroclastic currents. Multidisciplinary studies on the victims' skeletons and their biogeoarchaeological context shed light on the dynamic impacts of the 79 AD Plinian eruption on the area around the volcano and on the causes of death of its inhabitants. A recent unprecedented archaeological discovery revealed unique evidence of preservation of a vitrified brain from a human victim found in the town. SEM analysis of brain and spinal cord vitrified remains showed an integrally preserved central nervous system. Results from site research combined with lab analysis offer new insights concerning the unique conditions occurred during the 79 AD Vesuvius eruption, with crucial implications for the present-day risk of a similar outcome to around three million people living close to the volcano, including metropolitan Naples.

Keywords: Forensic Anthropology; Bioarchaeology; 79 AD Eruption Victims; Vesuvius

Introduction

Up to now, there have been sporadic discoveries of well-preserved cerebral tissue from ancient human remains [1]. Under certain taphonomic conditions that prevent soft tissue decomposition, these remains are typically saponified [2]. However, ancient brains reported in literature show only partial preservation of neuronal structures [3]. During our recent paleoforensic survey at the archaeological site of Herculaneum, we discovered glassy material within the cranial cavity from a victim of the 79 AD Vesuvius eruption, apparently derived from his brain. Recent examination by proteomic analysis and gas chromatography-mass spectrometry of this material allowed us to identify fatty acids of human hair fat and several proteins of human brain origin, thus indicating preservation of vitrified human brain tissue [4]. The conversion of human tissue to glass (vitrification) occurred due to the rapid cooling of the volcanic ash deposit after exposure to the burning ash cloud [5]. Previous heating bone experiments showed

temperatures of about 500 °C [6], as also confirmed by our recent reflectance analysis on carbonized wood from Herculaneum [4]. Recently, examination of the ultrastructure of vitrified human remains showed several typical features of the central nervous system (CNS) from brain and spinal cord tissue [7].

Historical Background

In AD 79, a major explosive eruption hit by hot ash-avalanches the Vesuvius towns up to 20 kilometres away, causing thousands of fatalities [8]. Herculaneum, located 6 kilometres from the volcano, was rapidly buried beneath 20 metres thick volcanic deposits. The environmental burial conditions ensured the town to be preserved intact until the first discovery of its theatre, in 1710. Some decades later, during the early Bourbon exploration, the first human victims were discovered on November 18, 1739. A whole urban settlement gradually revealed itself as it had been buried by volcanic ashes

and returned in its integrity. However, most exceptional has been the discovery of some hundreds of human victims, unearthed in several seafront chambers and on the beach during archaeological investigations in the second half of the 1900s [9]. The volcanic

deposits have preserved intact for centuries the corpses of these victims as time capsules, a source of unique bio-anthropological information [10].

The guardian of the collegium augustalium



Figure 1: A complete overview of the 79 AD eruption's victim. The original context of discovery (*Collegium Augustalium*, Herculaneum).

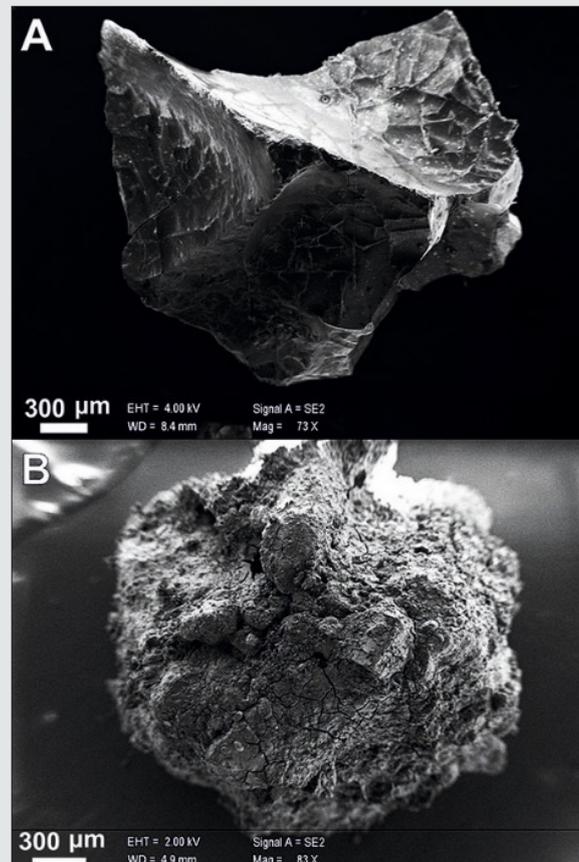


Figure 2: Vitrified human tissues from the central nervous system. A. brain fragment; B. spinal cord fragment (SEM, scale bars in micron).

In the mid-1960s, during the archaeological excavations of the town, in the Collegium Augustalium a small room was discovered containing a human victim lying on a wooden bed. This young adult male is believed to have been the guardian of the College, a centre of the cult of the Emperor Augustus. The victim is lying ventrally, face down in the volcanic ash (Figure 1). The skull and the post-cranial bones show complete charring and cracking induced by the hot pyroclastic ash surge [11], a high-speed turbulent cloud rich in hot gases, ash, and steam [12].

In a recent survey at the archaeological site we identified vitrified cerebral tissue within the skull of this 79 AD eruption victim [4]. Microspectroscopic analysis by Raman technique has

shown this tissue to consist exclusively of organic matter. We then carried out a scanning electron microscope (SEM) investigation on the vitrified tissue collected from the skull and the spinal cord (Figure 2A). Examination of the vitrified ultrastructure revealed several features, typical of the central nervous system (CNS) (Figure 2B). This type of remains is unique for the excellent quality of the tissue preservation. In literature several cases of ancient brains are reported which are preserved due to natural mummification or saponification, which allowed only partial preservation of neuronal brain tissue [1-3]. At Herculaneum, instead, the structures have been immobilized in their native condition, as a result of a natural process of vitrification induced by the local environmental conditions during the eruption [4,5].

Neuronal ultrastructures

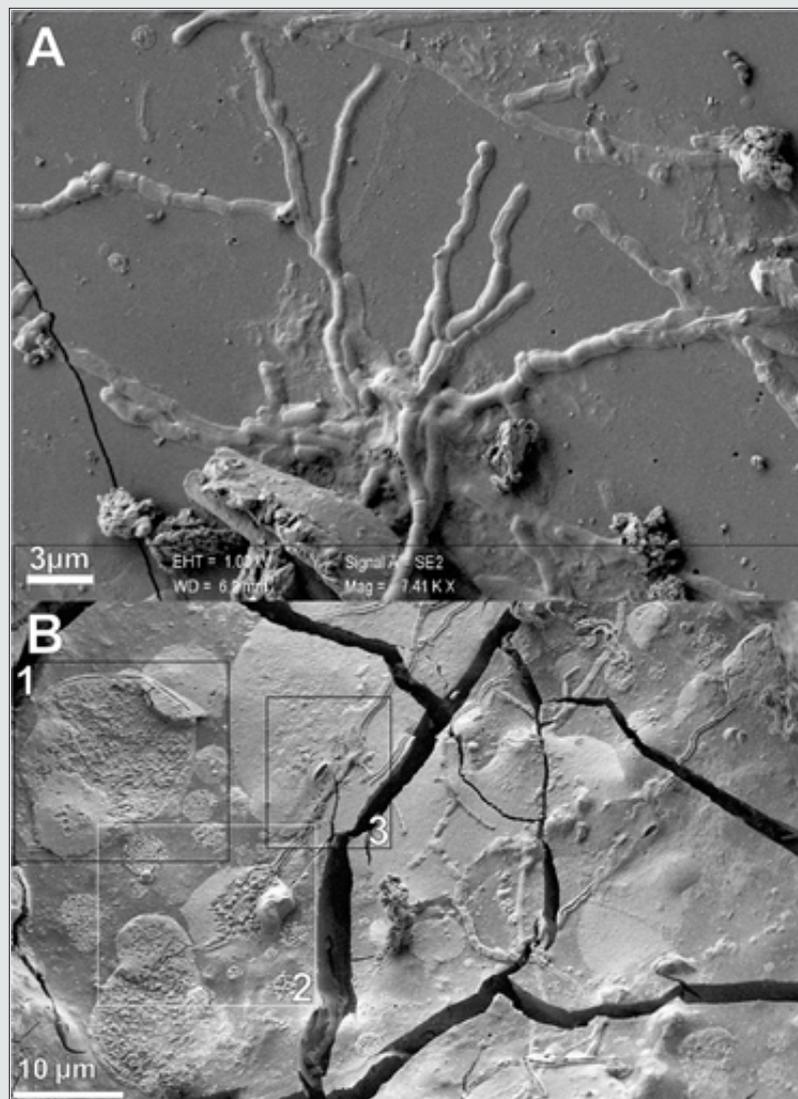


Figure 3: Structures of the central nervous system. A. SEM image of brain axons; B. SEM image of spinal cord axons intercepting cell bodies and sheath-shaped structures (scale bars in micron).

According to our SEM investigation, distinctive result of the CNS tissue vitrification has been the preservation of a system of axon-like tubular structures running across the cerebral matrix (Figure 3A) [7]. The observed neuronal architecture is analogous to that one detectable from biological samples after manual drop plunging protocol, or high-pressure freezing protocol followed by freeze-fracturing and cryo-SEM imaging [13]. These structures are mostly elongated and round in shape, as described in literature [14]. The mean diameter ranges between 550 and 830 nm, as expected for white matter axons which are in the medium 0.5 - 0.8 μm size range [15]. Such tubular structures are smaller in diameter than blood vessels in the cerebrovascular system [16]. A complex neuronal system was also identified in a glassy residue of spinal cord from the back bone. A basic structure is recurrent within the vitrified matrix: the neuronal architecture shows the preservation of a number of cellular bodies interconnected by a reticulum of multiple tubular structures (Figure 3B), whose morphology and size are analogous to those of neurons and axons. An image analysis procedure was used to extract quantitative information from SEM images. The round-shaped cell bodies, mean sized from 2.7 to 14.2 μm , show a cell membrane and an intracellular lumen filled by filamentous structures and nanovesicles. The sections of the neuronal cell body were classified in three groups depending on diameter (μm , mean \pm SEM) (14.17 \pm 1.66; 8.06 \pm 0.32; 2.72 \pm 0.28) and mean area (μm^2 , mean \pm SEM) (157.9 \pm 36.2; 53.2 \pm 6.8; 7v1 \pm 1.1).

We have also calculated the mean diameter (nm, mean \pm SEM) of 15 brain axons (717.7 \pm 24.0) and 15 spinal cord axons (672.1 \pm 20.2), values that are comparable to those of white matter axons. Furthermore, the free axons that we have identified in the vitrified brain tissue (Figure 3A) have proved to possess the typical myelin periodicity as resulting from neural network processing based on our specific mathematical method elaborated through MATLAB software [17]. Through this method, in two different vitrified brain axons we detected several different membrane layers that wrapped around individual axons and formed compact myelin: the pattern alternating darker lines and white spaces is the same as seen in vivo for mammalian CNS myelin [18]. In contrast with myelinated axons observed in the cerebral tissue, axons in continuity with cells detected from the spinal cord appear as non-myelinated, and characterized by a smaller mean diameter (452.5 \pm 14.5). We have also identified the presence of regular nanotubes inside the preserved cytoplasmic matrix of a neuronal cell body, as evidenced by our algorithm image processing [6]. The mean diameter of such nanostructures is of about 23 nm, value which is analogous to microtubules size [19]. Through the same procedure we discovered that the CNS matrix of both the brain and spinal cord is made up of recurring, similar nanostructures. This evidence suggests that the preservation process of neuronal tissue induced by vitrification is the same for all the CNS structural components. On the base of the ultrastructural features that we observed on archaeological vitrified human tissue, we have classified the cell bodies as neurons and the axon-like processes as axons. We hypothesize that the

unique natural process of vitrification occurred during the 79 AD eruption has locked the structure of the CNS, thus preserving intact its morphology. The detection of original neuronal structures of the central nervous system indicates the durable preservation of human brain and spinal cord ancient tissues.

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Author Contributions

P.P. designed and supervised the project; P.P. and F.S. designed and carried out the archaeological investigation; P.P., F.S., G.G., A.P. and A.V. performed data curation and formal analysis; P.P., V.G., G.C., M.P., F.S., G.G., A.P., A.V., E.C. and M.N. wrote the manuscript.

Competing Interests: The authors declare no competing interests.

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