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The Heslington brain: a challenge for analytical Raman spectroscopy†

Howell G. M. Edwards,[a]* Esam M. A. Ali[a] and Sonia O'Connor[b]

The survival of brain tissue in archaeological depositional environments is a very unusual occurrence that has generated much discussion and conjecture forensically. Here, we report the Raman spectroscopic analysis of biomaterial found in the cranial cavity of a decapitated skull dating from the Iron Age, some 2500 years ago, from which the presence of degraded protein consistent with it being naturally preserved brain is concluded. The novel observation of characteristic Raman spectroscopic signatures of biochemicals produced by cyanobacteria, namely carotenoids and scytonemin, both in the brain tissue and surrounding deposits from the cranium is consistent with the waterlogged depositional environment in which the human skeletal remains were found. The Raman spectral data are in support of biochemical, morphological and radiographic analyses of this biomaterial, which therefore can be described as brain that has been significantly reduced in volume inside the cranial cavity. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: Raman spectroscopy; prehistoric skeletal remains; archaeological brain; cyanobacteria; scytonemin

Introduction

The application of Raman spectroscopy to the analytical characterisation of archaeological biomaterials is now well established[1,2] despite the common occurrence of fluorescence emission backgrounds that arise from specimen degradation and the absorption of impurities from the depositional environment. In particular, novel information has emerged about the integrity and state of preservation of bioorganic compounds in human and animal tissues that have been exposed to degradative procedures; to a great extent, a major factor in this work has been long wavelength laser excitation of the Raman spectra in the near-infrared region at 785 and 1064 nm to minimise fluorescence generation. Thereby, Raman spectra of human skin from natural and chemically preserved mummies, bones, teeth, hair and nail[3–8] have been obtained successfully along with associated artefacts comprising ivories, resins, textiles and manuscripts. Comparison of the Raman spectra of these archaeological materials with their modern counterparts[9] reveals the extent of preservation or degradation that has been suffered by the artefact or archaeological specimen.

Generally, the soft tissues, including body organs, degrade quickly upon burial. Hair and nail may persist a little longer but eventually only skeletal bone survives, although this will also be degraded in extremes of pH. Preservation of soft tissues does occur where cryogenic protection has been effected environmentally and immediately in cold deserts; examples of this in the literature include the Qilakitsok Greenland ice mummies,[6] high-altitude Andean mummies[8] and a mummified penguin from Antarctica,[10] all of which share common factors in the preservation of their tissues through natural desiccation and low temperatures. In deliberate mummification processes such as those practised in Egyptian Dynastic funerary rituals,[5] the presence of exogenous chemical preservatives has sometimes caused post-mummification degradation through the excessive overuse of chemicals, desiccants and resins, and this can be evaluated using Raman spectroscopy to provide useful information for the conservation of the mummy.[11] In other cases, such as the natural tanning of soft tissues in acid bog burials, the preservation of human remains has been achieved despite degradation having occurred[12–14] Nevertheless, occasionally, otherwise skeletalised remains that seem to have preserved brain structures in the skull are discovered, which have created some controversial discussion relating to their attribution and occurrence in the depositional environment[15] During excavation of a mediaeval cemetery in Kingston upon Hull, UK, amorphous endocranial masses were noted within the skulls of some 25 of the bodies recovered, representing about 10% of the burials; what was surprising is that these organs had survived when other soft tissues had not.

In 2008, a human skull containing what appeared to be the remains of a brain was excavated[16] from a waterlogged pit at Site A, Heslington East, York, UK, during construction work being undertaken for a new campus at the University of York. The skull, with its articulated mandible and vertebrae C1 and C2, lay face down in a dark brown organics—rich, soft and sandy clay (Fig. 1). After recovery, it was noted that the skull contained a resilient mass that was not consistent with in-washed silt. Inspection of the cranial cavity through the *foramen magnum* showed the presence of an apparently yellow, convoluted material, which upon subsequent examination of a small sample (Fig. 2) proved to be consistent with brain masses seen previously in skeletalised human remains elsewhere.[15] A calibrated radiocarbon date of 673–482 BC (OxA-

* *Correspondence to: Howell G. M. Edwards, Chemical & Forensic Sciences, School of Life Sciences, University of Bradford, Bradford BD7 1DP, UK. E-mail: h.g.m.edwards@bradford.ac.uk*

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a *Chemical and Forensic Sciences, School of Life Sciences, University of Bradford, Bradford BD7 1DP, UK*

b *Archaeological Sciences, School of Life Sciences, University of Bradford, Bradford BD7 1DP, UK*

c *Department of Forensic Science, University of Sohag, Sohag, Egypt*

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Figure 1. The Iron Age skull found lying face down in a waterlogged pit at Heslington East, York, UK. (© York Archaeological Trust).

Figure 2. Portion of suspected brain tissue detached from the Heslington skull. (© Sonia O'Connor).

20677: 2469 ± 34 BP) was determined from collagen extracted from the mandible, placing the skull in the Iron Age. Following the removal of superficial sediments, the skull was interrogated by radiography, computed tomography (CT scanning) and magnetic resonance imaging, whereby the internal structure of the brain mass could be discerned (Figs 3 and 4). After a considerable period of storage in dark, wet and refrigerated conditions, the skull was cut open and the fragmented remains of the brain were removed with the aims of characterising the surviving material, understanding the circumstances of its preservation and its archaeological significance. To preserve as much of the anatomical structure and external morphology as possible, sampling of the brain was restricted to one fragment. The brain and sediments from within and around the skull were subjected to a comprehensive analytical study, including neuroimmunilogical tests, DNA analysis, scanning electron microscopy and polarised light microscopy, which have been summarised in a full report.[16]

Anthropological examination showed the skull to be that of a male aged between 26 and 45 years at death and cranial studies indicated that there was no evidence of disease. However, an examination of the vertebrae showed that the arch of C2 had been

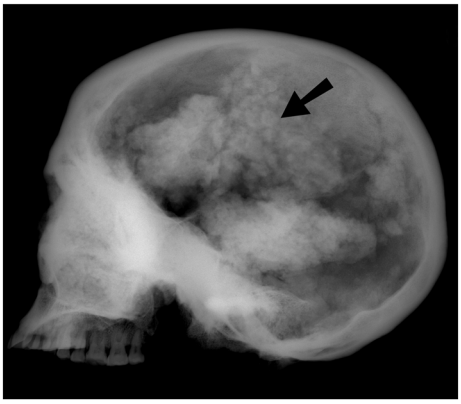

Figure 3. Radiograph of the Heslington cranium showing washed-in sediment and the preserved brain tissue (indicated by the arrow) (© Sonia O'Connor).

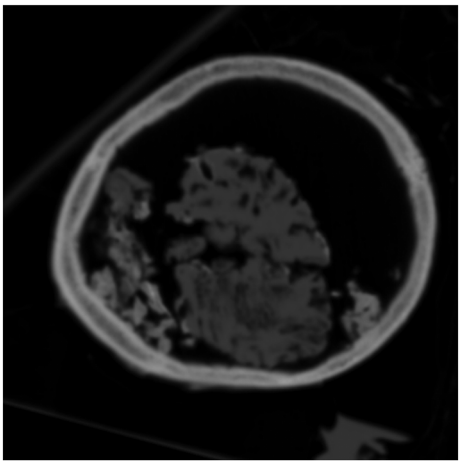

Figure 4. CT image of the Heslington cranium, showing distinct but rather shrunken brain lobes (© David King).

fractured on either side of the centrum and there is a cluster of nine transverse cut marks made with a thin-bladed instrument; both the fractures and the cuts were made *perimortem* indicating an abrupt trauma to the neck, such as is caused by hanging, followed by careful decapitation. The histological study of the skull shows cracking and erosive patterns in the bone indicative of intermittent periods of dry and wet burial conditions, which are conducive to microbial colonisation, although putrefaction does not seem to have occurred. The brain mass itself was odourless, yellow-brown with a resilient texture and of volume 250–300 ml, approximately 20% the volume of a full healthy adult brain. Electron microscopy of the brain histological sections has shown remnants of myelin structures, and although there is no cellular structure remaining, because of autolysis, there is little evidence for putrefaction. Biomolecular analysis of the brain tissue shows little protein has survived, no phospholipids or cholesterol, but microbial alteration products of cholesterol and lipids, with fatty acids, were detected. No biomarkers indicative of artificial preservation procedures or embalming were detected.

The scene is thus set to see what information Raman spectroscopy can provide from analysis of this exceptionally preserved brain material from 2500 years ago; despite the significant analytical successes demonstrated in the field of ancient biomaterials from archaeological and geological environments, this is the first time

that Raman spectroscopy has been applied to such a problem, and the analytical spectroscopic challenge is considerable.

Experimental

Specimens

Two specimens of material from the decapitated skull were presented for analysis, comprising the detached section of brain tissue and a specimen of dark brown/black sludge-like deposit from the surface of the fragment shown in Fig. 4. Specimens were hydrated when removed from the skull, and no further desiccation, chemical or mechanical treatment was undertaken.

Spectroscopy

Raman spectroscopy was effected using a Bruker IFS 66/FRA 106 FT-Raman spectrometer with an Nd^{3+}/YAG laser operating at 1064 nm with a spectral resolution of 4 cm^{-1} and between 500 and 4000 spectral scans accumulated to improve signal-to-noise ratio over a wavenumber range of 200–3500 cm^{-1}, each spectrum requiring between 10 and 80 min to record. A typical spectrum was recorded using minimal laser power of about 50 mW at the sample to avoid degradation. The spectral footprint in macroscopic mode was approximately 100 microns. Spectra were obtained from several regions of the specimens to ensure that reproducibility was achieved from potentially heterogeneous samples.

Raman spectra were also obtained using a DeltaNu 1064 Advantage System (DeltaNu, Laramie, WY, USA), a prototype portable Raman spectrometer, equipped with a 1064-nm diode laser giving a maximum laser power of 1000 mW at source. This is a dispersive reflective grating system giving Raman spectra in the wavenumber range 200–2000 cm^{-1}. The output optics provides a laser spot size of approximately 100 microns. The detector is an Intevac Photonics MOSIR 950 camera based on transfer electron photocathode and electron bombardment gain technology. The camera has a working range from 950 to 1650 nm and is thermoelectrically cooled to −40 C°. A knee-shaped optical head attached to the instrument allows for the flexible positioning of the sample relative to the instrument. The instrument is equipped with NuSpec software, which permits the selection of three steps of operable spectral resolution from 15 to 20 cm^{-1}. The DeltaNu software allows a five-step set-up of adjustable laser power from 800 to 30 mW. The spectral integration time and the number of accumulations are under the full control of the operator. For this application, 5-s integration time was used with the accumulation of five scans, using the lowest power setting and a spectral resolution of 15 cm^{-1}.

Results and discussion

With the use of the Raman spectrometers with long wavelength excitation in the near-infrared region at 1064 nm, spectral signatures of both the brain tissue and the surface sludge were obtained successfully with essentially fluorescence-free spectral emission backgrounds, as has been observed for other types of archaeological and biogeological specimen, and has been effectively applied to forensic analytical spectroscopy of archaeological biomaterials and tissues.

Figure 5 shows the Raman spectrum obtained from the specimen of the putative brain from the Heslington cranium,

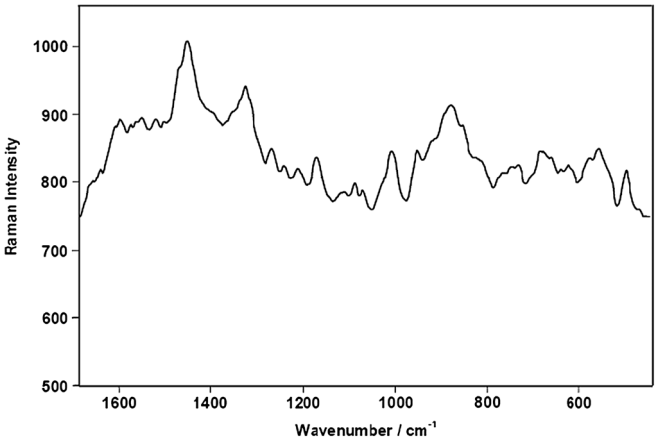

Figure 5. FT-Raman spectrum of Heslington brain specimen, 1064-nm excitation, wavenumber range 450–1700 cm^{-1}, 500 scans, 4 cm^{-1} spectral resolution.

which indicates the presence of degraded protein.[1,17] This can be evidenced by the presence of the amide I mode that can be seen as a weak, broad band at 1657 cm^{-1}. The low wavenumber components at 1640 and 1610 cm^{-1} are assignable to protein molecular degradation; the alpha-helical amide I protein structure characterised by the 1657 cm^{-1} band has significantly disappeared and has been replaced with β-sheet and random coil conformations occurring at lower wavenumbers. CH_2 deformation is also observed at 1425 cm^{-1} as a broad and asymmetric band. NH deformation is present as a broad band at 1295 cm^{-1}. In addition, in this specimen, there is a clear indication[18–20] of the presence of a carotenoid (probably beta-carotene, with bands at 1513, 1157 and 1004 cm^{-1}) and of the cyanobacterial protective biochemical scytonemin (with characteristic bands at 1590, 1554, 1327, and 1165 cm^{-1}). There is no evidence of minerals present in this specimen, as might perhaps have been expected from uptake of salts during its exposure to a waterlogged environment.[21] Isolated features at 1080 and 1067 cm^{-1} are tentatively assignable to CO stretching. A broad feature centred at 883 cm^{-1} with asymmetry on the high wavenumber side is assignable to CH_2 rocking modes coupled with CC stretching modes, and weaker bands at 683 and 550 cm^{-1} could arise from COC and CCC deformations.

Figure 6 shows the FT-Raman spectrum of the same specimen in the wavenumber range 2380–3750 cm^{-1}, which clearly indicates a rather broad CH stretching envelope with maxima at 2926 and

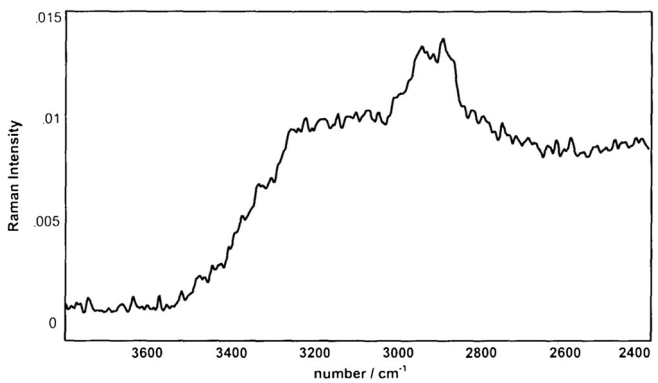

Figure 6. FT-Raman spectrum of Heslington brain specimen, 1064-nm excitation, wavenumber range 2380–3750 cm^{-1}, 500 scans, 4 cm^{-1} spectral resolution.

2877 cm^{-1}, which are assignable to CH_2 and CH_3 modes of aliphatic compounds; the very broad, diffuse band between 3000 and 3300 cm^{-1} is assignable to NH stretching modes, with possibly some OH stretching contribution.[17]

In conclusion, there is evidence in this specimen from the Raman spectra of the presence of degraded protein with cyanobacterial colonisation residues. This supports the visual assignment of the biomass as brain tissue from radiographical and CT images and also confirms the biomolecular analyses that significant protein degradation has occurred to nitrogen-containing aliphatic residues. However, the presence of a cyanobacterial protective chemical shown here for the first time is indicative of biodeterioative processes operational that require conservational attention for the preservation of the integrity of the specimen. This result also supports the conjecture that the skull had been exposed to dry and wet periods during its burial,[16] conditions that are ideal for nurturing cyanobacterial colonisation, as has been demonstrated recently in a similar Raman spectroscopic study of ancient mammoth ivory from a waterlogged gravel pit in which the same cyanobacterial pigment, scytonemin, was detected.[21] This pigment is manufactured exclusively by cyanobacteria under environmentally stressed conditions; the archaeal cyanobacterial colonisation of geological niches in the early Earth history required the production of specific chemical protectant biomolecules such as scytonemin for their survival strategies, and scytonemin with associated carotenoids have been discovered in ancient scenarios such as meteorite impact craters and stromatolites[22,23] from 3.7 Ga. In the case of the Heslington skull, the state of preservation of the brain and the survival of plant remains in the surrounding deposits suggest that the environment had been constantly wet and anoxic over the period of burial. It is most likely that the intermittent dry and wet conditions relate to the period of excavation of the skull. During this time, it was gradually exposed during some weeks of excavation in variable weather conditions. Photographic evidence shows that the back of the skull was uncovered for some time, whilst work continued in adjacent areas of the site, until it was removed to temporary storage before cleaning of the external deposits began and the brain was discovered.

The Raman spectrum (Fig. 7) of the sludge surrounding the brain tissue is very different from that of the brain tissue itself and does not show evidence of biodegraded protein but only exhibits the chemical signatures from cyanobacterial colonies, namely carotenoids and scytonemin. Also, there is no specific evidence of the presence of minerals in the sludge, as was also found for the brain specimen itself. These cyanobacterial signatures located in the cranial cavity but outside the brain tissue could lead to the conclusion that perhaps some microbiological protective role could have been operating here for protection.

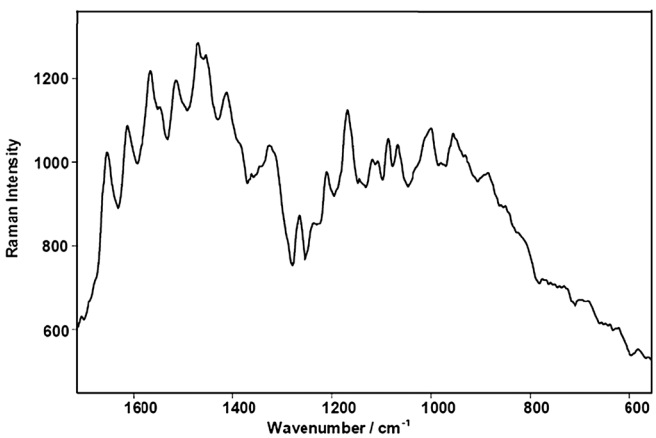

Figure 7. FT-Raman spectrum of the sludge from the surface of the brain, 1064-nm excitation, wavenumber range 450–1700 cm^{-1}, 500 scans, 4 cm^{-1} spectral resolution.

Raman spectra obtained using the portable spectrometer unit operating at the same near-infrared wavelength of excitation were identical with those obtained with the laboratory-based system; however, for the portable system, the CH stretching and OH stretching regions were not accessible because of the upper wavenumber limit of 2000 cm^{-1}, although this may be viewed as detrimental for the investigation of biological samples generally; in fact, most of the structural deductions formulated in this paper derive from the spectral wavenumber region below 2000 cm^{-1}. The poorer spectral resolution of the portable instrument also did not materially affect the results, which were nevertheless achieved in a significantly shorter acquisition time by using the portable spectrometer unit. The availability of such an instrument for on-site and in-field archaeological work, potentially as a screening device for materials of interest that could be further investigated under laboratory conditions is also to be noted,[24] so providing a noteworthy and documentary example for future archaeological applications of Raman spectroscopy.

Conclusions

The survival of brain tissue in archaeological depositional environments is a very unusual occurrence that has generated much discussion historically. The Raman spectroscopic study of biomaterial found in the cranial cavity of a decapitated skull dating from the early Iron Age, some 2500 years ago, has indicated the presence of degraded protein consistent with it being naturally preserved brain, in confirmation of other analytical studies. The novel observation of characteristic Raman spectroscopic signatures of biochemicals produced by cyanobacteria both in the brain tissue and in the surrounding sludge deposits is consistent with the waterlogged depositional environment in which the human skeletal remains were found and also suggests the possibility that there has been some microbiological preservation of the brain tissue, which needs further investigation. The recording of Raman spectral data from biological specimens without the need for desiccation or pretreatment is a critically important attribute that has been essential to this study; in addition, the successful observation of fluorescence-free Raman spectra using near-infrared excitation from ancient biomaterials directly removed from an archaeological excavation posed a challenge that has been met, and novel information thereby provided to inform the behaviour of such material in the burial environment for future forensic adaptation.

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