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Characterization of an unusual coating on funerary portraits from Roman Egypt circa 100-300AD

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Abstract

This paper details the investigation of a discrete coating observed on a group of Egyptian panel paintings, six mummy portraits and one funerary panel, dating from first-third century CE. Six mummy portraits in this group are encaustic, and the funerary panel is tempera using an animal glue binder. An accretion or coating has been observed on the surface and recesses of the paint layers on these panels. Examination of the portraits using ultraviolet radiation revealed an irregular visible fluorescence on the surface. On the mummy portraits, the fluorescence often extends only as far as where the linen wrappings would have secured the portrait to its mummy. Under magnification, the coating appears as a crizzled encrustation. Material exhibiting these characteristics was sampled from the surface of all seven panels. Initial analysis of samples from four panels by gas chromatography mass spectrometry (GC/MS) and enzyme-linked immunoassay (ELISA) revealed the presence of egg. Subsequent analysis of the coating from all seven portraits by peptide mass fingerprinting (PMF) and liquid chromatography with tandem mass spectrometry (LCMSMS) confirmed egg and further characterized the coating as highly deamidated, whole hen egg, or hen egg white in one instance. Importantly, the ^{14}C date of the coating from two portraits indicates the time of application as approximately 2000 years ago, implying that the coating, at least in those cases, is not a modern addition. This report summarizes the examination and analytical characterization of this unusual coating. Possibly applied as an aesthetic or protective layer, or a symbolic and ritual unguent, the principal function of this coating remains unknown.

Keywords Funerary portrait, Egg coating, Peptide mass fingerprinting, LCMSMS, Radiocarbon dating

Introduction

Ancient funerary portraits are individualized images of the deceased that are painted on wooden panels. They may have been placed in tombs in memory of family members, or secured over the face of a mummified body (mummy portrait). In this paper we use "portrait" or "funerary portrait" to include both funerary and mummy paintings, and "mummy portrait" when it is necessary to make a distinction. These personalized funerary images were created mainly during the Roman occupation of Egypt, dating from approximately first–third century CE, and were reserved only for those who could afford costly funerary expenses. The painting style and technique evolved from the Greco-Roman tradition of wall painting, and the quality of the portrait likely reflects the

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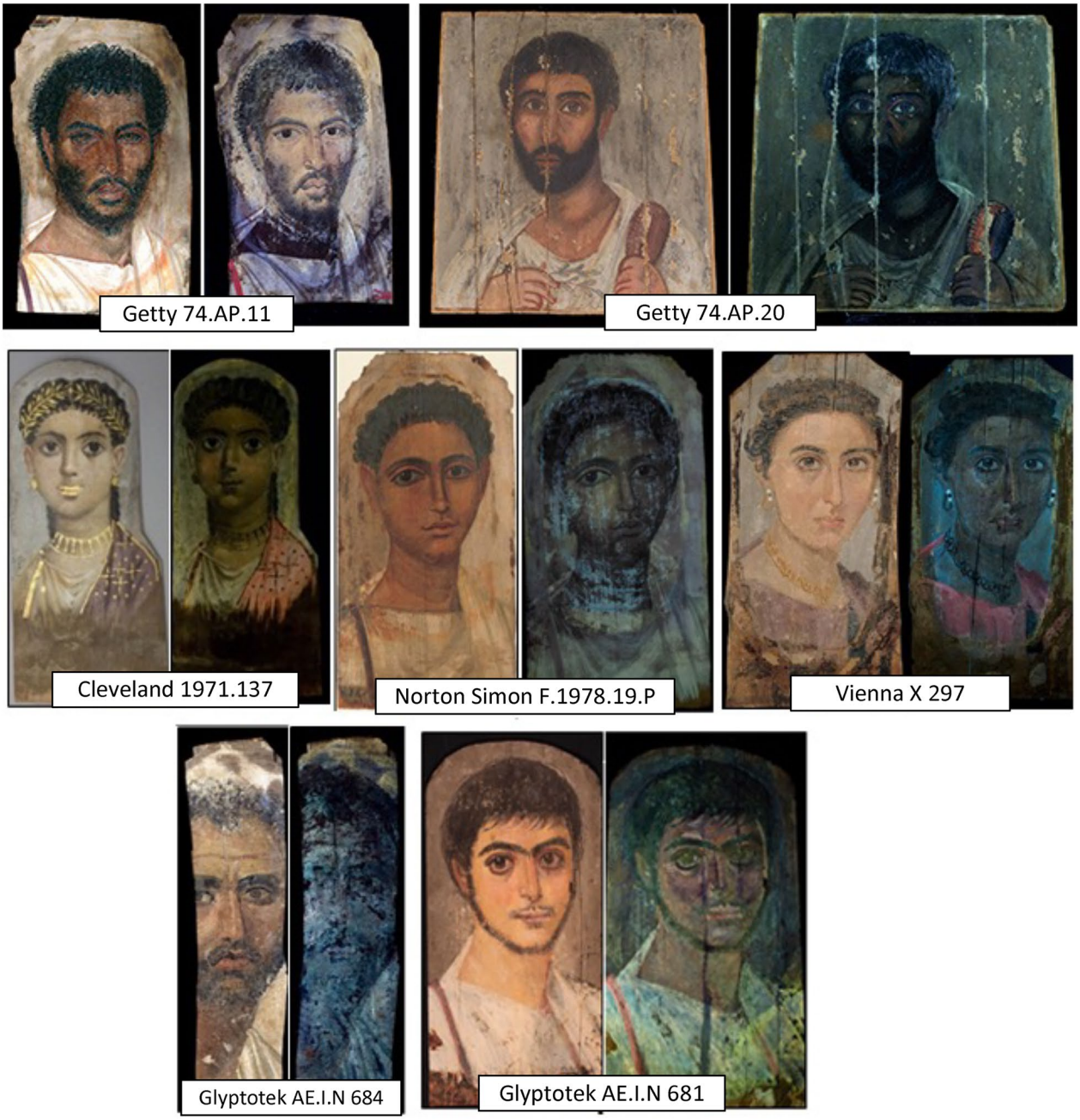

Fig. 1 The seven portraits analyzed in this study. Each portrait is shown with visible illumination (left) and ultraviolet-induced visible fluorescence (UVF) (right)

economic status of individuals represented [1]. The portraits were typically painted with a conventional pallet of pigments bound in encaustic (wax-based) or tempera (animal glue or plant gum) media, or a combination of both [2]. Mummy portraits were secured in place by linen or stucco wrappings covering their edges, framing the portrait. Portrait imagery reflects the diverse population that lived in Roman Egypt during this period–male and female, young and old–and most portraits were painted following a standardized and conventional layout, Fig. 1.

Approximately 1000 mummy portraits[1] are housed in museum collections worldwide, and there are about 100 intact portrait mummies that provide valuable information on their context and construction [3].

[1] This statistic includes both mummy portraits and non-mummy funerary portraits.

Ongoing research on funerary portrait production has focused on the pigments, binders and woods that were used [4]. Recently, however, an unusual coating has been observed on the surfaces of six encaustic portraits and one tempera (animal glue) portrait from collections in five museums. Whereas coatings (varnishes) and libations are known to have been applied on certain painted surfaces in ancient Egypt, coatings on funerary portraits have not previously been described in publications.

Significance

Numerous materials employed as paint binders, adhesives, and used in mummification procedures in ancient Egypt have been described [5–7]. Although egg has been noted as a binding medium in certain paintings around the ancient Mediterranean [8, 9], there have been no definitive identifications of egg as a binder or adhesive in ancient Egypt to date, nor have any uses of eggs other than dietary ones been suggested. The use an egg-based surface coating on several Romano-Egyptian funerary portraits housed in various museum collections represents a previously undescribed possible use of egg in the ancient world. Through the combination of highly sensitive and specific analytical techniques, the coating has been extensively characterized, and its application date determined. The data obtained provide evidence for the use of a whole hen egg or egg glair (white) coating discretely applied to funerary Romano-Egyptian portraits likely at the time of their manufacture, thus providing new insight into the production and ritual use of funerary portraits.

Study background

In 2013, a collaborative study and comparison of ancient funerary portraits from museum collections worldwide was initiated with the goal of identifying their material composition and manufacturing methods (APPEAR) [10]. The project has since expanded our understanding of the painted portraits thus enabling unique features and trends to be recognized [11].

At the first APPEAR meeting in 2013, observation of an unusual surface accretion on several. J. Paul Getty Museum (Getty) portraits was presented as a possible direction of study. Prior to this meeting, binding media analyses carried out on three wooden panel paintings (74.AP.20–22) in the Getty collection indicated the presence of egg. Initially presumed to be from a restoration treatment, cross sections later showed that it was a discrete surface layer [12]. Subsequent analysis of a similar accretion or coating observed on two mummy portraits in the Ny Carlsberg Glyptotek collection (Glyptotek) also revealed the presence of egg. During the examination of Glyptotek portrait AE.I.N 684 under UV radiation, conservators had noted a *"distinct fluorescent border at the bottom of the portrait and a faint one at its top. The coating is not present where the mummy wrappings would have covered the panel."* [13]

At this point, existing and new samples from the four Getty and Glyptotek portraits were analyzed by PMF and LCMSMS. Three additional portraits from other collections likely containing similar coatings were subsequently identified through the APPEAR database based on their visible appearance and fluorescence response, and samples from these were also analyzed by the same techniques.

Experimental background

GC/MS, ELISA

In the initial phase of the current study, coatings from the Getty and Glyptotek paintings were analyzed by GC/MS and/or ELISA. Quantitative amino acid analysis by GC/MS is a well-established method for identification of proteins such as animal or fish glue, egg and casein in artworks [14, 15]. The relative amounts of the standard twenty amino acids (or a subset of them) in a sample provide a fingerprint characteristic of a specific protein source. Mixtures of proteins and the effects of aging and pigment interaction may complicate confident identifications. GC/MS can distinguish between egg yolk and egg glair based on the presence or absence of yolk lipids (palmitic and stearic acids), but it is not clear how lipid material would behave as a coating over thousands of years, as fatty acids are volatile and evaporate with time. GC/MS cannot be used to establish the species from which the glue or egg proteins originated.

ELISA is an antibody-based technique commonly used in biological research to identify proteins or other biological macromolecules by means of a colorimetric assay. Since 2005, numerous ELISA techniques have been developed and applied specifically to works of art because they require small samples, are extremely sensitive, and can identify complex mixtures of proteins [16–21]. The methodology used in this study has been described previously [16]. In the case of the funerary portraits, ELISA was utilized primarily to identify complex mixtures of proteins such as egg and animal glue that could not be identified solely by GC/MS [8].

GC/MS analysis of the coating samples from the Getty and Glyptotek portraits showed that they best matched egg glair without added oil, wax, or tree resins (Table 1). The egg coating samples did not contain lipid components, such as glycerol, palmitic and stearic acids. ELISA also confirmed the identification of ovalbumin, the main protein found in egg glair. This was a significant finding since previous analyses had shown that the paint medium

Table 1 Summary of analyses of seven funerary portraits

Institution	Accession No	Medium	ELISA	GCMS	Sample Method	PMF Result*	LCMSMS Result*	Hen egg proteins found by Mascot	^{14}C Date
Getty	74.AP.11	Encaustic	Egg	Egg	Sample Stick	Whole hen egg	Whole hen egg, mammalian collagen	Oval, Vit2, Lysc, Apov1, Trfe, Vit1, Iovo, Apob, Ovaly	25–128 CE
Getty	74.AP.20	Tempera	Egg, glue	Glue	Sample Stick	Whole hen egg, trace collagen	Whole hen egg, mammalian collagen	Oval, Vit2, Lysc, Apov1, Trfe, Vit1	
Glyptotek	AE.I.N 681	Encaustic	N/A	Egg	Remnant from GCMS analysis	Whole hen egg	Whole hen egg, mammalian collagen	Oval, Vit2, Lysc, Apov1, Vit1, Trfe, Ovaly, Apob	
Glyptotek	AE.I.N 684	Encaustic	N/A	Egg	Remnant from GCMS analysis	Whole hen egg	Whole hen egg, mammalian collagen	Oval, Lysc, Trfe, Ovaly, Apov1, Iovo	
Norton Simon	F.1978.19.P	Encaustic	N/A	N/A	Sample Stick	Whole hen egg	Whole hen egg, mammalian collagen	Oval, Vit2, Vit1, Trfe, Lysc, Apov1, Ovaly, Iovo, Ovalx, Apob, Apoa1	
Vienna	X 297	Encaustic	N/A	N/A	Sample Stick	Whole hen egg	Whole hen egg, mammalian collagen	Oval, Lysc, Trfe, Apov1, Vit2, Ovaly	42 BCE-108 CE
Cleveland	1971.137	Encaustic	N/A	N/A	Sample Stick	Hen egg glair	Hen egg glair, mammalian collagen	Oval, Lysc, Trfe, Iovo, Ovaly	

Oval ovalbumin; *Vit2* vitellogenin-2; *Lysc* lysozyme; *Apov1* apovitellenin-1; *Trfe* ovotransferrin; *Vit1* vitellogenin-1; *Ovaly* ovalbumin-related protein Y; *ApoB* apolipoprotein B; *Iovo* ovomucoid; *Ovalx* ovalbumin-related protein X; *Apob* apolipoprotein B; *Apoai* apolipoprotein A-I

* All show keratin contamination

of three of these four portraits was encaustic, and the medium of the other was animal glue, leaving the importance of the finding of egg protein uncertain. These analyses provided a starting point for in-depth analysis of the egg coatings by PMF and LCMSMS, which is the focus of this paper.

PMF, LCMSMS

Heritage science has benefitted immensely from adoption and adaptation of analytical methods from biotechnology, particularly in regard to our understanding of the use of proteinaceous materials [22]. Two of these methods, PMF [23–25] and LCMSMS with protein database searching [26–28], have been applied to a variety of artworks and objects from cultural heritage and were used in this study to investigate the coating observed on the portraits.

PMF analysis involves the enzymatic digestion of proteins followed by Matrix Assisted Laser Desorption-Ionization Time of Flight Mass Spectrometric analysis (MALDI) of the resultant peptide mixture [29–32]. For each protein, the amino acid sequence is unique, thus, the mixture of peptides is unique—a "peptide mass fingerprint." Marker ions [33] in the MALDI spectra from known reference materials are compared with those from unknown samples for identification.

The use of PMF, also termed Zooarchaeology by Mass Spectrometry (ZooMS) [34] for identification of the sources of collagen-based materials has been well developed and applied successfully to a wide range of objects and artefacts from both cultural heritage and archaeology [35–37]. With PMF, some mammalian groups can be identified to the family level (*cervidae* (deer) and *canidae* (dogs), for example), whereas others may be further identified to species level (many *cetaceans*, for example) [38]. Minimal sample requirements plus high sensitivity and specificity have made this method a powerful tool for the conservator as well as the archaeologist, and there is ongoing activity to extend the methodology to keratin-based materials [39], as well as fish [40] and shells [41].

Proteinaceous materials commonly found in artworks, such as egg, animal glues and casein, are also amenable to analysis by PMF [42, 43], as is the case with the present work. Markers for common proteinaceous materials found in artworks, as well as keratin, which is often encountered as a surface contaminant, have been published [29, 44], and a list of those relevant to this study is shown in Table 2.

Table 2 Markers used to identify materials found in artworks and objects of cultural heritage

Egg Yolk	Egg White	Animal Glue	Keratin
1048.6*	1345.7*	1095.6*	1179.7*
1077.6*	1428.6	1105.6*	1235.6
1085.7*	1555.7	1241.8	1300.6
1164.5*	1687.8*	1267.8*	1475.8*
1324.7	1773.9	1427.7*	1493.8
1401.7*	1859.9*	1435.8*	1707.8
1406.6*	1913.0	1459.7*	1765.8
1445.7	2009.0*	1473.7	1791.8
1560.7		1586.9	2384.0*
1561.7*		1648.8*	2705.2
1591.7		1655.9	
1891.0		1923.0	
1892.0		1963.0	
1893.0*		1976.0	
2236.1		2705.3*	

Markers are collected and validated from several sources including published research data and protein sequences, LCMSMS data from known samples, and analysis of known reference samples. If a majority of the expected markers is observed in the MALDI spectrum, the indicated protein or material is considered to be positively identified. Egg yolk and white markers were derived from database searching of PMF spectra of authentic samples with Mascot, (www.Matrixscience.com) as well as LCMSMS data from known references. Keratin markers were also derived from database searching of PMF spectra with Mascot. The keratin markers noted here are a truncated list of the most intense and persistent ions (attributed to the Uniprot IDs: P35527, P13645 and P04264) (* usually more intense)

LCMSMS with database searching is a mainstay of biotechnology. Its sensitivity is ideally matched to the minimal samples generally available with cultural objects, and its specificity allows more precise identification of proteinaceous materials than may be possible with other techniques [45]. In the present work it is used either to identify proteinaceous materials and/or to confirm those found with PMF.

Methods and materials

Samples

Samples of the portrait coatings were taken by multiple conservators, and not all sample sites were documented with micrographs. All portraits had a clearly visible surface layer and characteristic visual appearance, and the UVF response guided the conservators to specific sample sites. Although the coating was sampled carefully under magnification, the possibility of unintended inclusion of underlying paint and/or ground layers cannot be excluded, and those layers could be potential sources of egg protein. However, a recent study of binding media in 61 Romano-Egyptian paintings, of which 51 were funerary mummy portraits, identified egg in only two paint layers by ELISA [8]. Thus, the paint binding medium seems unlikely to be the source of egg discussed in this paper.

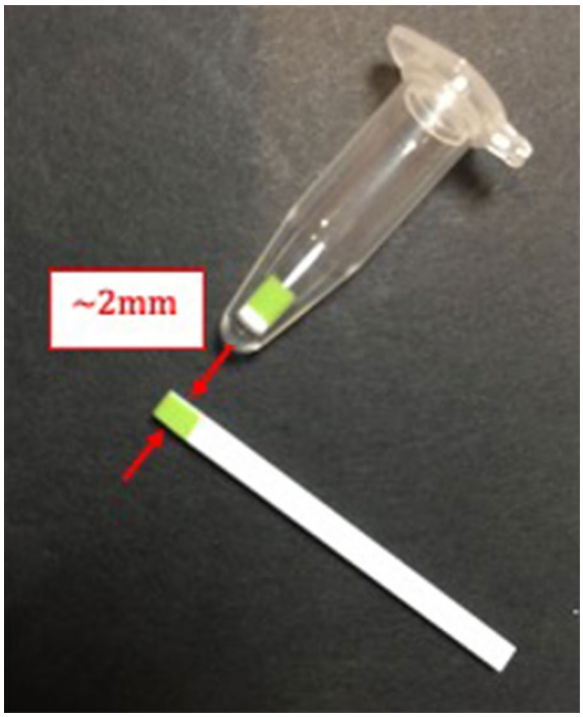

Fig. 2 Sample stick comprised of a polystyrene support with attached micro grit polishing film

Several recently introduced sampling methods using, for example, eraser crumbs, [46, 47] and fine abrasive films [42, 47, 48], can abrade and entrap small amounts of material from an object/surface for subsequent protein analysis. Eraser crumbs are more appropriate for friable surfaces or loosely adhered layers whereas abrasive films are more appropriate for solid objects or hard surface coatings. Even when surface alteration is not of critical importance, sampling with an abrasive film may be easier and more controllable than, for instance, excision with a scalpel. For the present work, samples from the Glyptotek portraits were remnants from previous analyses [13] received in Eppendorf tubes as a fine powder. All other samples were obtained by museum conservators using "sample sticks," which are small pieces of 30 µm alumina grit polishing film attached to a polystyrene support (Fig. 2). After sampling, the tip of the sample stick containing the abrasive film and entrapped sample is cut off, placed in an Eppendorf tube, and forwarded for analysis. Only a single sample was taken from each portrait. Portions of the sample prepared for PMF were also utilized for LCMSMS.[2]

[2] We estimate that this method removes a few 10's of micrograms of material from a hard surface. A quantitative study is forthcoming.

PMF

Digestion

60µL 50 mM ammonium bicarbonate (AMBI) was added to each sample and heated to 75 °C for 60 min. After cooling, 8 µL Promega Sequence Grade trypsin (0.02 µg/µL in 50 mM AMBI) was added and digestion proceeded overnight at 37 °C.

MALDI analysis

2µL of the digest were added to 20µL 40% acetonitrile (ACN), 0.1% trifluoroacetic acid (TFA) with saturated α-Cyano-4-hydroxycinnamic acid (CHCA) matrix. 0.65 µL of the mixture was spotted onto the MALDI plate. Spectra were obtained with an ABI/Sciex 5800 MALDI-TOF-TOF instrument operated in reflector mode. Calibration was done with a standard mixture of peptides: 757.3992 Da, 1046.5418 Da, 1296.6848 Da, 1347.7354 Da, 1619.8223 Da, 2093.0867 Da, 2465.1983 Da, and 3147.4710 Da. Spectra were coadditions of 1200–2000 laser shots. Acquired spectra were exported from ABI (Applied Biosystems) Data Explorer software as.txt files and imported into mMass [49] for analysis. Markers used to identify egg glair and yolk and animal glue as well as contaminant keratin are listed in Table 2.

LCMSMS

LCMSMS analyses were done on a Thermo Q Exactive-Orbitrap mass spectrometer interfaced with an UltiMate™3000 chromatographic system. The mass spectrometer was operated in data dependent mode (full scan 350-1800 Da at a resolution of 70,000, top 10 ions were selected for fragmentation at a resolution of 17, 500, detected peptides were placed on an exclusion list for 10 s.). The LC buffers were A: 0.1% formic acid and B: 0.1% formic acid in ACN, and a gradient from 2–50% B over 60 min. was used. One micro-liter of each of the digests that was analyzed by PMF was injected onto a nanocolumn that was 75 µm ID × 15 cm long, packed with Reprosil AQ C18 with 1.9 um beads (Dr. Maisch, Germany). The column had an integrated tip (pulled in-house at Northeastern University by laser) with a tapered internal ID of ~ 5–10 µm. The column was connected to the HPLC autosampler using a Pico View nESI source (New Objective, Woburn, MA) and high voltage was supplied to the back end of the column from the Pico View source using a conductive tee.

LCMSMS data files were converted to mgf format (mascot generic format, Matrix Science [50]) with msConvert software from ProteoWizard Tools [51] and searched on-line through Mascot. Mascot searches were against the SwisProt and contaminants databases, taxonomy: Chordata, enzyme: trypsin, one missed cleavage, and variable modifications: N, Q deamidation and M, K, P oxidation. Peptide tolerance was 0.3 Da and MSMS tolerance was 0.5 Da.

Radiocarbon dating

None of the analytical techniques that detected the presence of egg coating could indicate whether the coating was ancient or contemporary. As a result, samples from Getty 74.AP.11 and Norton Simon F.1978.19.P were submitted for ^{14}C dating [52]. The two samples submitted were small scrapings of the egg coating (~ 1 mg total each) obtained using a scalpel under a binocular microscope. The samples were combusted under vacuum with CuO at 900 °C in sealed quartz tubes, graphitized by Fe-catalyzed hydrogen reduction [53], and measured by Accelerator Mass Spectrometry (AMS) at the Keck AMS facility at University of California, Irvine [54]. Given the small sample sizes and the difficulty in separating egg protein from possible proteinaceous contamination by human keratin due to recent handling, no attempt was made to chemically clean the samples or to isolate protein. Instead, the samples were simply combusted as received and compared to results on ^{14}C-free wood to evaluate process backgrounds from the combustion and graphitization. The rationale for this decision was that since the mummies were not disturbed prior to excavation in the 19th and early twentieth centuries [55], any contamination would bias the samples to younger ages; hence an old date would be convincing evidence that the coating was applied prior to the mummy burial, i.e., during or shortly after the portrait painting, rather than post-excavation.

UVF

Getty and Norton Simon

Images were captured using a Canon 80D 24.2 megapixel modified camera with the UV-IR blocking filter removed, hence providing UV–VIS-IR functionality. The camera was outfitted with a Zeiss Milvus 50 mm macro lens. As control targets, a Labsphere Spectralon 99% reflectance target and Passport Color Checker were used.

Two Wildfire Long throw series (365 nm peak) UV radiation sources. UVF filters used were X-nite CC1, Peca 916 or 918 and Kodak Wratten 2e filters.

Glyptotek

Images were captured using a Canon EOS 5D Mark II, digital camera with a Tiffen 2A equivalent to Kodak Wratten 2A-filter, 400 nm UV-filter. Phillips UV bulbs. A Gretag Macbeth Munsell Color was used as control target.

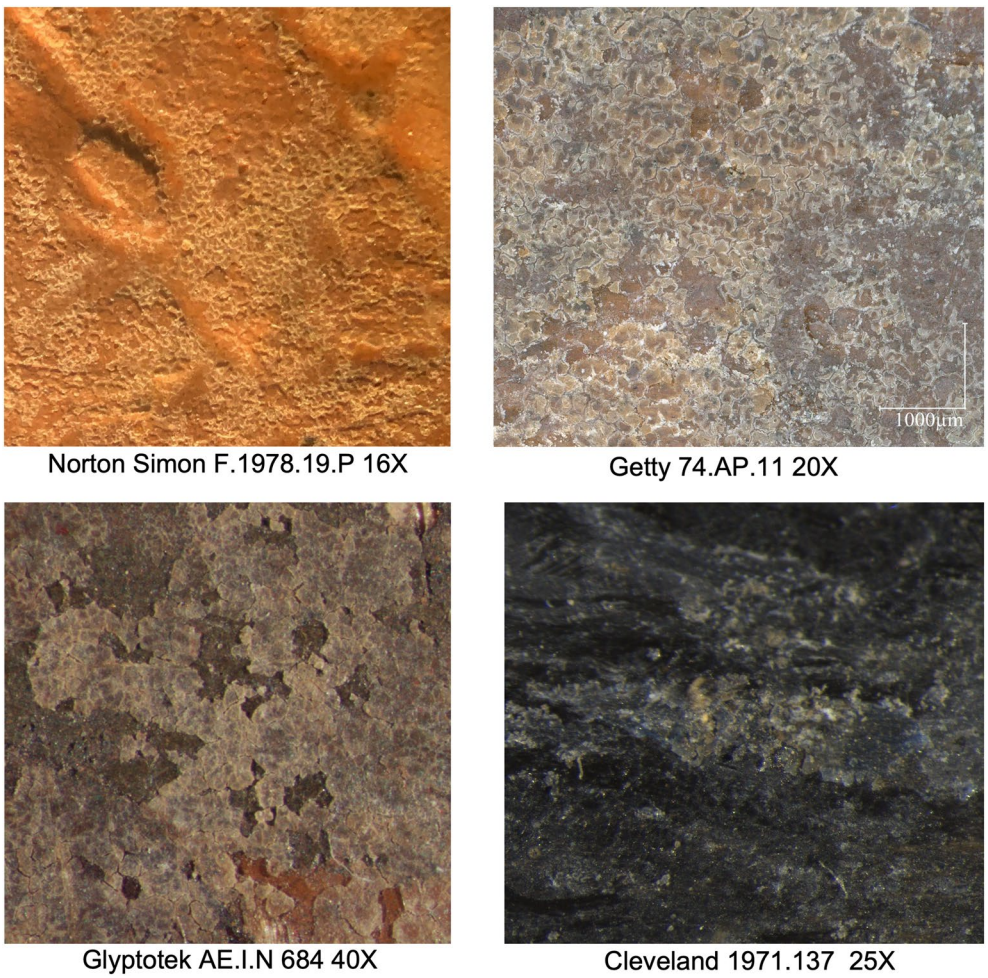

Fig. 3 Magnified surfaces from four of the portraits illustrating the characteristic surface appearance of the coating

Vienna

Nikon D80 (10 Mpixel, APS-C sensor); Nikon lens AF D Nikkor 28 – 105 mm f/3,5–4,5

UV lamps: UVLS- 28 EL 365 nm and Original Hanan, Fluorotest 366 nm.

Cleveland

1971.137 UVA visible fluorescence Camera: Canon 5DSR, 50 mm lens with X-Nite CC1, PECA 918 and 2E filters. Lighting: Two Wildfire long wave radiation lamps.

Materials

Ammonium bicarbonate (AMBI), trifluoro acetic acid (TFA) and formic acid (FA) were reagent grade from various suppliers and used without further purification; HPLC-grade acetonitrile and water were from ThremoFisher Scientific; Sequencing Grade Modified Trypsin was from Promega; α-Cyano-4-hydroxycinnamic acid (CHCA): Sigma-Aldrich #476870-10 g; Polystyrene strips were from Walthers, www.walthers.com; and aluminum oxide polishing film with pressure sensitive adhesive was from Precision Fiber Products, Inc., www.precisionfiberproducts.com.

Experimental results

UVF and visual examination

The paintings from which samples were analyzed—a single sample from each—are shown in Fig. 1, along with UVF images of each overall painting. All of the painted panels are mummy portraits except Getty 74.AP.20, which is a funerary portrait. Localized areas on all showed generally the same surface appearances: the coating layer looked visually like an irregular, distinct surface accretion that is often absent where the wrappings would have secured the panel to its mummy. Under magnification, the material can be described as "dried islands of a yellowed, glassy accretion," and it is usually pooled in the recesses of both encaustic and

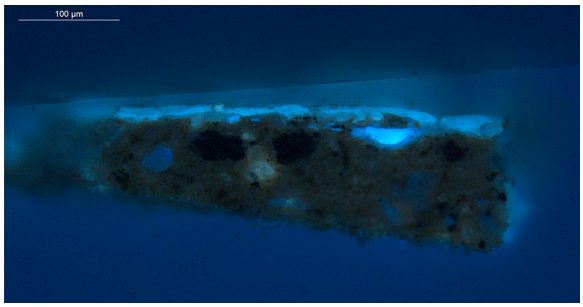

Fig. 4 UVF photomicrograph of a cross section from Glyptotek AE.I.N 684 showing characteristic fluorescence of egg on the top layer

tempera paint, Fig. 3. A blue or bluish/yellow visible fluorescence of the coating observed with ultraviolet radiation further connected these portraits and assisted with the identification of other potentially coated portraits. Often the coating had incrustations and surface deposits (sand/dirt) suggesting that it was not restoration material.

Cross sections from two of the paintings (Getty 74.AP.20 [12] and Glyptotek AE.I.N 684, Fig. 4) confirmed that the fluorescent material was definitely concentrated in a discrete surface layer.

Following the initial examination and analysis of the four painted panels from Getty and Glyptotek, three additional portraits displaying similar visual surface characteristics were identified in the APPEAR database. Based on the criteria described above, portraits from the Cleveland Museum of Art (1971.137), Norton Simon Museum (F.1978.19.P) and Kunsthistorisches Museum, Vienna (X 297) were sampled and analyzed, Fig. 1.

Initial analysis of four portraits from Getty and Glyptotek by GC/MS and/or ELISA [12, 13] indicated the presence of egg, even though the media had been determined to be encaustic and should therefore exclude egg, Table 1. A cross section from paint on Getty 74.AP.20 indicated that egg was present as a coating and not part of the paint [12], as did a cross section from Glyptotek AE.I.N 684, Fig. 4. Thus, it was assumed that the egg originated from a coating separate from the paint binding medium, and analyses by PMF and LCMSMS were undertaken to further characterize the coating on the Getty and Glyptotek portraits, as well as coatings on Norton Simon, Vienna and Cleveland portraits that were identified in the APPEAR database.

PMF analysis

Figure 5 (blue trace) is the PMF spectrum from coating sampled from Getty portrait 74.AP.11 compared with unaged, whole egg reference (red trace). Many of the ions in the Getty sample can be attributed to surface contamination (unlabeled ions are mainly contaminating human epithelial keratin), but a number of ions are similar to but not identical with those in egg glair and yolk, which are indicated in the red trace by green circles. Closer examination of the "near" matching ions led to the realization that the Getty sample was whole egg but likely highly deamidated. Several of the more intense egg ions observed in both the coating and reference spectra,

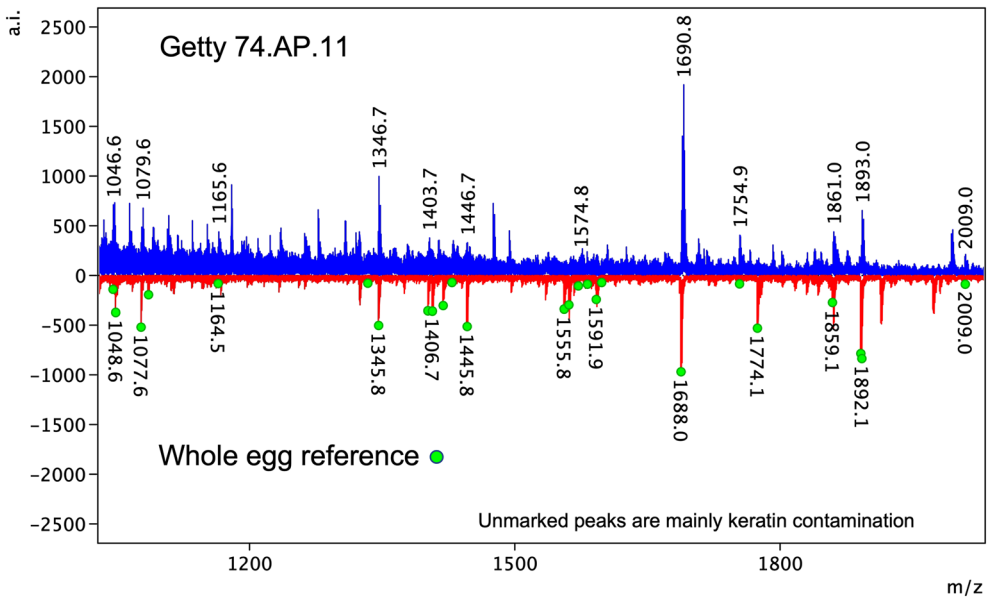

Fig. 5 PMF spectra from the coating on Getty 74.AP.11 (top) and whole egg reference (bottom). Egg yolk and glair markers (Table 2) are highlighted with green circles in the lower spectrum

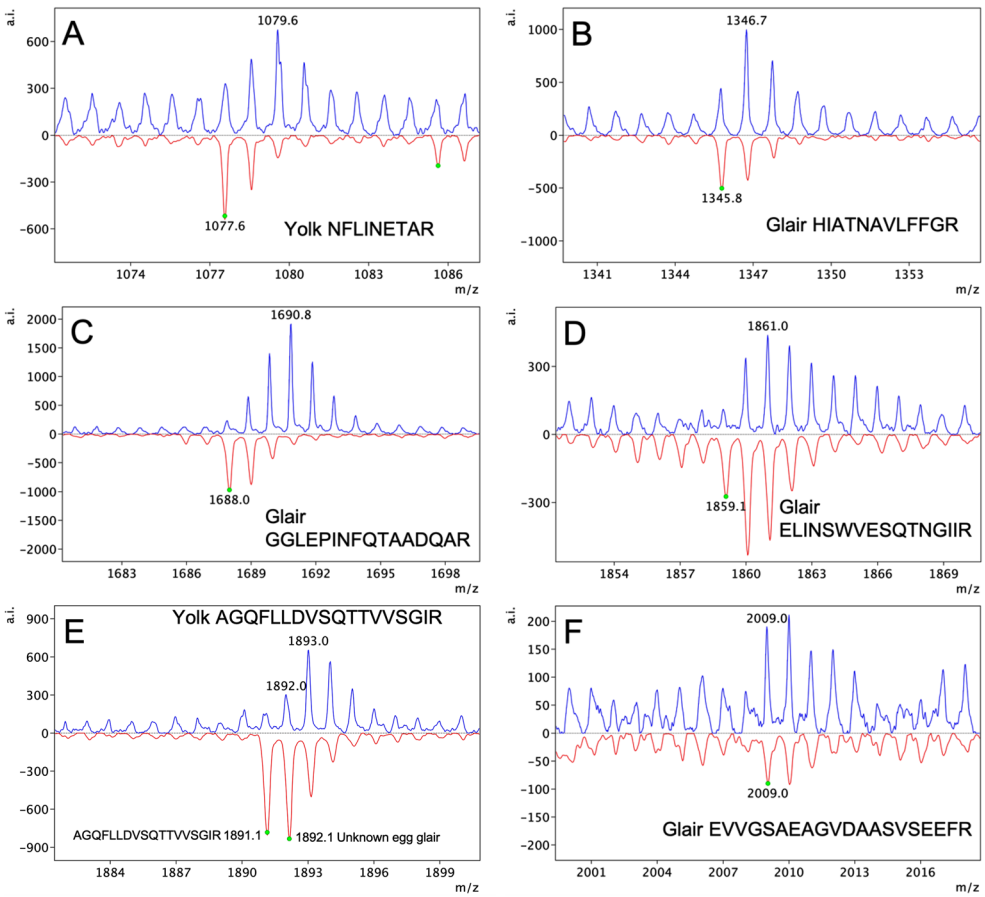

Fig. 6 Expanded spectra from selected ions in Fig. 5 (blue traces) and their sequences as determined by LCMSMS. Masses of the non-deamidated peptides from whole egg reference are shown in the red traces. Isotopic envelopes from the sample for sequences with N or Q residues are shifted to higher mass (**A**–**E**) whereas the sequence without N or Q residues is not (**F**). The sequence of the 1892.1 Da ion (**E**) observed in egg glair reference (not shown) is unknown

along with their sequences as determined by LCMSMS, are shown in Fig. 6A–F. Sequences 6A–E, all of which contain Q and/or N residues, are shifted to higher mass, likely due to deamidation. Sequence 6F, which contains neither Q nor N residues, is not shifted, supporting deamidation as the source of the mass shifts in the other spectra.

Deamidation is a chemical reaction of the amino acids with an amide side group (asparagine, N, and glutamine, Q) resulting in a 1 Da increase in mass from the loss of the amine side group and replacement with a carboxyl side group [34]. Deamidation can originate from several sources including age, chemical exposure, heat, and humidity and does not necessarily proceed to 100% completion, so there may be a mixture of amidated and deamidated species present resulting in a complex mixture and isotopic pattern. An example is shown in Fig. 7 for GGLEPINFQTAADQAR (Fig. 6C) to illustrate the interpretation of the isotopic pattern resulting from the overlap of amidated and deamidated forms of the same peptide.

Figure 8 compares spectra from all seven portraits that were analyzed by PMF. All exhibit both egg glair and yolk markers with varying degrees of intensity, with the exception of the Cleveland portrait, which has only egg glair markers. All spectra show highly deamidated peptides, as indicated by their isotopic patterns. Four ions common to all seven samples, and which were usually the most intense, are labeled.[3] Because of their relatively low intensity and the high keratin background, only the more intense egg-related ions were usually observed, and glair ions were the most abundant. PMF spectra are included in Additional file 3 as mMass (.msd) files. It should also be noted that the egg here is specifically from chicken,

[3] The 1892 Da ion cluster in Cleveland is from glair and is usually obscured by the 1893 Da cluster from yolk. See Fig. 6E.

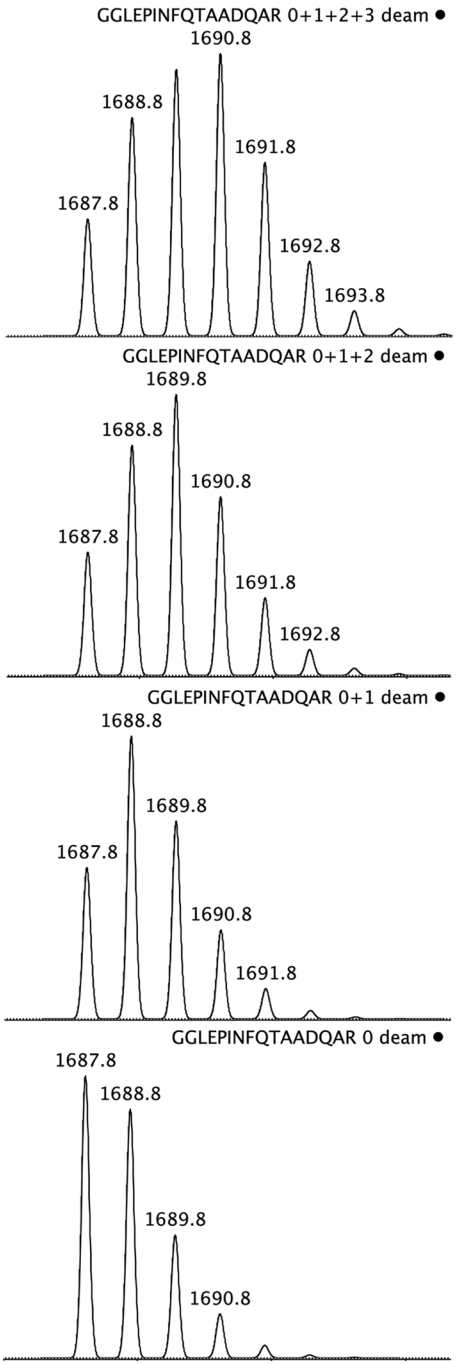

Fig. 7 Isotopic pattern formed by the overlap of amidated and deamidated species of the same peptide. From bottom to top, the peptide GGLEPINFQTAADQAR with 0, 1, 2 and 3 deamidations and the resulting overlapped spectra. Compare the top spectrum here with Fig. 6C

Gallus gallus domesticus, a domesticated fowl used mostly for egg and meat production and which is widely distributed over the globe.

LCMSMS analysis

Digest aliquots from all seven portraits analyzed by MALDI were subsequently analyzed by LCMSMS, and the MSMS spectra were searched through Mascot to corroborate the PMF identifications and their degree of deamidation. Data from all portraits provided similar, consistent results confirming the interpretation of the PMF spectra as being indicative of highly deamidated, whole chicken egg.

The presence of egg glair was indicated primarily by identification of ovalbumin, lysozyme, and ovotransferrin; egg yolk was indicated primarily by identification of vitellogenin-1, vitellogenin-2, and apovitellenin-1. These were usually the main egg proteins found in the samples, and, as noted with the PMF spectra, abundances varied from sample to sample. Consistent with the PMF results, both amidated and deamidated peptides were identified, and high levels of contaminating human epithelial keratin were also present.

The PMF spectrum from Getty 74.AP.20 was the only result that indicated the presence of a trace of mammalian collagen, consistent with ELISA and GC/MS results, Table 1. The LCMSMS results from all seven portraits, however, indicated the presence of low levels of mammalian collagen, although the data were insufficient to conclusively determine the species. The presence of collagen is surprising in that, except for Getty 74.AP.20, the portraits are encaustic. Collagen was observed in only one PMF spectrum likely because of its low level and the high levels of keratin contamination in all samples. Many of the proteins identified by Mascot were contaminating human epithelial keratin types, also consistent with the high background observed in the PMF spectra.

LCMSMS data (.mgf format) and Mascot Summary Reports for all samples are included in the Additional file 1, 2.

Mascot search results, examples

Table 3 is the Mascot summary of the ions used to identify ovalbumin (egg glair, *Gallus gallus*) in Getty 74.AP.20, and it includes several of the ions shown in Fig. 6. As expected, based on the PMF data, the 1345 Da peptide (HIATNAVLFFGR), the 1688 Da peptide (GGLEPINFQTAADQAR) and the 1859 Da peptide (ELINSWVESQTNGIIR) show deamidation, whereas the 2009 Da peptide (EVVGSAEAGVDAASVSEEFR) ion does not. Peptides are represented multiple times in the table because of the short exclude time between MSMS spectral acquisitions.

Figures 9 and 10 are examples of MSMS spectra that were used to identify ovalbumin in the Mascot analysis, Table 3. Figure 9 is the ovalbumin 1687 Da ion identified

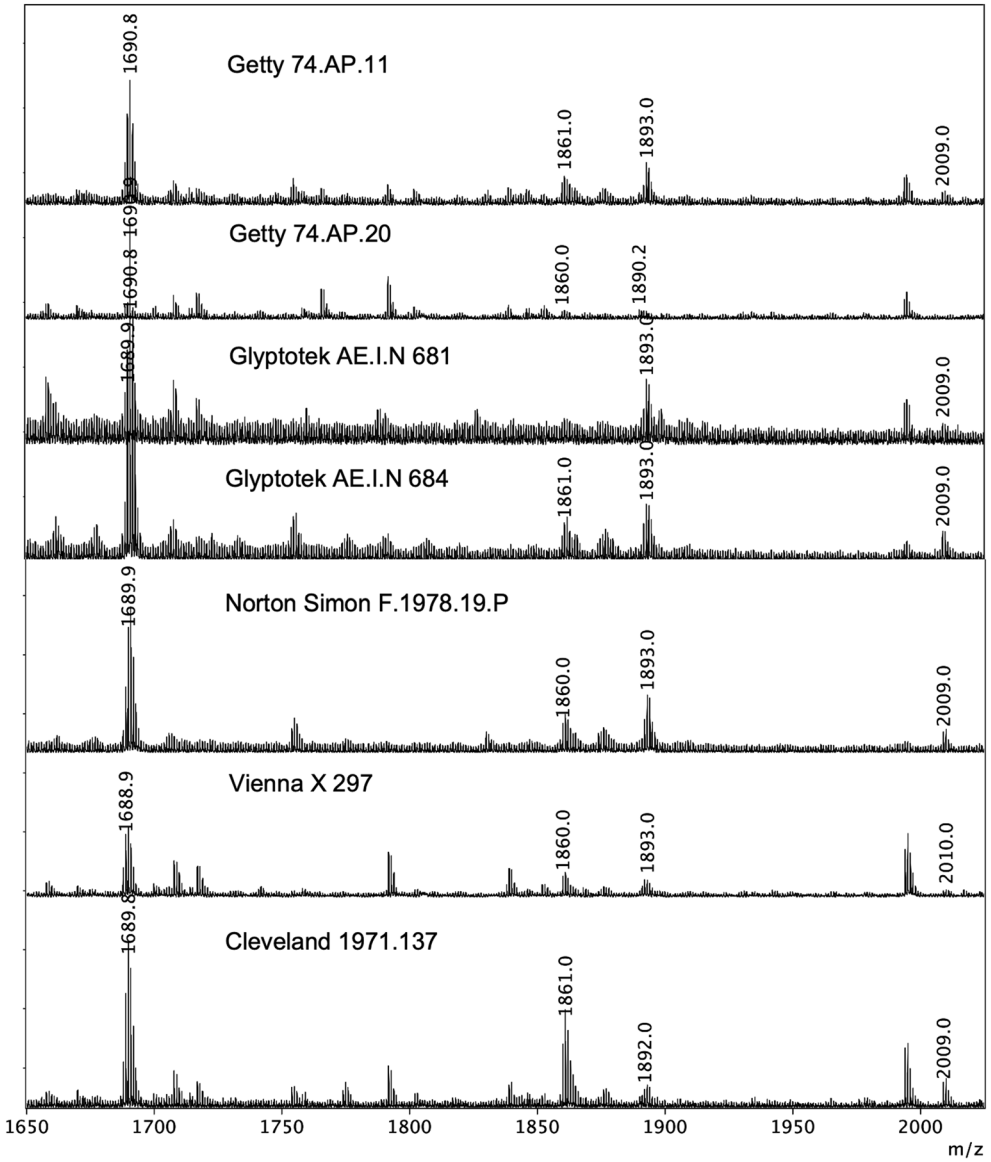

Fig. 8 PMF spectra from coatings on the seven funerary portraits. Labeled ion clusters were usually the most intense and were common to each sample. Ions indicative of both egg glair and yolk were detected in all of these samples except for Cleveland, where only glair was observed

as non-deamidated GGLEPINFQTAADQAR, and Fig. 10 is the ovalbumin1690Da ion identified as triply deamidated GGLEPINFQTAADQAR at N7, Q9 and Q14.

Table 4 is the Mascot summary of ions used to identify apovitellenin (egg yolk, *Gallus gallus*) in Getty 74.AP.11. Consistent with the PMF data, both are deamidated.

Figures 11 and 12 are examples of MSMS spectra used to identify apovitellenin in the Mascot analysis, Table 4. Figure 11 is the apovitellenin 1078 Da ion identified as NFLINETAR deamidated at N5. Figure 12 is the apovitellenin1893Da ion sequence identified as AGQFLLDVSQTTVVSGIR deamidated at Q3 and Q10.

^{14}C analysis

Although visual examination of the portraits with egg-containing coatings suggested that the coatings were not from recent restorations, analyses of samples from them provided no direct support for this conclusion. In order to establish their age, samples of the coatings from two paintings were analyzed by ^{14}C dating.

The radiocarbon age for the whole hen egg coating on Getty portrait (74.AP.11) was reported as UCIAMS-232793: 1935 ± 15 BP (^{14}C years before AD 1950). The calibrated age range, determined using the Calib 8.2 software with the IntCal20 dataset [56],

Table 3 The Mascot summary of peptides used to identify ovalbumin (*Gallus gallus*) in the LCMSMS analysis of the sample from Getty 74.AP.20 including HIATNAVLFFGR, GGLEPINFQTAADQAR, ELINSWVESQTNGIIR and EVVGSAEAGVDAASVSEEFR shown in Fig. 6

Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
449.5806	1345.7201	1345.7143	0.0058	0	32	0.011	1	U	K.HIATNAVLFFGR.C + Deamidated (NQ)
524.9058	1571.6956	1571.6926	0.0030	1	35	0.045	1	U	K.AFKDEDTQAMPFR.V + Deamidated (NQ); Oxidation (M)
799.3630	1596.7115	1596.7090	0.0026	0	53	0.00032	1	U	K.LTEWTSSNVMEER.K + Oxidation (M)
799.8538	1597.6930	1597.6930	0.0001	0	37	0.014	1	U	K.LTEWTSSNVMEER.K + Deamidated (NQ); Oxidation (M)
844.4242	1686.8338	1686.8325	0.0013	0	70	5e-06	1	U	R.GGLEPINFQTAADQAR.E
844.9118	1687.8091	1687.8165	-0.0075	0	76	1.1e-06	1	U	R.GGLEPINFQTAADQAR.E + Deamidated (NQ)
844.9131	1687.8117	1687.8165	-0.0049	0	66	7.1e-06	1	U	R.GGLEPINFQTAADQAR.E + Deamidated (NQ)
844.9147	1687.8149	1687.8165	-0.0017	0	54	0.00045	1	U	R.GGLEPINFQTAADQAR.E + Deamidated (NQ)
844.9234	1687.8322	1687.8165	0.0157	0	76	1.9e-06	1	U	R.GGLEPINFQTAADQAR.E + Deamidated (NQ)
845.4084	1688.8023	1688.8006	0.0018	0	77	1.3e-06	1	U	R.GGLEPINFQTAADQAR.E + 2 Deamidated (NQ)
563.9415	1688.8025	1688.8006	0.0020	0	76	4.4e-06	1	U	R.GGLEPINFQTAADQAR.E + 2 Deamidated (NQ)
845.4092	1688.8038	1688.8006	0.0033	0	108	3.7e-09	1	U	R.GGLEPINFQTAADQAR.E + 2 Deamidated (NQ)
845.4094	1688.8043	1688.8006	0.0038	0	56	3.7e-05	1	U	R.GGLEPINFQTAADQAR.E + 2 Deamidated (NQ)
845.4096	1688.8047	1688.8006	0.0041	0	77	6.4e-07	1	U	R.GGLEPINFQTAADQAR.E + 2 Deamidated (NQ)
563.9423	1688.8051	1688.8006	0.0045	0	80	4.9e-07	1	U	R.GGLEPINFQTAADQAR.E + 2 Deamidated (NQ)
845.4103	1688.8060	1688.8006	0.0054	0	95	2.7e-08	1	U	R.GGLEPINFQTAADQAR.E + 2 Deamidated (NQ)
845.4115	1688.8085	1688.8006	0.0080	0	76	1.1e-06	1	U	R.GGLEPINFQTAADQAR.E + 2 Deamidated (NQ)
845.4118	1688.8090	1688.8006	0.0084	0	83	2.2e-07	1	U	R.GGLEPINFQTAADQAR.E + 2 Deamidated (NQ)
845.4121	1688.8096	1688.8006	0.0090	0	99	4.1e-08	1	U	R.GGLEPINFQTAADQAR.E + 2 Deamidated (NQ)
845.4126	1688.8106	1688.8006	0.0101	0	89	9.8e-08	1	U	R.GGLEPINFQTAADQAR.E + 2 Deamidated (NQ)
845.4147	1688.8149	1688.8006	0.0143	0	93	7.5e-08	1	U	R.GGLEPINFQTAADQAR.E + 2 Deamidated (NQ)
845.4155	1688.8164	1688.8006	0.0159	0	72	1.9e-05	1	U	R.GGLEPINFQTAADQAR.E + 2 Deamidated (NQ)
845.8958	1689.7770	1689.7846	-0.0075	0	108	2.4e-09	1	U	R.GGLEPINFQTAADQAR.E + 3 Deamidated (NQ)
845.8996	1689.7846	1689.7846	0.0000	0	90	7.1e-08	1	U	R.GGLEPINFQTAADQAR.E + 3 Deamidated (NQ)
845.9004	1689.7863	1689.7846	0.0018	0	107	1.8e-09	1	U	R.GGLEPINFQTAADQAR.E + 3 Deamidated (NQ)
845.9021	1689.7896	1689.7846	0.0050	0	77	2e-06	1	U	R.GGLEPINFQTAADQAR.E + 3 Deamidated (NQ)
930.4763	1858.9381	1858.9425	-0.0043	0	69	4.4e-06	1	U	R.ELINSWVESQTNGIIR.N + Deamidated (NQ)
930.4804	1858.9462	1858.9425	0.0037	0	86	9.3e-07	1	U	R.ELINSWVESQTNGIIR.N + Deamidated (NQ)
670.3183	2007.9331	2007.9385	-0.0054	0	44	0.00034	1	U	R.EVVGSAEAGVDAASVSEEFR.A
670.3193	2007.9360	2007.9385	-0.0026	0	48	0.0017	1	U	R.EVVGSAEAGVDAASVSEEFR.A
1004.9781	2007.9416	2007.9385	0.0031	0	89	4.8e-08	1	U	R.EVVGSAEAGVDAASVSEEFR.A
1004.9786	2007.9426	2007.9385	0.0041	0	70	5.7e-06	1	U	R.EVVGSAEAGVDAASVSEEFR.A
1004.9787	2007.9429	2007.9385	0.0044	0	90	5e-08	1	U	R.EVVGSAEAGVDAASVSEEFR.A
1004.9794	2007.9442	2007.9385	0.0057	0	121	1.9e-11	1	U	R.EVVGSAEAGVDAASVSEEFR.A
670.3223	2007.9452	2007.9385	0.0067	0	65	1.9e-05	1	U	R.EVVGSAEAGVDAASVSEEFR.A
1004.9799	2007.9453	2007.9385	0.0067	0	75	7.6e-07	1	U	R.EVVGSAEAGVDAASVSEEFR.A
1004.9821	2007.9497	2007.9385	0.0112	0	84	8.1e-08	1	U	R.EVVGSAEAGVDAASVSEEFR.A
1004.9852	2007.9559	2007.9385	0.0173	0	72	1.4e-06	1	U	R.EVVGSAEAGVDAASVSEEFR.A

As observed in the PMF spectra, HIATNAVLFFGR, ELINSWVESQTNGIIR and GGLEPINFQTAADQAR are deamidated whereas EVVGSAEAGVDAASVSEEFR is not, consistent with the presence or absence of N and Q residues

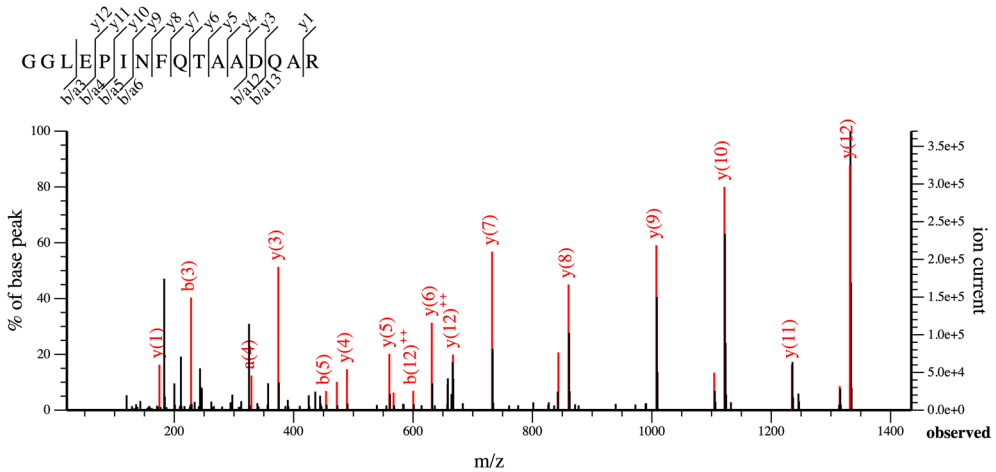

Fig. 9 MSMS spectrum from the Getty portrait 74.AP.20 sample that identifies the non-deamidated ovalbumin 1688 Da ion sequence as GGLEPINFQTAADQAR

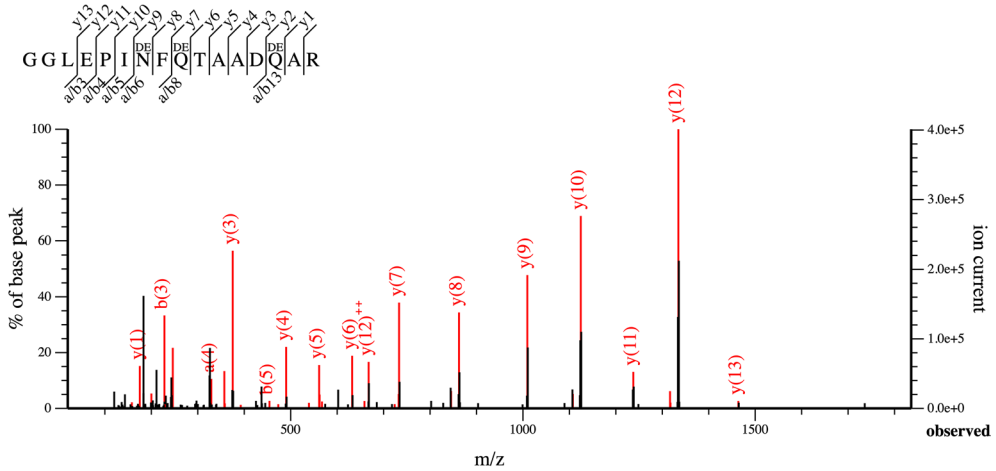

Fig. 10 MSMS spectrum from the Getty portrait 74.AP.20 sample that identifies the ovalbumin 1690 Da ion sequence as GGLEPINFQTAADQAR deamidated at N7, Q9 and Q14

Table 4 The Mascot summary of peptides used to identify apovitellenin (*Gallus gallus*) in the LCMSMS analysis of the sample from Getty 74.AP.11 including NFLINETAR and AGQFLLDVSQTTVVSGIR shown in Fig. 6

Observed	Mr(expt)	Mr(calc)	Delta	M	Score	Expect	Rank	U	Peptide
540.2703	1078.5260	1078.5294	-0.0034	0	43	0.045	1	U	R.NFLINETAR.L + 2 Deamidated (NQ)
631.6712	1891.9918	1891.9891	0.0027	0	56	0.0024	1	U	R.AGQFLLDVSQTTVVSGIR.N + 2 Deamidated (NQ)
947.0042	1891.9939	1891.9891	0.0048	0	67	5e-05	1	U	R.AGQFLLDVSQTTVVSGIR.N + 2 Deamidated (NQ)

As observed in the PMF spectra, both are deamidated

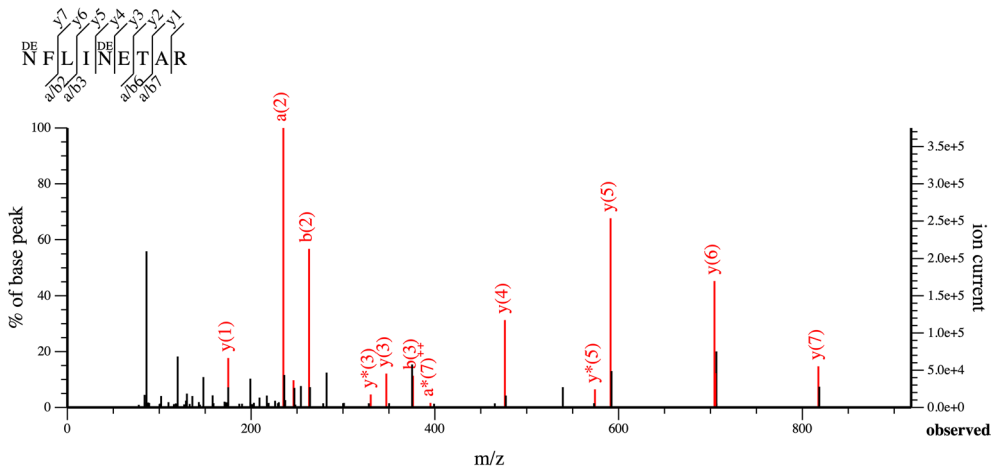

Fig. 11 MSMS spectrum from the Getty portrait 74.AP.11 sample that identifies the apovitellenin 1079 Da ion sequence as NFLINETAR deamidated at N1 and N5

indicates a date of around 92 CE (median probability), with a full 2 sigma (95% probability) age range of 25–128 CE. This date is consistent with the age range of the portrait's linden wood panel, ^{14}C dated in the same facility as UCIAMS-128007: 1925 ± 20 BP, corresponding to a median probability calibrated age of 103 CE, 2 sigma calibrated age range 28–204 CE.

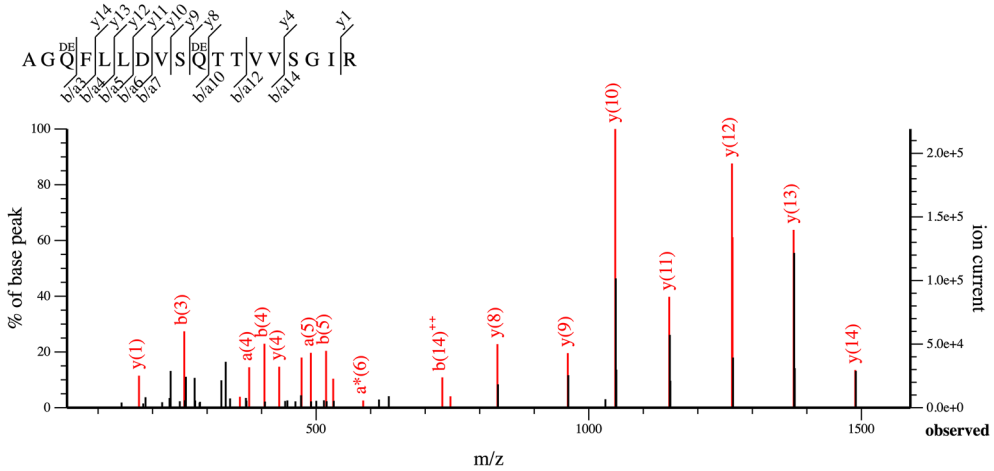

Fig. 12 MSMS spectrum from the Getty portrait 74.AP.11 sample that identifies the apovitellenin 1893 Da ion sequence as AGQFLLDVSQTTVVSGIR deamidated at Q3 and Q10

The radiocarbon age for the egg coating on the Norton Simon portrait (F.1978.19.P) was UCIAMS-237981: 1990 ± 20 BP, with a median probability calibrated age of 24 CE and a full 2 sigma age range of 42 BCE-108 CE.

The dates established for both portraits support that the application of the egg coatings is ancient and not a recent intervention. Although the coatings on only two of the portraits have been ^{14}C dated, it is likely that the coatings on the others were also applied in antiquity based on their similar visual and UVF characteristics and the detection of highly deamidated hen egg on all of them.

Discussion

Egg, binding media and coatings in antiquity

The chicken was not native to Egypt and is believed to have arrived from Asia via Europe around the seventh century BCE, possibly earlier, in sporadic appearances and disappearances [57, 58]. Artistic representation supports the presence of the chicken between 1425–1123 BCE [59], primarily for sport, such as cock fighting, or as an auspicious symbol and sign of victory going into battle [60]. Due to a lack of archeological evidence (bones and shells), it is thought that the chicken as a source of food was only exploited much later. Pliny the Elder, AD 23/24—79, discusses both the use of chickens for cock fighting, food and possible artificial hatching [61]. An increase in both written and archaeological evidence during the Roman period further supports the domestication of the chicken and egg not only as a source of food but also as an industry in Egypt through the invention of egg incubators [62].

The identification of ancient binding media is complex, and the absence of published information or the presence of dubious results to date supports this. There are a few comprehensive studies on the chemistry of egg and numerous overviews of the use of egg, yoke or glair, added to binders and used historically as a varnish [63–65]. A 2016 compilation of paint media analysis from a group of ancient paintings reported the identification of egg, yoke or glair, typically mixed with glue on 12 out of 14 panels analyzed [66]. The use of egg glair as a binder on medieval manuscript illuminations is well known [67]. Medieval treatises also mention that egg glair varnishes were applied to prevent materials from sticking to freshly painted surfaces providing protection from external forces [65, 68]. This benefit might have been useful to the ancient portrait painters before burying mummies in the hot Egyptian dessert sand. Egg glair has been thoroughly documented from the Renaissance to the nineteenth century, primarily as a coating to provide luster and gloss to painted surfaces. Various types of coatings have been identified on late period funerary painted artifacts, such as sarcophagi, including oils, *pistachia spp*.resins, pine resins, and bitumen, alone or modified [6, 8, 69, 70]. While these unguents had a practical and aesthetic purpose, their primary function was part of the funerary ceremony. The use of egg associated with this ritual has not, to the authors' knowledge, been documented to date.

The egg as a symbol of life and rebirth was of cosmic significance to the ancient Egyptians. Representing renewal and everlasting light, the egg was believed to be the center of the creation myth spanning into the Christian Era [71, 72]. The profound symbolism of the egg in combination with esteemed ancient Egyptian funerary practices may support the application of an egg coating as a ritual function, thus providing compelling evidence for its use beyond practical or aesthetic reasons. Our

confirmation of the existence of this intentional coating may also provide clues to how ancient artists worked and define workshop and/or regional methods. As we continue to characterize materials with sensitive analytical techniques, we will gain more insights into when and how such coatings were applied and a better understanding of the ancient artistic and ceremonial customs of which they were a part.

Unexplained observations

LCMSMS data consistently point to the presence of low levels of animal glue in the coating. Whether this is admixed material from a ground, although unlikely based on the thickness of the paint layers, or it was intentionally added to the egg mixture, cannot be determined from the present data. Its consistent appearance in all samples included in this study, however, may argue against it being due to a later conservation intervention.

The egg coating on all seven portraits examined here was highly deamidated. While these coatings are all believed to be ancient, and age can be a factor in deamidation, the extent of deamidation still seems out of place, especially when the underlying paint layers are visually unaffected. Further study will be needed to determine the exact circumstances behind the coatings' physical and chemical characteristics.

Finally, the addition of the coating *after* the portrait has been affixed to its mummy is significant, but unexplained. If the coating were applied to the entire panel as part of its manufacture, it might suggest a protective "varnish." However, application after insertion into the mummy broadens the possibilities of its function or intent, and at this point we can only speculate as to what the original purpose might have been.

Conclusions

This study has focused on the characterization of a unique coating observed on a group of Romano-Egyptian funerary portraits. The possible presence of such a coating can be strongly suggested by close visual observation, or more importantly, by observation of UVF. A unique feature of the coatings on the mummy portraits is that they seem to terminate where the mummy wrappings secured the panels in place, suggesting that the coating was applied after the portrait had been placed on the mummy. The coating on six funerary portraits confirmed the presence of deamidated whole hen egg and on one portrait, egg glair alone. As noted above, only a single sample was analyzed from each painting so, whether the glair-only result for the Cleveland portrait is representative of its overall coating cannot be determined without additional sampling. Significantly, since the LCMSMS data fully confirmed the interpretation of the PMF data, future analyses will be possible with PMF alone.

The identification of whole hen egg is quite unusual as egg has not to date been confirmed as the sole binder for funerary portraits, and literature on historic egg coatings, from well after ancient times, discusses only the use of egg glair alone.

^{14}C dating results from samples taken from two portraits confirm that their coating was applied approximately 2000 years ago, most likely at the final stage of the mummification ritual. While the ^{14}C date for only two portraits does not confirm when the others in this group were coated, the presence of deamidated hen egg on the group studied may suggest that the coating was applied in antiquity for those exhibiting similar characteristics.

Understanding the purpose of the coating remains enigmatic. It is evident that hen egg was selected as a material distinct from the common materials used for painting the portraits. Whether this was a part of the funerary ritual, or a protective measure to preserve the painted portrait is uncertain, but, as more examples are identified and dated, a better understanding of ancient Egyptian funerary and artistic practices will be possible.

The work presented here required the collaboration of a multidisciplinary team employing complementary analytical techniques to recognize, identify, date and better understand a discrete surface coating observed on a group of funerary portraits from Egypt during the Roman period. This study was made possible by our ability to visually compare portraits from various museum collections through the APPEAR project as well as the opportunity to pursue in-depth technical studies to confirm unique, parallel features. This project provided the very rare opportunity to discover and characterize the application of a hen egg coating in antiquity. Although the comparative data is not extensive—seven confirmed egg coatings, six as whole hen egg and one as hen egg glair and two with confirmed ancient dates—this study presents new information about the manufacture of funerary portraits and underscores the need for further investigations and an expanded exploration of ancient coating materials and practices. Thanks to analytical investigations, these studies are possible and "the history of ancient media is only starting to be written."

Photo credits

Ny Carlsberg Glyptotek

Portrait of a bearded young man AE.I.N 681 and Portrait of a bearded man. AE.I.N 684: Images: Maria Louise Sargent.

J. Paul Getty

Mummy Portrait of a Bearded Man, Romano-Egyptian, AD 150–170. Encaustic on linden panel, 37 × 21 cm (14 9/16 × 8 1/4 in.). Los Angeles, J. Paul Getty Museum, 74.AP.11.

Portrait of a Bearded Man, Romano-Egyptian, AD 100. Tempera on panel,

36 × 37.5 × 0.3 cm (14 3/16 × 14 3/4 × 1/8 in.) Los Angeles, J. Paul Getty Museum, 74.AP.20.

Norton Simon

Portrait of a Man, second century AD. F.1978.19.P. Encaustic on wood, 12–1/8 × 6–5/8 in. (30.8 × 16.8 cm).

The Norton Simon Foundation, Gift of Mr. Norton Simon.

Cleveland

Funerary Portrait of a Young Girl, c. AD 25–37. Egypt, Roman Empire, late Tiberian. Encaustic on wood; overall: 39.4 × 17.4 cm (15 1/2 × 6 7/8 in.). The Cleveland Museum of Art, John L. Severance Fund 1971.137.

For the overall and UV images.

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Photomicrograph

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Vienna

Portrait of a Lady, Romano-Egyptian, AD 117–138. er-Rubayat. Encaustic on wood, 40 × 20 cm (15 ¾ × 7 7/8 in.). Vienna, Kunsthistorisches Museum, Antikensammlung, X 297. KHM-Museumsverband.

Abbreviations

AMS	Accelerator mass spectrometry
APPEAR	Ancient Panel Painting: Examination, Analysis and Research Project
ELISA	Enzyme-linked Immunosorbent assay
GC/MS	Gas chromatography coupled with mass spectrometry
LCMSMS	Liquid chromatography coupled with tandem mass spectrometry
PMF	Peptide mass fingerprinting
ZooMS	Zooarchaeology by mass spectrometry
MALDI	Matrix assisted laser desorption-ionization mass spectrometry
UVF	Ultraviolet-induced visible fluorescence

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40494-023-00908-5.

Additional file 1: Mascot Summary Reports.

Additional file 2: LCMSMS .mgf files.

Additional file 3: mMass .msd files.

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Author contributions

Conceptualization, preliminary investigation and imaging, MS, LRS; methodology and data analyses, JM, DK, JS; writing—original draft preparation DK, MS; writing review and editing, DK, MS, JM, LRS, JS. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and its additional files.

Declarations

Competing interests

The authors declare no competing interest.

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