Journal of Cultural Heritage xxx (2017) xxx–xxx

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Original article

Characterization of membrane metal threads by proteomics and analysis of a 14th c. thread from an Italian textile

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ARTICLE INFO

Article history:
Received 22 January 2018
Accepted 9 March 2018
Available online xxx

Keywords:
Metal threads
Membrane
Collagen
Cyprus gold
Proteomics
nanoLC-Orbitrap MS/MS

ABSTRACT

Beginning in the 13th century, membrane metal threads – made out of animal skins (leather, parchment, and vellum) or membranous material (e.g., stomach, intestine) coated with metal – were the most popular variety of decorative metal threads used in European textiles. This work provides the proteomics groundwork for the identification of the species and the type of membrane used in the manufacture of a 14th century membrane gilded thread. A protocol for small sample extraction and nanoLC-Orbitrap MS/MS analysis was first tested on standards of pig peritoneum and cow intestine metal-coated with or without the presence of an egg adhesive. The proteomes of each membrane were characterized and compared by qualitative and quantitative bioinformatics; in addition to the predominant collagen proteins in each membrane type, minor tissue-specific proteins (e.g., smooth muscle proteins from intestine standards) were detected. Species-specific collagen peptides (i.e., from collagen I and collagen III) were confidently identified to determine the species of origin, regardless of the application of metal and egg-based adhesives. Likewise, the thin layer of egg adhesive was successfully characterized with the detection of egg white (ovalbumin, ovotransferrin, lysozyme) and egg yolk (vitellogenin I, II, III) proteins. When applied to the thread from a 14th century Italian textile, this comprehensive methodology resulted in the identification of seven collagen I and III peptides specific to cow, as well as other proteins suggesting that the ancient thread was made with intestine or stomach membrane without the use of an egg-based adhesive.
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1. Introduction

Decorative metal threads have been extensively used for the embellishment of textiles since ancient times. Many examples of metal threads exist in artifacts of cultural importance, and even earlier references to lavish gold and silver textiles can be found in ancient texts, including a description of an ephod containing gold in the Old Testament of the Bible (Exodus 39:2–3 "They hammered out thin sheets of gold and cut strands (. . .)"). The popularity of textiles woven or embroidered with metal threads persisted, and their use is frequently associated with textiles intended to portray wealth or symbolic importance [1]. Metal threads were most often made of gold, silver, or their alloys, often gilt, with the fabrication method differing by region and changing over time [2]. Five categories describing the use of metals in textiles have been defined: I. Metal applied with adhesive to already woven fabrics, II. Metal wire or flattened strips used directly in weaving, III. Metal wire or strips wound around a fiber core, IV. Metallic surface applied to organic wrappings (cellulosic or proteinaceous) wound around a fiber core, and V. Metallic surface applied to organic strips (cellulosic or proteinaceous) without a fiber core [2–6].

Protein metal threads (Categories IV and V) were made from membranous tissues (e.g., stomach or intestinal walls of animals), although skin has also been used as a substrate [2,7]. Research at the end of the 19th century suggested that gilt membranes were made using the intestines from slaughtered animals [8]. Cow intestine was similarly used in the manufacture of gold foil, also called goldbeater's skin, during the same time period. Metal was applied to the membrane with metal leaves or by mottle gilding, using either the natural exudates of the organic membrane or an additional adhesive [7]. Reports have described the use of egg white, egg yolk, animal fat, animal and fish glues, gums, and clays as adhesives but to date, no scientific investigation has been carried to substantiate the presence and nature of the adhesives [4,7,9]. In category IV threads, also known as gilt membranes or Cyprus gold, the gilded membrane was cut into thin strips and wound around a silk or linen core [2]. No adhesive was used between the gilded organic wrapping and the

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https://doi.org/10.1016/j.culher.2018.03.007
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Please cite this article in press as: A.K. Popowich, et al., Characterization of membrane metal threads by proteomics and analysis of a 14th c. thread from an Italian textile, Journal of Cultural Heritage (2017), https://doi.org/10.1016/j.culher.2018.03.007

fiber core; instead, the twist of the metal membrane thread around the core was sufficient to keep the material in place. Migration of adhesive and wrapping material into the core fiber has however been observed [5]. In category V threads the metal was applied to membrane strips and used in textiles without being wound [4].

Membrane metal threads were first used in the 11th century by Levantine traders in Cyprus [1]. Beginning in the 13th century, gilt membranes became the most common variety of metal threads used in European textiles, especially those from Italy and Spain, as well as Western Asia because they were flexible, lightweight, and inexpensive [7]. Substantial focus has been given to identifying the metals used in metal threads, as studies show that the composition of the metal and gold/silver ratios are suggestive of geographic origin of the metal thread [6,7,10]. Recently, a range of electron microscopy techniques and micro-Raman spectroscopy have been used to characterize cross-sections of metal threads revealing details of the production technology through study of the 3D texture [11]. Conversely, little attention has been given to identifying the membrane type or animal species used in organic metal threads, although the elucidation of this information would provide valuable insight into the origin, production technique, and use of membrane metal threads in the medieval period [1]. Identification of the membrane portion of the substrate poses difficulty, especially when a textile is in a deteriorated state. Using morphology on the microscopic scale, differences between leather, membranous material, parchment, and vellum have been made [1,2]; however, the appearance of the substrate can be drastically altered from its original state because of decomposition, embrittlement, wear, and treatment damage, making visual identification indefinite and oftentimes impossible. In contrast to morphological identification, DNA amplification and molecular biology techniques have been used to identify the animal species in organic metal threads found in textiles from the 11–15th centuries [9]. Unfortunately, this study could only narrow down the identification to a few candidate species. Here, we have adopted a bottom-up proteomics approach to analyze a 14th c. membrane thread: the entire membrane sample is characterized through a single extraction and digestion of the whole extract, including the membrane proteins and other binding proteins if present. The complex mixture is separated by liquid chromatography before analysis by an Orbitrap Velos mass spectrometer and the data obtained are searched against a public database. In the absence of historical information on the membrane's animal origin, any species can be targeted through a proteomics database search.

2. Research aims

To resolve the identification issue of the organic substrate of a metal thread several centuries old, a proteomics approach was devised. Proteomics has been used successfully to identify binders [12] and collagen-based substrates such as parchment [13], but has never been used to characterize membrane metal threads. The aim of this project is to show that a complex organic substrate, composed of a membrane base and protein binders, can be comprehensively characterized, including the type and species of the membrane and presence of proteinaceous adhesives. To test the applicability of proteomics to ancient samples, two series of standards (Table 1) were prepared at the University of Applied Arts Vienna (Austria) from the abdominal membrane of pig and from intestine of cow, then subjected to different treatments (heat fixation, egg white, or egg yolk used as adhesives for the metal coating). Pig and cow were chosen as species for their commonality and easy availability, and as probable source obtained in the past from butchered animals. The proteome of each membrane was determined by extracting proteins in samples of less than one milligram, thus revealing tissue-specific proteins, while peptides specific to the membrane species and the egg adhesive were characterized in untreated and treated samples. Finally, the developed protocol was applied to a membrane metal thread from a 14th century Italian textile (Fig. 1), thought to be made from an animal's internal organ and not skin.

Table 1
Reference standards.

Reference	Membrane	Adhesive	Fixation	Metal leaf type
Pig untreated	Peritoneum	None	None	None
Pig gilt	Peritoneum	None	None	Gilt silver
Pig gilt heat	Peritoneum	None	Heat	Gilt silver
Pig gilt egg white	Peritoneum	Egg white	None	Gilt silver
Pig gilt egg yolk	Peritoneum	Egg yolk	None	Gilt silver
Cow untreated	Gut	None	None	None
Cow silver	Gut	None	None	Silver
Cow silver heat	Gut	None	Heat	Silver
Cow silver egg white	Gut	Egg white	None	Silver

Fig. 1. 14th century Italian textile (#21714-1) from the History Museum in Graz, Austria, ©Universalmuseum Joanneum, Museum für Geschichte, Kulturhistorische Sammlung/Institute of Conservation, University of Applied Arts Vienna/Elisabeth Delvai 2017. The arrow points to where the thread was sampled. In the bottom right, the image of the thread was acquired with HIROX KH-8700 3D digital microscope (Hirox-USA, Inc., NJ), courtesy of Thomas Lam (Smithsonian's Museum Conservation Institute). Software: PowerPoint 2013.

3. Materials and methods

3.1. Standards

The gilt membrane reference standards were prepared at the Universität für angewandte Kunst (University of Applied Arts Vienna, Austria) based on what is known of traditional protocols [14,15]. Pig peritoneum, which is the membrane sack containing the abdominal organs, was obtained from the Veterinary University of Vienna (Vienna, Austria). Cattle gut was obtained from a local butcher. The fat was scraped off the membrane and the membrane washed and rinsed, then stretched and pinned. Gilt silver leaves or silver leaves were immediately applied on the damp membrane using egg yolk, egg white, or no adhesive (Fig. S1). The standards were allowed to dry overnight. Select standards were subjected to heat fixation with a heated spatula at approximately 80 °C. Reference standards are listed in Table 1.

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3.2. Historical sample

The original sample (#21714-1) comes from the collection of the History Museum at the Universalmuseum Joanneum (Graz, Austria). The textile, a silk fabric with metal fibers woven into it, was produced in Italy in the 14th century, depicts dogs or cats alongside birds. The sample was taken from a large piece of fabric that is well preserved. Scanning electron microscopy showed that the membrane was coated with gilt silver, and wound around bast fibers [16], although most of the metal has worn away from the sampled thread, as seen in Fig. 1.

3.3. Reagents

Iodoacetamide (IAM) and tris(hydroxymethyl)aminomethane hydrochloride (TRIS HCl) were obtained from Sigma–Aldrich (St. Louis, MO); guanidine hydrochloride (GuHCl), methanol (MeOH), acetonitrile (ACN), and Optima grade formic acid from Fisher Scientific (Fair Lawn, NJ); ammonium bicarbonate (ABC) from VWR International LLC (West Chester, PA); tris(2-carboxyethyl)phosphine (TCEP) from Thermo Scientific (Rockford, IL). Sequence grade modified trypsin from Promega (Madison, WI). Quantitation of protein was determined with the Pierce BCA Protein Assay Kit from Thermo Scientific (Rockford, IL).

3.4. Sample preparation

Standards were cut with a razor in squares of less than 1 mg and were solubilized in 600 μL of 4 M GuHCl and 50 mM Tris HCl at pH 8, using five cycles of 30 s homogenization with a Bead Ruptor Elite (Omni International) at 7 m/s, with 5 min dwell time at −20 °C between each cycle and following the final cycle. Protein extraction was allowed to continue by shaking the samples overnight at room temperature. All samples were centrifuged at 10,000 rpm for 20 min and the protein concentration of the supernatant was measured by the Pierce BCA Protein Assay. The supernatant was reduced with 500 mM TCEP for a final concentration of 50 mM TCEP and pH adjusted to 8.0. A 250 μL aliquot was alkylated with 40 mM IAM and vortexed for 45 min in the dark. This was followed by buffer exchange with 100 mM ABC using 3000 MWCO Amicon Ultra-0.5 mL centrifugal filters. Samples were digested with 0.5 μg of trypsin (trypsin:protein ratio 1:100), overnight at 37 °C. For peptide cleanup, the samples were loaded on a C18 stage tip made from an Empore SPE Extraction Disk (3 M) [17]. The tips were washed with 0.1% FA solution. Peptide mixtures were then eluted in 2× 20 μL of 80:20 (v/v) acetonitrile:0.1% FA. All samples were then dried down to a final volume of 10 μL.

Soluble proteins were extracted from 100 μg of an ancient 14th century thread sample, with the fiber core already removed, using the above described protocol, with the entire 600 μL extraction solution being reduced and alkylated. In addition to the soluble extracts, the insoluble fraction that was pelleted by centrifugation was retained and diluted in 100 mM ABC, after which both soluble and insoluble fractions underwent the sample preparation steps as above.

3.5. Protein analysis by nanoLC-Orbitrap MS/MS

The desalted samples were diluted and 0.5–2 μg of total protein was injected and analyzed by LC-MS/MS. The peptides were first loaded onto an in-house packed Thermo BioBasic C18 precolumn (30 mm × 75 μm i.d.) after which they were separated on an in-house packed analytical column (210 mm × 75 μm i.d.) made of the same stationary phase, using a Thermo Scientific Dionex Ultimate 3000 UHPLC system with the following gradient: 2% B 0–8 min, 55% B 98 min, 90% B 100–103 min, 2% B 104–120 min, where buffer A is 0.1% FA in H_2O and buffer B is 0.1% FA in ACN. The UHPLC was directly coupled to a Thermo Scientific LTQ Velos Dual Pressure Linear Ion Trap mass spectrometer which analyzed the peptides in positive mode using the following parameters: MS1 60,000 resolution, 100 ms acquisition time, 1×10^6 automatic gain control (AGC), MS2 15,000 resolution, 250 ms acquisition time, 5×10^5 AGC, top 8, 30 normalized collision energy (NCE) higher-energy collisional dissociation (HCD).

3.6. Bioinformatics analysis

PEAKS 8.0 (Bioinformatics Solutions Inc.) was used to search the RAW data for matches against the UniProt database (www.uniprot.org) of publicly available sequences. The mammalian database was imported on June 15th, 2017, while a customized database was created containing the complete Uniprot databases of *Sus scrofa* and *Bos taurus* to which were added *Gallus gallus* egg proteins (Timestamp 14th July 2017). Searches were carried out using trypsin as enzyme, three missed cleavages, peptide mass tolerance (PMS) of 10 ppm, fragment mass error tolerance (MS/MS) of 0.02 Da, carbamidomethylation as a fixed modification, and the following variable modifications as configured in PEAKS: deamidation (NQ), hydroxylation/oxidation (RYFPNKD), and oxidation (M). PEAKS PTM and SPIDER were both enabled to identify unspecific PTMs. Results were filtered using an FDR of less than or equal to 1% for peptide spectrum matches, a protein score of $-10\lg P \geq 20$, and 1 unique peptide.

Quantitative bioinformatics analysis was performed using Proteome Discoverer 2.2 (Thermo Fisher). Searches were carried out by MS Amanda 2.0, using the same search parameters used in PEAKS. Protein quantification was performed in Proteome Discoverer 2.2, based on summed abundance of unique plus razor peptides, with data normalized to collagen I alpha 1 (COL1A1).

Collagen peptides from pig and cow standards identified as “unique” to a protein group by PEAKS 8.0 software, when searching against the Mammalia protein database, were further investigated using the Basic Local Alignment Search Tool (BLAST) available online from both Uniprot (http://www.uniprot.org/blast searched against UniProtKB protein database) and the National Center for Biotechnology Information NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi searched against NCBI Mammalia database).

4. Results and discussion

4.1. Membrane characterization

The proteomes of peritoneum and intestine were examined to evaluate their differential composition, and were further analyzed using quantitative bioinformatics to search for protein markers that could potentially specify the membrane type. The peritoneum is a continuous membrane that lines the abdominal wall (parietal peritoneum) and covers the abdominal organs (visceral peritoneum); it is composed of a thin layer of mesothelium, which is made of a monolayer of squamous epithelial cells on a basement membrane, supported by a layer of connective tissue [18]. The basement membrane is composed of a specialized layer of extracellular matrix protein, with type IV collagen being the most abundant protein [19–22]. Other predominant protein constituents in basement membrane include laminin, nidogen (NID), and perlecan (HSPG) [18,23,24]. Although intestinal tissue also contains a basement membrane, in contrast to peritoneum, intestine has a layer of muscle tissue, specifically smooth muscle tissue. Smooth muscle proteins include calponin-1 (CNN1) and myosin-11 (MYH11) [19,25–27].

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Analysis of the peritoneum (Table S1) and intestine (Table S2) standards showed that there was minimal difference between the collagen proteins, other extracellular proteins, blood and serum proteins, and cellular protein content of these two membranes (see full results in SI files S1 and S2). As expected, multiple smooth muscle proteins were found in the intestine samples only, which correspond to the muscular layer present in intestine. Basement membrane specific proteins were, interestingly, not detected in peritoneum samples, but very low levels of NID1, HSPG, and collagen IV alpha 1 (COL4A1) were observed in the intestine samples (Table S2). These proteins are difficult to extract because of their low solubilities [22,28]. The collagen IV network for instance contains disulfide bonds making it highly insoluble and extraction with reducing agents, such as TCEP used here, is required to break disulfide bonds and can somewhat improve extractability [29–31]. However, here the top layer of the membrane was scraped to remove fat during preparation and most likely the mesothelial layer was removed alongside the fat. The basement membrane is also much thinner than the connective or muscle layers, so lower abundance of these proteins is expected.

The abundances of collagen and muscle proteins, normalized relative to collagen I alpha 1 (COL1A1), are shown in Fig. 2 using the gene names to allow for interspecies comparison. Collagen III alpha 1 (COL3A1), and to a lesser extent collagen I alpha 2 (COL1A2), were found in higher abundance in the intestine than the peritoneum. Of all the muscle proteins identified in the intestine, actin, desmin, and myosin-11 were found with the highest abundances in all cow standards and as such represent useful markers for membrane differentiation.

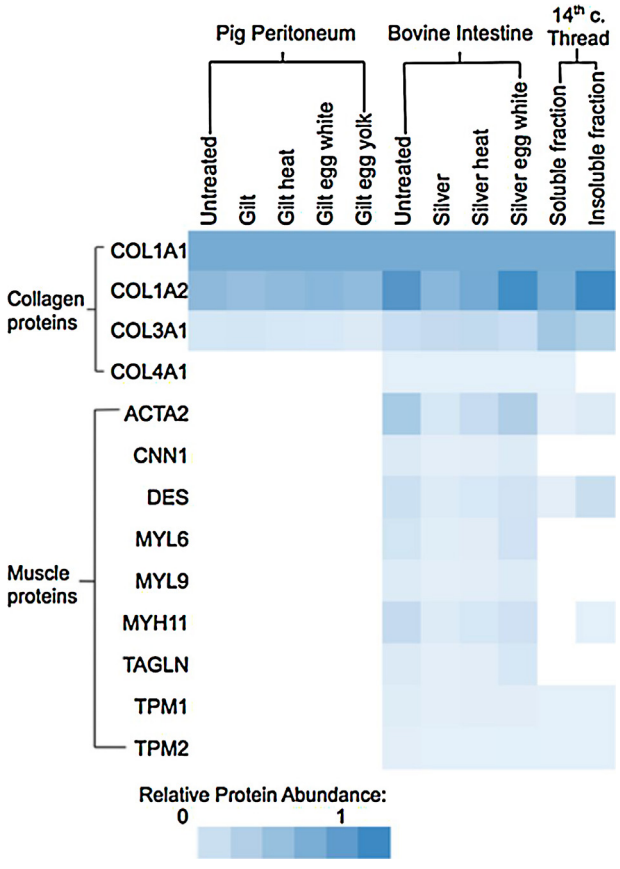

Fig. 2. Normalized relative abundance of collagen and muscle proteins (indicated by their gene codes) in peritoneum, intestine, and a 14th century membrane thread. Corresponding protein names: COL1A1, collagen I alpha 1; COL1A2, collagen I alpha 2; COL3A1, collagen III alpha 1; COL4A1, collagen IV alpha 1; ACTA2, actin, aortic smooth muscle; CNN1, calponin-1; DES, desmin; MYL6, myosin light polypeptide 6; MYL9, myosin regulatory light polypeptide 9; MYH11, myosin 11; TAGLN, transgelin; TPM1, tropomyosin alpha 1 chain; TPM2, tropomyosin beta chain. Software: Excel2013 and PowerPoint 2013.

4.2. Species identification

The long-term survival of collagen proteins makes them excellent markers for species identification in ancient artifacts. Previously, collagen peptide markers have been identified by peptide mass fingerprinting for species identification of bones [32] and skin substrates [13,33]. In the analysis of archeological animal glue, often these proteins are the only proteins detected in sufficient abundance to allow species identification [34–36]. In artworks where animal glue was commonly used as binder in paintings and frescoes, collagen peptides specific to bovine have previously been identified in an 18th century gilt sample [37].

As shown in Fig. 2, COL1A1 and COL1A2 were the most abundant collagen chains found in the intestine and abdominal membranes, followed by COL3A1. Several more collagen proteins (types IV, V, VI, and XIV) were identified from the LC–MS/MS analysis of the untreated (adhesive and metal free) pig and cow standards (Tables S1 and S2), but with much lower coverages. Having further established the abundance of collagen I and III chains in the membrane standards, species identification was focused on these proteins: the species markers identified in the standard membranes were also used as a proxy to determine whether the collagen chains, and by consequence species identification, would be affected by the manufacture processes.

Distinctive markers for pig and cow were identified by searching against the Uniprot Mammalia protein database in the untreated pig (SI file S1) and cow (SI file S2) membrane samples, and by selecting peptides uniquely found in the proteins and species of interest (COL1A1, COL1A2, and COL3A1 of *Sus scrofa* and *Bos taurus*). The selected peptides were further identified using BLAST (Uniprot and NCBI) as being specific to the *Sus scrofa* species (Table 2 for pig samples) or the bovid clade (Table 3 for cow samples) that includes the *Bos*, *Bubalus*, and *Bison* genera. There are indeed no peptide specific to the *Bos taurus* species in the targeted proteins. All collagen peptides are shown in SI File S3.

No COL1A1 specific peptides were found in either pig or cow: unsurprisingly, cow COL1A1 shares 97% of its sequence with that of pig, for instance. COL1A2 and COL3A1 have greater degrees of sequence variation between animal species. Six COL1A2 and nine COL3A1 peptides were identified in the untreated pig sample (corresponding to respectively five and six different sequence segments, Table 2), and six COL1A2 and three COL3A1 peptides were identified in the untreated cow sample (corresponding to respectively four and one different sequence segments, Table 3), after manual inspection of the data in Peaks.

With 15 and 9 peptides identified in the collagen chains for pig and cow respectively, species markers were successfully identified in both membrane types. The addition of the gilt or silver layer did not significantly affect the detection of the marker peptides and no degradation on the protein chains was observed because of the metal. Metal adducts on peptides were searched for but none were found; the metal seems to wear off easily (a phenomenon also observed on ancient textiles) and was completely filtered after solubilization of the proteins. Because of the presence of the egg yolk binder, some of the collagen peptides in the pig were not detected (five markers were not identified at all in the pig sample with egg yolk). A similar result was observed for the egg white in both the pig and cow, but to a lesser extent.

Overall, there was no dramatic loss of collagen peptides after treatment, suggesting that species identification should be possible in ancient samples using collagen peptides only.

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Table 2
Specific collagen peptides identified for the *Sus scrofa* (pig) standards, with peaks scores given in −10lgP.

Protein	Peptide sequence	Position	Standards				
			Untreated	Gilt	Gilt heat	Gilt egg white	Gilt egg yolk
	GNDGSVGPVGPAGPIGSAGPPGFPGAPGPK	235–264					
	+2 Hpro		53.33	29.74	62.93	34.58	
	+ 3 Hpro		65.91	45.91	68.18	26.13	
	GPTGPAGVR	424–432	32.71	31.52	33.19	31.13	33.97
COL1A2: A0A1S7J1Y9_PIG	GPTGDPG**K**NGE**K**GHAGLAGAR	499–519					
	+ 1 Hpro + 1 deam				52.20		52.83
	+ 2 Hpro + 1 deam		54.85	44.77	58.02	29.46	48.44
	IGPPGPSGISGPPGPPGPAGK	795–815					
Sus scrofa domesticus	+ 3 Hpro		55.28	61.94	57.17	60.78	58.03
	+ 4 Hpro		32.49	34.73	33.84	29.58	28.41
	+ 5 Hpro		29.41		32.28	21.26	
GenBank: BAX02569.1	GYPGNPGPAGAAGAPGPQGAVGPAGK	949–974					
	+ 2 Hpro		63.31	74.20	65.44	70.27	76.47
	+ 3 Hpro		65.50	76.37	67.61	82.88	75.54
	PGPAGAAGAPGPQGAVGPAGK	954–974					
	+ 1 Hpro		57.89	48.23	51.82		59.98
	+ 2 Hpro		61.39	67.05	60.37	63.22	61.06
	GEVGPAGSPGPSGSPGQR	352–369					
	+ 2 Hpro		51.76	57.04			54.05
	GVAGEPG**R**DGVPGGPGLR	520–537					
	+ 2 Hpro		40.98	42.81	39.54	39.81	40.43
	+ 3 Hpro		40.82	42.65	43.86	40.22	43.83
	GDSGAPGE**R**GPPGAVGPSGPR	676–696					
	+ 2 Hpro		42.37	51.10	50.62	41.45	47.46
	GPPGAVGPSGPR	685–696					
	+ 1 Hpro		39.00	39.76	37.97	41.53	
	+ 2 Hpro		25.83			41.45	
COL3A1: F1RYI8_PIG	GAPGE**K**GEGGPPGIAGQPGGTGPPGPPGPQGVK	829–861					
	+ 4 Hpro		32.24	20.28	40.95		
Sus scrofa	+ 5 Hpro		17.36		32.64	18.25	
	+ 6 Hpro		27.57	19.91	31.68		
RefSeq: NP_001230226.1	GEGGPPGIAGQPGGTGPPGPPGPQGV**K**GER	835–864					
	+ 4 Hpro		35.04		33.69		
	+ 5 Hpro					25.78	
	GEGGPPGIAGQPGGTGPPGPPGPQGVK	835–861					
	+ 3 Hpro		62.51	64.77	64.68		
	+ 4 Hpro		34.83	33.35	39.03	26.88	
	GSPGPQGPPGAPGPGGISGITGAR	934–957					
	+ 2 Hpro			65.66	63.52	56.79	68.32
	+ 3 Hpro		58.40	65.56	60.57	39.16	56.83
	NGD**R**GETGPAGPAGAPGPAGSR	1062–1083					
	+ 1 Hpro + 1 deam		46.17	29.06	55.25	15.67	56.69
	Total # peptides		**25**	**22**	**25**	**21**	**16**

4.3. Characterization of the egg adhesive

Egg white is composed of the proteins ovalbumin (50%), ovotranferrin (12–13%) and lysozyme (3%) [38,39], while egg yolk's primary protein components are the proteins vitellogenins (I, II, and III), with vitellogenin II being the most abundant [40]. The results of both pig and cow standards containing egg white or yolk adhesive (Table S3) identified the corresponding egg white proteins and egg yolk proteins. Overall, proteomic analysis of the standards demonstrated that the presence of vitellogenins in a sample indicates the use of egg yolk, whereas the presence of ovalbumin, ovotransferrin, and lysozyme, without any vitellogenins, indicates the use of egg white alone. Ovomucoid (11% of total egg white protein) was also detected with 61% coverage and 16 peptides in the cow intestine egg white standard, but with only 3% coverage and 1 peptide in the pig peritoneum egg white standard. In addition, standards with egg yolk adhesive contained small quantities of egg white proteins (SI Files S1 and S2 and Table S3 for quantitative data), most likely resulting from incomplete separation during the preparation of the adhesive. The capabilities of quantitative bioinformatics analysis to distinguish between yolk and whole egg based on the levels of egg white proteins has yet to be examined. In addition to detecting these characteristic egg proteins, the egg containing standards did not experience any reduction in collagen or tissue protein signal (Fig. 2).

4.4. Analysis of a 14th century membrane metal thread

The proteomics methodology was applied to the analysis of a membrane metal thread from a 14th century Italian textile and the results searched against the entire Mammalia protein database (SI File S4). Several proteins specific to smooth muscle tissue were detected in the ancient sample, including MYH11 and desmin (Tables S4 and S5). The normalized relative abundances of collagen and muscle proteins are shown in Fig. 2. Overall, the presence of multiple proteins found only in muscle tissue suggest that the membrane may be from intestine or stomach, both of which have a muscular layer and are known to have been commonly used in medieval gilt membranes [2,7].

COL1A1, COL1A2, and COLIIIA1 were detected with the highest number of peptides from *Bos taurus* (45, 42, and 38 peptides, respectively). In spite of an average loss in protein coverage of 16% on the main three collagen chains, most bovine markers identified in the reference samples were observed in the ancient sample: seven out of nine of these peptides (e.g., COL1A2 peptide GAP*GAIGAP*GPANGDR [Fig. 3]) were detected in the soluble

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Table 3
Specific collagen peptides identified for the *Bos Taurus* (cow) standards, with peaks scores given in −10lgP.

Protein	Peptide sequence	Position	Standards				14th c. thread Sol.	14th c. thread Ins.
			Untreated	Silver	Silver heat	Silver egg white		
	GAPGAIGAPGPAGANGDR[a,b,c]	674–691						
	+ 2 Hpro + 1 deam		54.48	58.18	55.47	53.06	44.55	16.37
	GAPGAIGAPGPAGANGD**R**GEAGPAGPAGPAGPR[a,b,c]	674–706						
	+ 2 Hpro + 1 deam		63.42	62.63	58.48	68.55	33.75	72.00
COL1A2: CO1A2_BOVIN	SGETGASGPPGFVGEK[a,b,c]	829–844						
	+ 1 Hpro		48.91		46.16			45.48
Bos Taurus	GYPGNAGPVGAAGAPGPQGPVGPVGK[a,b,c]	947–972						
	+ 2 Hpro		78.21	78.88	72.41	80.92	59.44	47.41
RefSeq: NP_776945.1	+ 2 Hpro + 1 deam				51.76			
	AGPVGAAGAPGPQGPVGPVGK[a,b,d]	952–972						
	+ 1 Hpro		61.47		43.40	48.78		
	IGQPGAVGPAGIR[a,b,c,d]	1066–1078			39.13			
	+ 1 deam						36.14	33.59
	+ 1 Hpro		42.92	42.94	38.72	41.03	40.43	37.86
	+ 1 Hpro + 1 deam						32.48	34.05
	+ 2 Hpro						32.15	
COL3A1: Q08E14_BOVIN	GAPGE**K**GEGGPPGAAGPAGGSGPAGPPGPQGVK[a,b,c,d,e]	828–860						
	+ 3 Hpro		80.88	75.98	45.82	68.08	61.29	
	+ 4 Hpro		38.30	36.80				
Bos Taurus	GEGGPPGAAGPAGGSGPAGPPGPQGVK[a,b,c,d,e]	834–860						
	+ 2 Hpro		86.72	84.30	59.22	48.45	39.79	
RefSeq: NP_001070299.1	GEGGPPGAAGPAGGSGPAGPPGPQGV**K**GER[a,b,c,d,e]	834–863						
	+ 3 Hpro		79.56	81.63	46.83	64.53	39.64	
	Total # peptides		**10**	**8**	**11**	**8**	**10**	**7**

Sol.: soluble fraction; Ins.: insoluble fraction.
[a] *Bos taurus*.
[b] *Bos mutus*.
[c] *Bos indicus*.
[d] *Bubalus bubalis*.
[e] *Bison bison bison*.

fraction and five in the insoluble fraction, as shown in Table 3. Peptide IGQPGAVGPAGIR, frequently used to identify bovine by peptide mass fingerprinting because of its two characteristic peaks at *m/z* 1192 (no hydroxyproline) and *m/z* 1208 (one hydroxyproline), was observed in the historic sample with deamidation on the glutamine residue Q. This modification, commonly found in ancient proteins, was not observed in the standard samples. Finally, no egg proteins were detected, suggesting that the membrane was likely prepared without the use of an egg-based adhesive.

5. Conclusions

The analysis of a 14th century thread demonstrates for the first time the capabilities of proteomics for the analysis of ancient membrane metal threads, despite small sample sizes and complex matrices.

In addition, the study was successful in:

1- Finding protein markers to differentiate the type of membrane made from an internal organ. The possibility that animal skins (leather, parchment) were used for metal threads will be further explored, and in particular the susceptibility of each type of membrane to degradation.
2- Identifying collagen species markers independently of the presence of binders and metal. While bovine was clearly identified in the ancient sample, there is, to our knowledge, little information regarding the species used in membrane threads. The methods of fabrication and species used are likely to vary by geographic origin or for other reasons such as stylistic, quality of materials, cost, etc. Knowledge of the species could have important implications for determining the provenance of the threads.
3- Identifying egg-based adhesives alongside the membrane proteins. The complete absence of egg peptides in the ancient sample indicates that such a binder was likely not used. A collagen-based glue, whose proteins would be undistinguishable from the membrane collagens, would require careful separation during sample preparation. Although beyond the scope of this study, the present work will need to be broadened to the analysis of different combinations of membrane + protein binders to best characterize membrane metal threads in ancient textiles.

The proteomics study of membrane metal threads represents a new application to the field of cultural heritage. The expertise and care necessary to make textiles with these threads, as well as the high cost of the materials used (e.g., gold, silk) make them some of the most valuable items of ancient textiles. The implementation of proteomics to determine the choice of membrane type, animal species and use of protein binder, in conjunction with the analysis of the metal layer, will result in a more thorough understanding of the origin and technology of these metal threads.

Funding

Aleksandra K. Popowich was supported by an Alberta Smithsonian Internship Program Scholarship from the Government of Alberta.

Please cite this article in press as: A.K. Popowich, et al., Characterization of membrane metal threads by proteomics and analysis of a 14th c. thread from an Italian textile, Journal of Cultural Heritage (2017), https://doi.org/10.1016/j.culher.2018.03.007

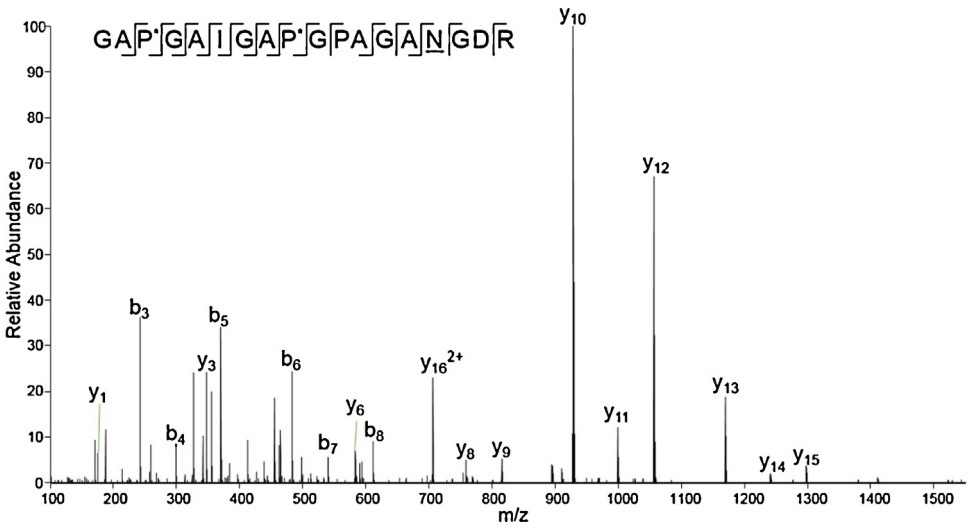

Fig. 3. Spectrum of the unique cow COL1A2 peptide (GAP*GAIGAP*GPANGDR) from the 14th century membrane metal thread. Hydroxylated proline is denoted by * and deamidated asparagine is underlined. Software: PowerPoint 2013.

Disclosure of interest

The authors declare that they have no competing interest.

Acknowledgments

We particularly thank Elisabeth Delvai who provided samples during completion of her master degree at the Institute of Conservation, University of Applied Arts Vienna, as well as the Museum für Geschichte, Universalmuseum Joanneum. The authors also thank Dr. Bernhard Pichler, head of Archaeometry Department, University of Applied Arts Vienna, Dr. Robert Koestler, director of the Museum Conservation Institute and Mary Ballard, textile conservator at MCI for their help in coordinating the project and valuable discussions on metal threads. Finally Dr. Thomas Lam and Asher G. Newsome, MCI, are thanked for their technical support.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.culher.2018.03.007.

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