

The Medallion of Dr Rémy Guillard (1799-1869) Contains Well Epidermis of Napoléon the First

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Abstract : Objective: We report results obtained on mtDNA sequence of the epidermal cells contained in the Guillard medallion. Methods: SEM-EDX analyses and DNA sequence of mtDNA obtained from the epidermal cells were realized. Results: The epidermal cells (keratinocytes) are well conserved. The mtDNA HVS1 sequence obtained from them comprises the 16184 T mutation only, which is highly characteristic of Napoléon the First.

Keywords : Guillard Medallion, SEM-EDX Analysis, Keratinocytes, mtDNA 16184T Mutation, Napoléon the First

In 1840, an expedition starting from Toulon was organized to exhume the remains of Emperor Napoléon the First (who died on May 5, 1821 in Saint Helena), and the surgeon major Rémy-Julien Guillard (1799-1869) embarked on “La Belle Poule” [1]. In Saint Helena, the exhumation had to begin at midnight on October 15, but finally it was in fact around 6 a.m. that the coffin was released and reassembled ; it was 1 p.m. when the last cover gives way.

Docteur Guillard lifts a white veil that reveals the Emperor’s intact body (his faithful companions present recognize him). Putting the veil back on the Emperor’s body, Dr Guillard discreetly recovers a fragment of skin.

In 1936 the Musée des Armées (Paris) received a medallion containing a fragment of “epidermis” of Napoléon. This medallion, named then Guillard medallion [2], is a double-face medallion (Figure 1) of oval form (dimensions 13x18 mm) ; it is constituted of two glasses , lightly bulging, set on two metallic rings. At the anterior face is the piece of epidermis, whitish in colour, that is put down on a black hexagonal sheet, itself stuck up to the paper piece that is loaded on the medallion bottom.

At the posterior face (on the paper) is written on five lines (in French) : Epidermis, of the forehead, of, the Emperor, Napoleon, 1st.

The goal of this publication is, for external samples, to study the metal, the glass and minerals deposited on the glass surface, and for the internal sample to study the epidermal piece for morphology, chemical composition and DNA.

Material and Methods

The metal and the glass of the medallion were scratched with a sterile Gillette blade (in positions of 1 and 2 of the Figure 2, respectively) and then transferred to dedicated sticky papers for SEM observations and analyses. The mineral material on the glass (in position 3 of Figure 2) was directly scotched on a sellotape, that scotch-tape being turned over for SEM observations and analyses.



Figure 1 : Anterior Face (F.A.), above, and posterior face (F.P.), below, of the medallion ; distances are in mm.

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Figure 2 : Sample location seen on the surface of an enlarged view of the anterior face of the medallion. 1 : metal ; 2 : glass ; 3 (on a scotch tape) : on the glass surface.

Samples were then observed and analysed by SEM (Scanning Electron Microscopy) – EDX (Energy Dispersive X-Ray). The observations were conducted by SEM (named SEM-1), using a Philips XL30 instrument (environmental version) ; GSE (Gaseous Secondary Electrons) and BSE (Back Scattering Electrons) procedures were used, the last one to detect heavy elements.

Elemental analysis were realized by X-ray micro-fluorescence, this SEM microscope being equipped with a Bruker AXS energy dispersive X-ray ; the system of analysis is PGT (Spirit Model, of Princeton Gamma Technology). Each elemental analysis is given in the form of a spectrum, with Kilo-electrons / Volts (ke / V) on the abscissa and elemental peak heights (cps / eV) in ordinates.

The epidermis (and cotton) were observed by another SEM (SEM-2) : an Auriga FEG-FIB (Zeiss), analyses being performed on secondary electrons (SE).

The epidermal fragment removed was carefully cleared off the cotton fibers and, in a sterile PCR hood, was decontaminated to avoid any risk of infection by exterior DNA molecules. This decontaminated epidermal fragment was washed several times, and then cut in four approximately equal parts.

DNA extraction for each part of this decontaminated epidermis was conducted using a standard method (0.5 M EDTA, sarcosyl 20% and proteinase K 10 mg/ml) ; the genomic DNA so obtained was purified using a commercial kit (NucleoSpin ® kit ; Macherey-Nagel, Duren, Germany), in accordance with the manufactured instructions.

The mtDNA (mitochondrial DNA) genomic sequence interval of HVS1 from positions 15,991 to 16,390 was amplified by PCR with primers F15,971 and R16,410. For each PCR, the DNA extract for eyebrow specimen was amplified in a 12.5 µl reaction mixture (2 mM MgCl₂, 50 mM KCl, 10 mM Tris/HCl pH=9, 0.1% Triton X-100, 0.2mM of each dNTPs, 0.1 µM of each primer) and 2.5 U of DNA polymerase (Ampli Taq Gold ; Applied Biosystems, Foster City, CA, USA). The amplification was carried out with an initial denaturation step at 95°C for 6 min, followed by 35 cycles at 95°C for 1 min, and 72°C for 1 min.

PCR products were purified from agarose gel (QIAQuick PCR purification kit, Valencia, CA, USA). Both strands of all the amplified mtDNA fragments eluted from agarose gel slices were directly sequenced (Big Dye Terminator Cycle Sequencing kit, Applied Biosystems) and separated (ABJ PRISM3130X1 Genetic Analyser, Applied Biosystems).

The sequences obtained were aligned against the Revised Cambridge Reference Sequence, to identify polymorphic sites. SeqScape software (Applied Biosystems) and Clustal analysis were used for pairwise alignments.

Results

1. External samples

1.1 The metal of the medallion.

One example of scale scratched on the surface of the metallic part of the medallion is illustrated on the SEM (3000X, in BSE) photograph of **Figure 3**. Its EDX analysis shows that it is mainly composed of gold (77.7%) ; it is in fact an alloy of gold, silver (13.3%) and copper (9%).

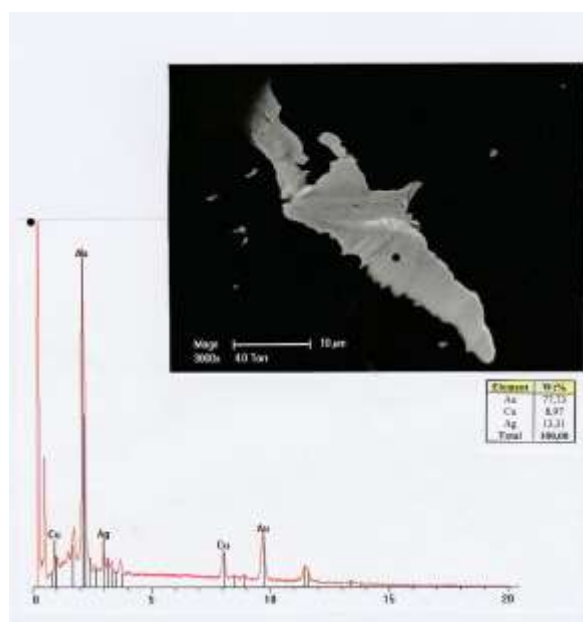


Figure 3 : An example of analysis of a metallic particle of the medallion. *Above* : SEM-1 photograph (3000x, in BSE) of a scale of the metal ; the black point indicates the location where the EDX analysis was realized. *Below* : spectrum at the black point. Cu (two mean peaks) : copper ; Au (two mean peaks) : gold ; Ag : silver. *Insert* : normalized compositions in gold , copper and silver.

Because that the gold content in these sorts of samples are superior to 75%, it is a gold of 18 carats ; in jewel trade, that is considered as pure gold.

1.2. The glass of the medallion.

One example of a typical glass debris scratched on the medallion surface is illustrated on the SEM (2500x, in GSE) photograph of **Figure 4**. It is a flat mini-scale, with a smooth surface and rectilinear and angular outlines. Its EDX analysis shows that it is mainly composed of silicium (64.3%) and oxygen (SiO_2), with calcium (20.6%) and sodium (15.2%).

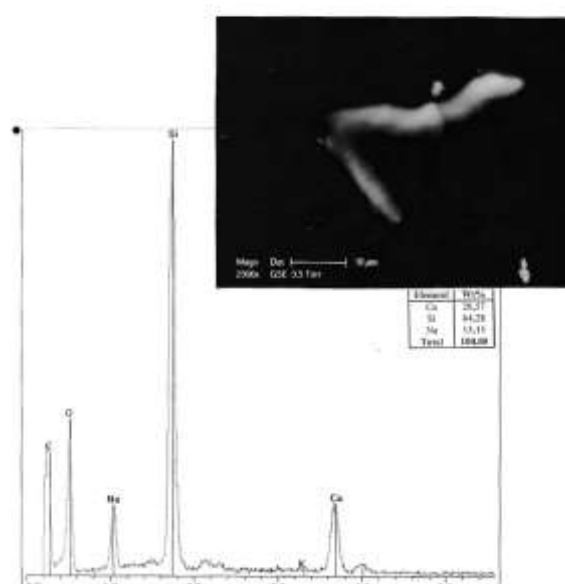


Figure 4 : An example of analysis of a glass particle of the medallion. *Above* : SEM-1 photograph (2500x, in GSE) of a glass particle. *Below* : spectrum at the black point. C : carbon ; O : oxygen ; Na : sodium ; Si : silicium ; K (traces) : potassium ; Ca (two peaks) : calcium . *Insert* : normalized composition in calcium ; silicium and sodium.

All the samples of glass debris tested have a similar composition : that of a calco-sodic glass, with a very little proportion of potassium. This sort of glass was used for flat glasses since in France since the beginning of the Middle-Age.

In this glass, there is no added aluminium : that proves that the silicium component is of pure silica (of a quartz type). Absence of iron (and of zinc, copper, cobalt and manganese) confirms that it is not a coloured glass.

1.2 Mineral deposits at the glass surface.

There are many deposits (traces of glue, minerals, vegetal debris, textile fibers...) on the glass surface. The most important are the traces of glue (**Figure 5**). The most plentiful are the mineral deposits : the upper photograph of **Figure 6** shows an example of a pile of deposited mineral particles. EDX analyses show that most of them are some forms of alumina-silicate, rich in iron and in titanium.



Figure 5 : Examples of traces of glue on the glass. *Upper photograph* : SEM-1 (300x, in BSE) photograph of a glue bubble. *Lower photograph* : SEM-1 (150x, in GSE) of a point of glue. *Below* : spectrum at the circle. C : carbon ; O : oxygen ; Al : aluminium ; S : sulphur ; Cl : chlorine ; Ca : calcium . Elevated contents of carbon and oxygen in the spectrum show that it is an organic glue ; presence of sulphur indicates its animal origin.

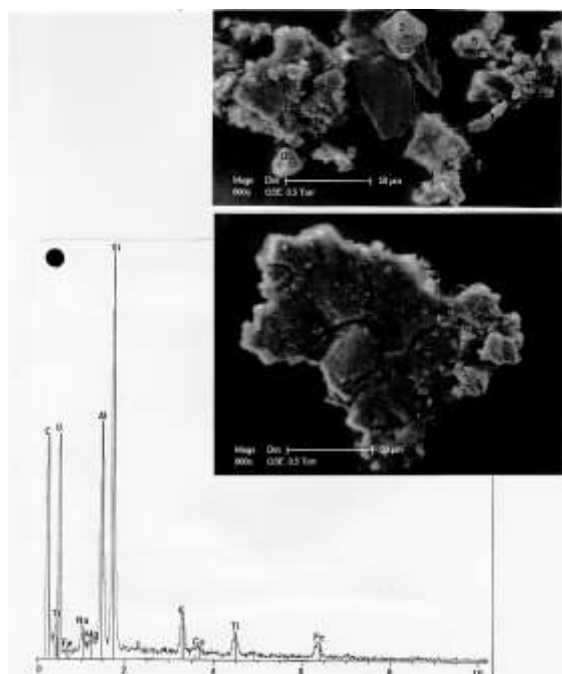


Figure 6 : Some examples of mineral particles loaded on the glass surface of the medallion. *Upper photograph* : SEM-1 photograph (800x, in GSE) of these particles. **O** : an illite ; **1** : illite and montmorillonite mixt ; **2** : titanium ; **3** : gold ; **4** : illite , montmorillonite and kaolinite mixt ; **5** : iron oxide ; **6** : silicate ,

with titanium and iron ; **7** : talcum . *Lower photograph* : SEM-1 photograph (800x, in GSE) of an alumina-silicate micro-conglomerate. *Below* : spectrum at the black point. C : carbon ; Ti (two peaks) : titanium ; Fe (two peaks) : iron ; Na : sodium ; Mg (traces) magnesium ; Al : aluminium ; Si : silicium ; K : potassium ; Ca : calcium.

The lower photograph of figure 6 shows a conglomerate of alumina-silicate particles, for which EDX analysis is given on the bellow of the figure : it is well a compound of alumina (Al_2O_3) silicate (silicium and potassium), with elevated contents of titanium and of iron.

Such mineral particles are characteristic of the lavas of the Saint Helena island [3].

2. The internal sample

The medallion can be easily opened by pressing on the little button of aperture (**Figure 7**). One of us (G.L.) opened the medallion , and took a sample (with a sterile micro-point) a little part of the epidermis ; the location of this micro-sample on the epidermis is indicated on the optical photograph of **Figure 8**.



Figure 7 : Lateral Face (F.L.) of the medallion (height of 4 mm) ; the arrow point indicates the button of aperture of the medallion.

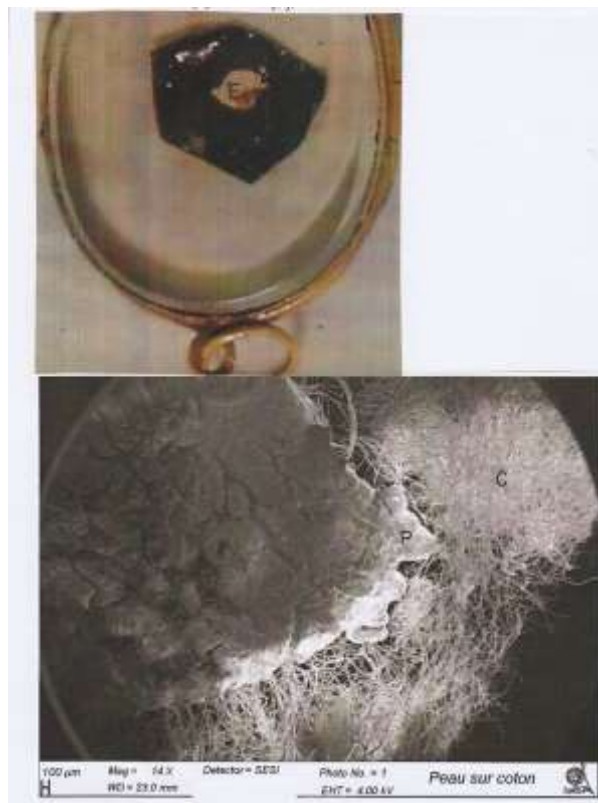


Figure 8 : Above : Enhanced optical photograph of the lower part (transversal view) of the medallion showing the epidermis (E) ; arrow point indicates the location where the micro-sample was made. Below : SEM-2 photograph (14x, in SE) of some part of this micro-sample, located at the limit between cotton (C) and skin (P).

2.1. Epidermal morphology

The SEM (14X, in SE) photograph of figure 8 shows a portion of the micro-sample located at the limit between the epidermal layer and the coating cotton.

The SEM (100x above, and 253x below) photographs of **Figure 9** show, above some part of the cotton tissue, and below an example of a portion of a detached cotton fiber . This fiber portion is one of cotton, because it is s a fine (of about 10 μm of width) ribbon with characteristic twist and regularly placed-alternating along the fiber length. Enlargement of the ribbon hem indicates that they are notably hydrated fibers.

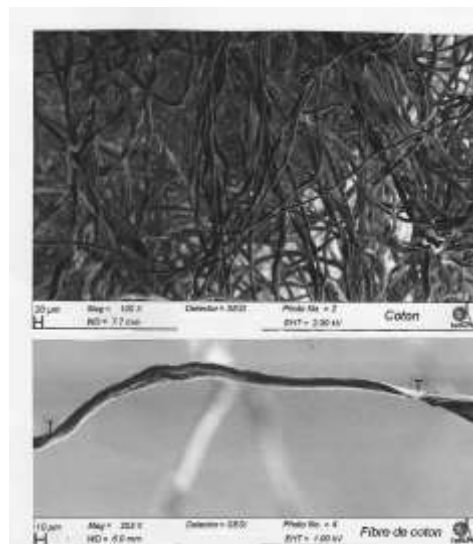


Figure 9 : Above : SEM-2 photograph (100x, in SE) of the cotton. Below : enhanced (253x) SEM-2 photograph of a portion of a cotton fiber (T : twists).

The SEM photograph (24X, in SE) of **Figure 10** is an enlargement of some part of the epidermal portion. Epidermal cells are well visible on the skin layer : they are flat cells of about several hundred μm^2 of surface, well defined from adjacent cells by angular outlines. EDX analysis of these cells (figure 10, below) shows that they are composed of organic matter (carbon, nitrogen, oxygen) only.

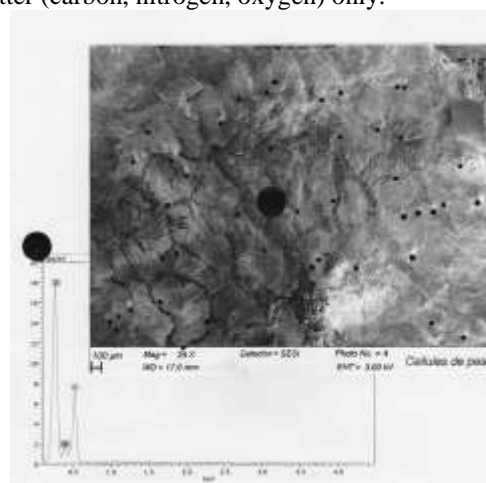


Figure 10 : Above : Enhanced (24x) SEM-2 photograph of epidermis (E) cells ; C : cotton . The little black dot indicates keratinocyte granules . The big black dot indicates the location where the EDX analysis was realized. Below : spectrum at the black dot. C : carbon ; N : nitrogen ; O : oxygen.

2.2. DNA analysis of the epidermis.

A first quarter of the cut epidermal sample was used for DNA extraction and mtDNA sequencing. We found only one mutation in the HVS1 corresponding sequence : in position 16,184, the cytosine (C) is replaced by a thymine (T) ; this transversion 16, 184 C>T , named 16184T, is the HVS1 mtDNA mutation characteristic of Napoléon [4].

Discussion

The writing in the medallion is surely that of Dr Guillard : we have compared (courtesy of J.Macé) the forms of the e, r (terminal) and d letters (in the words “Excellence”, “rappeler” and “épidémie”) of some written letters addressed by Guillard to his minister in 1836 (kept in the military archives of Vincennes), and they are exactly the same than those written in the medallion.

There is no visible hallmark on the surface of the metallic part of the medallion. The date of the 9th of November 1797 is that of the obligatory marking of golden jewels in France ; so the medallion dating is obviously anterior to that date.

Together with the obtained results concerning the glass composition (a calco-sodic glass) it is likely that the medallion is a precious Middle-Age to the Renaissance object (probably initially devoted to religious functions), salvaged by Guillard and then used for conversation and presentation of the Emperor epidermis.

Guillard noted Napoléon “1st” on the medallion paper ; so the use of this object as a container of the Napoléon epidermis dates necessarily from at least 1852 (the date of the beginning of Napoléon’s III reign).

The 1936 gift to the Musée des Armées was carried out by Miss West, who was the authorized representative of Félix Rimbeaux, himself the son of Firmin Rainbeaux, who was squire of Napoléon III. Except for the medallion, it consists (courtesy of D. Guillet) of four other objects : a piece of fabric which lined with the underneath of Napoléon’s coffin at Saint Helena ; a lead fragment , taken in the coffin at the time of the exhumation ; a ground sample , and dissected flowers collected at the bottom of the vault.

Because the medallion glass is covered with the characteristic alumina-silicate of the ground of Saint Helena, it is possible that it could be contaminated by the ground of this third sample. Another possibility is that Guillard himself, proceeding to some sort of ritual inhumation , had buried the medallion containing the relic of the Emperor epidermis in some sample of the Saint Helena ground.

Traces of glue are well visible, even to the naked eye (see photographs of Figure 1), on the two faces of the medallion glasses. This establish that the written face of the medallion was stuck up to a solid substratum (a cardboard or a piece of wood) , for previous exhibition.

The cotton fibers are probably those of the (non-weaved) textile-named “ouate”- that padded the interior of the last coffin (that containing the Emperor body).

Epidermis cells contained in the medallion are remarkably well conserved (see SEM photographs of figures 8 and 10) ; that it is certainly due to the keratinisation of their membranes. In fact, we observed previously well conserved human keratinocytes / corneocytes in some very ancient relics [5, 6] .

Examination of the morphology of these cells (flat, but thick surfaces ; numerous granules on the cell surface ; jointive cells...) shows that they are more keratinocytes than corneocytes. Consequently they must contain DNA (and mtDNA), because the normal terminal differentiation of epidermal keratinocytes leads to the loss of all cellular organelles (including the nucleus) during the conversion of living cells to corneocytes [7] .

Conclusion

We have studied by SEM-EDX the external parts of the Guillard medallion : its frame is of gold, the glasses are of a calco-sodic glass and there are numerous minerals characteristic of the Saint Helena island ground at the glass surface.

These studies show that the medallion contains numerous epidermal cells (corneocytes) well conserved, packed up by a sort of cotton wool.

Mitochondrial DNA (mtDNA) of these cells corresponds to that of Napoléon the First, as previously characterized.

Acknowledgments

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Conflict of Interest: The authors declare no conflicts of interest.

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