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Ongoing tissue changes in an experimentally mummified human leg

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DOI: <https://doi.org/10.1002/ar.24333>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-179236>

Journal Article

Accepted Version

Originally published at:

Morozova, Irina; Öhrström, Lena M; Eppenberger, Patrick; Bode-Lesniewska, Beata; Gascho, Dominic; Haas, Cordula; Akgül, Gülfirde; Neukamm, Judith; Röthlin, Kim A; Imhof, Alexander; Shved, Natallia; Papageorgopoulou, Christina; Rühli, Frank J (2020). Ongoing tissue changes in an experimentally mummified human leg. *Anatomical Record*, 303(12):3085-3095.

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Ongoing tissue changes in an experimentally mummified human leg

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Running Title: Tissue changes in a mummified human leg

Grant Sponsor Swiss National Science Foundation (SNF): Swiss Mummy Project: Correlative radiological and immunohistochemical-genetic assessment of modern and ancient mummified human tissues. (grant-no. 120662).

Swiss National Science Foundation (SNF): The Canopic Jar Project: interdisciplinary analyses of ancient human soft tissues, (grant no: 162803)

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/ar.24333

Abstract

Artificial mummification has been used since antiquity and is best known from ancient Egypt. Despite ancient Egyptian mummies being studied for several decades, the mummification techniques of that time are not well understood. Modern mummification experiments involving animal and human tissues have contributed additional insights relevant to a broad field of research. In the current study, we present follow-up results of an experiment on artificial mummification, which began in 2009. A human leg was artificially mummified and monitored during almost a year with histological, molecular, and radiological techniques. Since then, it has remained in a dry, natron salt blend for nine years. The current analyses show further progression of dehydration and tissue alterations, as well as DNA degradation, suggesting an ongoing process. Our results add new insights into the mechanisms of tissue mummification. Taking into account that the process is still ongoing, further research is required, including a re-evaluation of the human leg in the future.

Introduction

The scientific study of mummies is of broad interest with impact in various research fields including archeology, physical anthropology, paleogenetics, medicine, and forensics. Mummies are not only fascinating historical and cultural artifacts, but also a valuable source for the study of human evolution and disease (Aufderheide, 2000; Thompson et al., 2013).

Natural mummification can occur under particular environmental conditions, such as in deserts, salty areas, crypts, or moors. Artificial or anthropogenic mummies are known from several cultures and periods, the best known of which are those of ancient Egypt. The ancient Egyptians treated the bodies of the dead, convinced of their need in the afterlife. Despite ancient mummies having been studied for several decades, the mummification techniques of this period are not well understood. The only written sources date back to Herodotus (c. 484 BC – c. 425 BC) (Herodot, 2000) and Diodorus Siculus (c. 90 – c. 30 BC) (Diodorus, 1985), and were written at the end of the Egyptian kingdom. In addition, much of our knowledge about mummification comes from tomb inscriptions and preserved papyri that deal with ritual aspects (Ikram and Dodson, 1998). Still today, there is a broad interest, also in forensics, to gain more knowledge about those techniques. Several experimental mummification studies have been performed with animal models (Garner, 1979; Notman and Aufderheide, 1992; Oh and Shin, 2014), but only a few human mummification studies have been completed so far. A whole body was successfully experimentally mummified by Brier and Wade (Brier and Wade, 1997); however, no continuous radiological, histological, or biochemical analyses have been undertaken during the mummification process.

In 2009, we started an experiment on artificial mummification of a human leg (Panzer et al., 2013; Shved et al., 2014; Papageorgopoulou et al., 2015). The experiment aimed to track tissue

alterations during the mummification process in real time. The focus of the experiment was on the desiccation process in a salt-rich environment, either under natural conditions or in anthropogenic mummification, as exemplified in ancient Egypt. Macroscopic, molecular, histological, and radiological alterations were assessed. At the end of the first 322 days of the experiment, a very advanced state of mummification was found. At the molecular level, continuous degradation of DNA was observed, histologically, the tissues underwent continuous degradation over time, and radiologically, progressive dehydration accompanied by varying tissue alterations were seen. Although certain regions did not fully desiccate, no macroscopic tissue destruction or decomposition was noticed.

In this article, we present the results of a follow-up analysis nine years after the beginning of the original experiment. The leg has remained preserved in a dry, natron salt blend up until now. To identify any further tissue alterations, we have now re-examined the leg using molecular, histological, and radiological methods.

Materials and Methods.

Materials

In the previously performed experiment in May 2009, a left lower leg (with intra-vitam body declaration and ethics committee approval) was amputated 24h postmortem and artificially mummified. The desiccation process was simulated according to the technique reportedly used in ancient Egypt. Seventy kilograms of a blend of natron salts, a naturally occurring mixture of sodium chloride, sodium sulfate, sodium carbonate, and sodium bicarbonate, were used. In

ancient Egypt, natural natron used for the mummification process was mostly collected from the Wadi Natrun desert in present day northern Egypt. Chemically pure components were powdered and dehydrated at 100°C, then mixed according to the formula outlined in Brier and Wade: 54 % NaCl, 16 % Na₂SO₄, 18% Na₂CO₃, 12% NaHCO₃ (Brier and Wade, 1997). Radiological, histological, and molecular analyses were performed repeatedly, documenting the mummification process (Panzer et al., 2013; Shved et al., 2014; Papageorgopoulou et al., 2015). The original experiment was closely monitored for 322 days, after which the leg was mostly mummified and showed a leathery appearance. However, particular areas, such as the thigh and foot, did not completely dehydrate (Fig. 1 a & b). This circumstance could be explained by the climate (i.e. cooler environment, also not as arid as in Egypt) and some lack of ventilation. Since then, the leg has been kept under stable conditions (including humidity and temperature), covered with a blend of natron salts, in a laboratory at the Institute of Evolutionary Medicine. In the current study, similar analyses have been conducted between eight and nine years after the end of the original 322-day experiment.

Methods

Radiological Analyses

Radiological analyses were performed in 2017 at the Forensic Institute of the University of Zurich, using a clinical CT Scanner: Somatom Definition Flash (Siemens Healthcare, Forchheim, Germany) as well as a clinical MRI scanner: 3 Tesla Achieva (Philipp Healthcare, Best, The Netherlands) using an XL Torso coil. The CT scan parameters were as follows: 120 kVp (tube voltage) and 860 mAs (tube current) reconstructed with a slice thickness of 0.6 mm (increment: 0.4 mm) in an adjusted field of view. The MRI examination included T1-weighted sequences

(TR: 636.2 and TE: 6.9) and T2-weighted sequences (TR: 4141.8 and TE: 60.0) with a slice thickness of 5 mm. Fifty slices in transversal orientation were acquired in both weightings for the distal part and the proximal part of the leg. IR sequences were not performed for comparison with Panzer et al., since their selected inversion time (TI) was not indicated. Additionally, ultra-short echo (UTE) sequences using vendor specific software patches were also deemed unnecessary with sufficient signal contrast in the T1-weighted and T2-weighted images. Furthermore, Panzer et al. did not evaluate their UTE sequences.

The resulting images were qualitatively analyzed, and the main tissue-types, that is skin, muscle tissue, compact bone, and cartilage, were quantitatively evaluated as was performed during the original study (Panzer et al., 2013). Dedicated software (OsiriX–MD 64 bit, version 8.0.1; Pixmeo, Switzerland) was used for image evaluation. Hounsfield units (HU) for CT and signal intensity (SI) for MR were measured within defined regions of interest (ROIs) by two individual readers (LO and PE), both physicians with several years of experience in paleoradiological research. For each tissue-category, three measurements were taken by each reader; mean values were then calculated for every three corresponding measurements. Following the original study, all soft tissue structures - apart from cartilage - were analyzed at the level of the middle third of the lower leg. The cartilage was analyzed at the level of the patella (Fig. 2). Care was taken to reproduce ROI size and placement as close to the original publication as possible. Where possible, results were then compared to the findings of the original study.

Histological analyses

Two small samples for histological analyses were taken at the distal third of the medial lower leg, punch biopsy (internal No. 100), as well as in the mid of the lower leg, medial (internal No. 104). Histological analyses were performed at the Institute of Pathology and Molecular Pathology of the University Hospital, Zurich, Switzerland. The samples were rehydrated, fixed by immersion in buffered 4% formalin and embedded in paraffin. Two μm thick sections were cut and stained with hematoxylin and eosin (HE) and Elastica-van-Gieson (EvG) according to standard routine procedures.

Molecular analyses

The samples from previous stages of the experiment were kept at -20°C since the beginning of the experiment until the present time. The new samples (muscle and skin) were taken in October 2017 from the mid-calf sampling area as previously described (Shved et al., 2014).

DNA extraction: DNA was extracted from muscle and skin samples from the following time points: Stage 0 (beginning of the experiment; M0 and S0, respectively), Stage I (end of the first stage of the experiment; MI, SI), Stage II (current status, eight years after the beginning of the experiment, MII, SII). Therefore, DNA from seven samples in total (six experimental plus a negative control) was extracted using the same method as described in Shved et al., 2014 using QIAamp DNA Mini Kit (Qiagen) following the manufacturer's protocol. 23-31 mg of tissue was used for extraction. In the end, DNA was dissolved in 60 μl of elution buffer. DNA quantity was assessed using Qubit fluorometer (Thermo Fisher Scientific).

DNA fragmentation analysis: DNA fragmentation in samples from Stage II was assessed, first, via gel-electrophoresis (1 µl of DNA extract in 0.8% agarose gel), second, using Agilent 2200 TapeStation System (with High Sensitivity D1000 Screen Tapes).

Autosomal STR analysis: STR analysis was performed at the Zurich Institute of Forensic Medicine, University of Zurich. DNA extracts from Stage II samples were amplified using the AmpFLSTR™ NGM Select™ PCR Amplification Kit (Thermofisher) according to the manufacturers' instructions. Thirty amplification cycles were run, respectively. PCR products were separated and detected with a Genetic Analyzer 3130xl. Amplicons of the STR loci were up to 450 bp in length. Raw data were analyzed with the Genemapper ID-X Software Version 1.4 (Thermofisher). A threshold of 50 RFUs was used for peak detection.

Next Generation Sequencing (NGS) analysis: To assess the changing of DNA damage profile during the eight years, the skin and muscle samples from Stage 0, Stage I, and Stage II were analyzed using high-throughput sequencing. The library preparation was performed according to the protocols for ancient DNA (Meyer and Kircher, 2010; Kircher et al., 2012). Since the DNA concentrations in samples M0, S0, SI, and SII were relatively high in comparison to other samples (Table 1), the 1:10 dilutions were used to make libraries from these samples; for other samples, non-diluted DNA was used. Before the library preparation, DNA was sheared by ultrasound using a Covaris sonicator according to the manufacturer protocol. The resulting fragments were about 300 bp in length. The sequencing was performed at the Functional Genomic Centre Zurich, Switzerland, on Illumina HighSeq4000 with 2*150+8+8 cycles. The sequencing data were analyzed using EAGER v.1.92.33 software (Peltzer et al., 2016).

Bacteriology

Standard bacteriological analyses of one muscle sample (middle lower leg, fronto-laterally; punch biopsy) was performed at the Hospital Langenthal, Switzerland.

pH measurements and temperature

Measurement of pH was done with indicator strips (universal indicator 0-14; Merck, kGaA, Darmstadt, Germany) on the molecular samples (muscle and skin), which were taken from the mid-calf sampling area. The temperature was measured with an electronic digital thermometer "MULTI", Huberlab, Switzerland. Circumstance conditions were stable with an external temperature of 22°C and a relative air humidity of 56%.

Results

Macroscopically, the leg has changed in size, however the appearance in terms of color and consistence did not significantly change since the end of the first phase of the experiment after 322 days. While most parts are now fully mummified, the thigh and foot in particular still feature certain areas that are not entirely dehydrated. However, no signs of decomposition of the sample were noted in any area (Fig. 1). Over all, the leg lost a substantial amount of weight over the past 9 years. While the leg initially weighed 6 kg, and the weight dropped to 3.5 kg after the first 208 days of the experiment; currently, the leg weighs 2.4 kg. Thus, the weight of the leg dropped to 58% of its initial value by the end of the first phase of the experiment and has since decreased further by 31.5%, now only weighing 40% of its initial weight.

pH-Measurements

The pH values did not change significantly over the past nine years. The current measurement indicated an alkaline pH of 9 for the muscle sample and a slightly higher pH of 10 for the skin sample. At the beginning of the experiment, the pH values of the fresh tissues were all in the neutral range of 6-7, and then, with some initial fluctuations, increased steadily during mummification in the alkaline natron salt blend throughout the first phase of the experiment (final values: pH of 9 for muscle and pH of 10 for skin).

Radiology

Overall, the radiological examinations show further tissue shrinkage (Fig. 3). Interpretation of anatomical structures is mostly complicated by additional air inclusions and further tissue-contractions. Particularly, the differentiation of myofascial structures presented some difficulties. In addition, several hyper-dense areas (approx. 700 HU) of unclear origin, measuring up to max 10x20mm, were identified in muscles and soft tissues on the CT scans. The sampling areas, i.e. the places where earlier samples had been regularly taken, also altered the surface structure over time in certain cases. On MRI-scans, distinguishing between different tissues, especially between different muscles or muscle groups, was even more difficult.

CT-Measurements:

In the following, the HU values from our ROI measurements were compared with the HU values originally published by Panzer et al.

Cutis: Mean HU is 639 (606 and 672 (interpreter 1 and interpreter 2, respectively)). The value decreased further from the end value of 737 on day 60, which is congruent with the trend seen before.

Subcutis: Mean HU value is -52 (-56 and -48 (interpreter 1 and interpreter 2)). The value increased further from -78 on day 60, which is also similar to the trend seen before.

Cartilage: Mean HU value is 377 (interpreter 1:272; interpreter 2: 483). The value decreased from end value of 340 on day 60, which is counter to the trend seen before. However, significant interobserver differences were noted.

Compact bone: Mean HU value is 1630 (interpreter 1: 1567; interpreter 2: 1693). The value decreased from the final value of 1760, which is counter to the trend seen before.

Muscles: The mean HU values vary widely, ranging from 95 – 140. The mean value of all measured muscles is 132 and 199 (interpreter 1 and interpreter 2, respectively). The value decreased from the final mean value of 223, which is clearly counter to the trend seen before.

Magnetic Resonance Imaging (MRI) Measurements:

For a qualitative comparison, we repeated the approach of Panzer et al., according to which, over the course of the experiment, signal intensities of different anatomical structures were measured across several MRI scans. The SI measurements differ widely across tissue as well as between observers. However, since SI values in standard T1- or T2-weighted MRI constitute qualitative values, they cannot be reliably compared between different acquisitions (Bergeest and Jäger, 2008). Therefore, only a qualitative assessment could be conducted:

Tissues are still identifiable, while being further shrunken and show more air inclusion.

However, the exact identification of various muscles is not guaranteed. In conclusion, the MR images are comparable to other examples of artificial and natural mummifications.

Histology

The microscopical appearance of the tissue samples after nine years are quite similar to the findings at the end of the previous stage of the experiment, on day 208 (Papageorgopoulou et al., 2015), suggesting constant preservation of the histological details by the mummification process during this period. The fibers of the skeletal muscles are well preserved, even after 9 years (Fig. 4, a & b). The nuclei cannot be seen; however, the striation of the skeletal muscle cytoplasm can still be identified. The supporting soft tissue consists of necrotic adipocytes, collagen, and blood vessels. The microscopical appearance of the skin after 9 years reflects the changes described at day 208 of mummification (Fig. 4, c & d). The epidermis shows variable preservation of the stratification and variable planes of detachment, either within the stratum corneum or deeper epidermal layers. Hardly any nuclear details, of both epidermis or papillary dermis can be observed. Interestingly, the structure of some larger blood vessels (vein in Fig. 4, e & f) is remarkably well preserved, allowing for the identification of the vascular wall layers and showing the staining of the nuclei.

Molecular analyses

DNA concentration: The concentration of DNA extracted from samples from three different time points (Stages 0, I, and II) demonstrated different patterns for skin and muscle tissues. For skin samples, after eight years of mummification DNA concentration stayed unchanged (Table 1). At

the same time, there is a significant decrease in DNA quantity in muscle samples: starting from the same concentration as for the skin sample at Stage 0 (89.9 and 89.7 $\mu\text{g/ml}$, see Table 1), the DNA quantity decreased to 5% after nearly one year of mummification and decreased to 0.4% during the next seven years (Table 1). This confirms the previous observation of Shved et al. (Shved et al., 2014) about more intensive DNA damage in muscle tissues compared to skin.

DNA fragmentation: Gel electrophoresis analysis shows a strong DNA smearing at Stage II, the pattern which was previously observed at the end of the first stage of the experiment (Shved et al., 2014). TapeStation analysis revealed peaks of fragment sizes of about 1,500-2,000 bp (taking into account the resolution of the assay) (Fig. 5). Therefore, the average fragment lengths decreased from 8,000 bp for skin and 2,000-4,000 bp for muscle (Shved et al., 2014) to 1,500-2,000 bp in both tissues (Fig. 5), although the longer fragments still can be observed, but in much smaller amounts (Fig. 5).

It is worth noting that muscle DNA degraded to a greater degree than skin DNA. This is seen on the gel electrophorogram where muscle DNA is literally invisible (Fig. 5). It was previously observed that DNA fragmentation began earlier in muscle samples (Shved et al., 2014). This trend continued, as muscle DNA degraded more rapidly.

It is clear that the process of DNA degradation continues in skin: on the TapeStation electrophorogram, a long smear is visible, which demonstrates that DNA fragments with lengths up to 100 bp are present in the extract.

STR analysis: STR analysis demonstrated no allelic dropout of STR markers up to 350 bp (Table 2), which is expected, given the results of the fragmentation assessment. The STR profiles of the

samples from the first two stages of the experiment and the current stage are identical, confirming the absence of contamination during the present round of sampling and consequent molecular analysis.

NGS analysis - DNA damage: Six DNA samples analyzed by NGS (skin and muscle taken on Stage 0, Stage I, and Stage II) demonstrated the same whole-genome coverage (Suppl. Table 1). DNA damage profiles for skin and muscle samples showed different patterns. Both groups demonstrated a tendency of increasing C>T changes at the ends of DNA fragments with time (Fig. 6), which is a distinctive criteria for characterizing ancient DNA (Hofreiter et al., 2001; Gilbert et al., 2003). However, for muscle samples this tendency is more pronounced: the average frequency of C>T changes in the last seven nucleotides increased only 1.1x between Stage 0 and Stage I in the skin samples, while for the muscle samples, the misincorporation frequency increased nearly 2x (Suppl. Table 2). This process is still ongoing: the number of C>T changes at the ends of DNA fragments increased 1.4x in skin samples and 2.5x in muscle samples during the following eight years of mummification (Suppl. Table 2). It should be noticed that these numbers can be underestimated. C>T misincorporations occur more often in single-stranded overhangs arising as the results of DNA degradation *post mortem* (Hofreiter et al., 2001; Gilbert et al., 2003). In our case, as it was shown above, the average length of DNA fragments was about 1,500-2,000 bp. To prepare the DNA fragments for high-throughput sequencing, DNA was sheared artificially. Therefore, not only were the DNA ends resulting in natural DNA degradation analyzed, but also the ends derived during the sonication and therefore do not possess DNA overhangs. Nevertheless, based on the observed tendency, one

can expect further DNA fragmentation and more pronounced damage patterns in the samples with time.

Bacteriology

The analyzed muscle sample did not show any bacterial or fungal growth.

Discussion

Macroscopically, the mummification status of the human leg did not substantially change over the years. Although certain areas did not yet fully dehydrate, no signs of putrefaction are observable, and the experimentally mummified leg remains in a stable condition.

However, the weight further decreased significantly, which indicates substantial ongoing dehydration. The pH value remained stable with a slightly alkaline value. The surrounding natron salt blend may explain this. As a result of this, the alkaline environment is of significance for molecular analyses since DNA can be better preserved in such a setting. The absence of bacteria and fungi also indicates a stable environment without ongoing putrefaction.

Several years after the end of the first stage of the experiment, DNA degradation is still ongoing, although at a slower pace compared to the first year of mummification. DNA degradation is much more pronounced in muscle tissues in comparison to the skin. Therefore, we observed the DNA in skin to be more resistant to environmental influences. As we demonstrated in our experiment with artificially mummified pig organs (Öhrström et al., submitted), DNA preservation positively correlates with tissue density. Moreover, the upper layers of the

mummified leg dried out quicker during the natron treatment in comparison to the deeper layers, which likely protected DNA in the skin from hydrolysis and oxidation and, as a result, from chemical transformation. Based on the results of our experiment, we recommend considering mummified skin samples as a valuable resource for ancient DNA work since it may yield higher quantity and better quality DNA in comparison to muscle samples. Since skin is directly exposed to the environment, it should be noted that these tissues often have been contaminated over the years. However, it is essential to remember that work with ancient skin samples requires strict precautions to avoid modern contamination.

All tissues are preserved and identifiable on CT and MRI scans, however further altered in comparison to the initial publication of Panzer et al., mainly showing more tissue-shrinkage and more air inclusions. The appearance now resembles ancient examples of artificially or naturally mummified human remains.

The CT measurements demonstrated an increase in HU in the subcutis, and a decrease in the cutis, cartilage, and compact bone. The increase of HU values in the cutis is a common phenomenon, as reported by other radiological investigations of mummified tissues. A likely explanation is direct contact with the natron salt blend and therefore, a more significant dehydration effect region. Whereas the change of HU was consistent with the trend seen before regarding the cutis and subcutis, the values for cartilage and compact bone instead demonstrate an opposite trend.

Compared to the histological examination at the end of the first phase of the experiment, both muscle and skin show a similar spectrum of microscopic alterations, but there is some evidence

of an increased level of decay. In particular, the structure of the epidermis is further altered and partially destroyed, while the subcutis and dermis appear similar in terms of preservation. The muscle tissue can be clearly identified and its anatomical features described, but in comparison, the structure is clearly altered, and no cell nuclei are visible. In some structures, such as the medium-sized vein examined, microscopic features are remarkably well preserved also after nine years of mummification.

Conclusion

This follow-up study shows that the experimentally mummified human leg has remained in a mummified condition over several years, and although it did still not completely dehydrate, no decomposition occurred. However, the tissue structure has further altered in the meantime on a molecular, radiological as well as on a histological level, and even several years after the end of the mummification experiment, the process is likely still ongoing. As a result of this, our experimental setup simulates an essential step of artificial as well as certain natural mummification processes, namely dehydration. While we could show that putrefaction was successfully inhibited, the dehydration process did surprisingly not cease, even after several years. This observance may be explained by the laboratory environment with relatively high level of humidity. Furthermore the ancient Egyptians might have periodically exchanged the natron, which was not the case in our experiment. Based on the results of our experiment, mummified skin samples may constitute a valuable resource for ancient DNA since it can yield higher quantity and better quality DNA in comparison to other soft tissue samples, if contamination can be avoided.

Acknowledgement

The authors thank Prof. Dr. med. Michael Thali (Institute of Forensic Medicine, University of Zurich) for approving the CT and MR scans and the Balgrist University Hospital for performing the previous CT and MR Scans and Abigail Breidenstein, Institute of Evolutionary Medicine, University of Zurich, for proofreading the manuscript.

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Tables

Table 1: Concentration ($\mu\text{g/ml}$) of DNA extracted from muscle and skin samples during three stages of the mummification experiment.

Table 2: Results of STR analysis.

Figure legends

Figure 1: Photo of the human lower leg, a) day 0 (co Heinz Sonderegger); b) day 208 (co Heinz Sonderegger); c) now, after 9 years (co Lena Öhrström)

Figure 2: Location of the radiology measurements, illustrated on CT. a) axial slice at mid lower leg; 1) Tibialis anterior muscle 2) Tibialis posterior muscle 3) Soleus muscle 4) Peroneus muscle 5) Compact bone 6) Subcutis 7) Cutis; T: tibia, F: fibula. b) slice location (violet line). c) axial slice at the knee level, for cartilage measurements (red circle), P: patella. d) slice location (violet line).

Figure 3: Radiological alterations over time at selected dates (start of experiment - left column; end stage of first experiment- middle column; and after 8-9 years - right column. The alterations are presented on CT (axial view) at the level of the patella (a-c) and the mid lower leg (d-f) as well as on T2 weighted MRI (axial view) at the corresponding mid lower leg (g-i). Note the augmentation of density in the cutis on CT. On MRI, the tissue differentiation becomes more and more vague.

Figure 4: Histological analyses:

Skeletal Muscle in (a) HE and (b) EvG staining. The muscle fibers are well-preserved, however no nuclei can be seen. The stratification of the cytoplasm is well visible (red rectangle). The supporting soft tissue (asterisks) consists of small blood vessels, necrotic fatty tissue ,and collagen. (Original magnification 400x).

Skin samples in the HE staining (c/d) demonstrate variable quality of the epidermis with some areas showing better preservation of the squamous stratification and intact basal layers seated on the basal membrane (white arrow) (c), while hardly recognizable stratification in others (d).

There are variable areas of epidermal detachment, either in the stratum corneum (black arrow in panel c) or in basal layers (red arrow in panel d). (Original magnification 400x).

A well-preserved vein in (e) HE and (f) EvG stains. In the HE stain, some nuclei of the smooth muscle cells in the tunica media of the vessel wall can be seen (white arrows). The structure of the vessel wall is well recognizable in the EvG stain (f) including smooth muscle (red arrow) and elastic fibers (black arrow). (Original magnification 400x).

Figure 5: Analysis of DNA fragmentation at three main stages of the experiment. A) PCR amplification analysis at Stages 0-I (Shved et al., 2014) and TapeStation analysis at Stage II; B) Gel electrophoresis of genomic DNA at Stage II (M – Muscle; S – skin; L – ladder (kB)).

Figure 6: Frequencies of 5'C>T changes in NGS reads.

	Stage 0	Stage I	Stage II
Muscle	89.7	4.51	0.359
Skin	89.9	76.2	88.6

Table 1: Concentration ($\mu\text{g/ml}$) of DNA extracted from muscle and skin samples during three stages of the mummification experiment.

Marker	Muscle			Skin			Size Allele 1	Size Allele 2
	Stage 0	Stage I	Stage II	Stage 0	Stage I	Stage II		
vWA	16	16	16	16	16	16	171.5	
D16S539	11, 12	11, 12	11, 12	11, 12	11, 12	11, 12	251.5	255.4
D2S1338	17, 25	17, 25	17, 25	17, 25	17, 25	17, 25	295.8	328.2
AMEL	X	X	X	X	X	X	98.6	
D8S1179	13	13	13	13	13	13	142.5	
D21S11	30, 31	30, 31	30, 31	30, 31	30, 31	30, 31	207.1	211.0
D18S51	15	15	15	15	15	15	293.2	
D19S433	13, 14	13, 14	13, 14	13, 14	13, 14	13, 14	141.2	145.2
TH01	7	7	7	7	7	7	191.1	
FGA	24, 25	24, 25	24, 25	24, 25	24, 25	24, 25	259.0	262.8
D3S1358	15, 18	15, 18	15, 18	15, 18	15, 18	15, 18	145.4	157.6

Table 2: Results of STR analysis