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Titelpage

Title

Histology and Raman spectroscopy of limed human remains from the Rwandan Genocide

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Abstract

The Murambi Genocide Memorial Centre is one of the major centres in Rwanda that commemorate the 1994 Rwandan genocide. Seventeen months after the genocide, about 1000 excavated human remains were put on display in Murambi Technical School. Repeated efforts were made to desiccate the human remains with lime for educational reasons. The aim of this study was to assess their state of preservation and understand the extent of degradation of the tissue. Limed soft tissue samples from four individuals were examined with light and electron microscopy, and subjected to histological analysis. Raman spectroscopy at 785 nm and 1064 nm provided information about the impact of environmental conditions on the extent of deterioration to these samples, the presence of organics and the conversion of the associated lime from calcium hydroxide to calcium carbonate. While visual degradation of the bodies in Murambi has been reported, this study confirms deterioration at a microscopic and molecular level. Both histology and Raman spectroscopic analysis revealed that the limed bodies in Murambi were deteriorating at the time the samples were collected. The results of this study will inform future decisions regarding the long-term conservation of those human remains.

Keywords

Rwandan genocide; Histology; Raman spectroscopy; Limed human remains; Desiccation; Artificial mummification

1. Introduction

When forensic specialists investigate human remains, the aims of the investigation are often of medico-legal significance. In other cases, however, an interdisciplinary team of scientists can be asked for a conservation assessment of preserved human remains. These so called 'mummies' are often encountered in both bioarchaeological and forensic contexts, either preserved by natural processes or by artificial anthropogenic methods. This study presents the analysis of artificially preserved, limed human remains from the Rwandan Genocide (1994).

On the 21st April 1994 a large-scale massacre took place in Murambi Technical School near the town of Gikongoro in the southwest of Rwanda. Whilst thousands of Tutsi were sheltering in the technical school, Hutu militiamen and soldiers went from building to building killing between 20 000 to 70 000 individuals [1]. The next day, the perpetrators buried the deceased in several mass graves [1-3]. In 1995 and 1996, about 20 000 bodies were exhumed by the Amagaju local association of survivors and the Murambi Initiative Committee for the Burial of Victims (CIEM - Comité d'Initiative pour l'Enterrement des victimes de Murambi). About 1000 of the human remains that were not claimed by surviving relatives were desiccated with lime and put on display to show the world what happened [1, 3-6]. Nowadays the Murambi Genocide Memorial Centre is one of the major centres in Rwanda that commemorate the Rwandan genocide. The former school is laid out in blocks of classrooms. Some contain piles of skulls and skeletal remains, ordered per bone. Other rooms display human remains with surviving soft tissue, preserved by several layers of lime (Fig. 1). Traces of violence on the displayed individuals can be clearly observed.

The use of lime in this context is perhaps understandable, since lime has often been used as a disinfectant in burials associated with conflict, such as World War I [7, 8], World War II [9, 10], the Spanish Civil War [11-14], the Portuguese Colonial War [15] and the conflicts in the former Yugoslavia [16]. In the case of Murambi, the lime was not added to the burial. Once exhumed, lime was deliberately applied to the human remains, attempting to halt further decay and preserve the bodies. Over the years, the human remains had been re-limed several times, although none of the Rwandans the authors spoke to, could remember how many times. Neither could they confirm if quicklime or hydrated lime was used.

Lime, derived from limestone (calcium carbonate CaCO_3), is a generic term used to cover calcium oxide (CaO - variously known as quicklime, unslaked lime or burnt lime) and calcium hydroxide (Ca(OH)_2 - variously known as hydrated lime or slaked lime). Calcium oxide is hygroscopic. It absorbs atmospheric moisture rapidly and reacts with water in a process of hydration to form calcium hydroxide [17]. Lime has an alkaline pH (pH 12-14) which limits bacterial proliferation, hence its use as a disinfectant with the assumption that the application of lime to corpses might preserve them.

However, Schotsmans et al. [17-19] showed that lime, when deposited in the direct depositional environment, does not stop decomposition but only retards the rate of decay. Since lime is only applied to the outside of a body, putrefactive decay can continue from within by micro-organisms derived mainly from the body itself, especially the abdominal cavity. Bacterial populations on the exterior of the carcass are reduced by the lime, but not eliminated. A superficial desiccation takes place, but the surface area to volume ratio is too large to desiccate a full body with internal organs and their associated host bacterial community. The end result is skeletonisation [17-19].

Indeed, despite efforts to preserve the remains with several layers of lime at the Murambi Technical School, it did not stop them from deteriorating. For this reason The National Commission for the Fight against Genocide (CNLG – la Commission Nationale de Lute contre le Genocide), a government appointed Rwandan organisation responsible for the preservation of all genocide sites throughout Rwanda, requested Inforce Foundation and the Cranfield Forensic Institute to assess the remains and the environmental conditions in which they were displayed. In addition to local assessments of the premises, permission was given by the Rwandan government and The National Commission for the Fight against Genocide to analyse four lime samples and twelve small tissue samples in the United Kingdom (Table 1).

In order to assess these sixteen samples, it was decided to conduct a microscopic and molecular analysis. The technique of histology has been used for more than a century throughout the world forming the basis of modern day pathology, but also for the examination of ancient tissues, known as 'palaeohistology'. Whereas modern histology is a diagnostic tool, different information is sought from the histological examination of ancient tissues, such as assessing the state of preservation or detecting diseases [20]. To investigate molecular changes, Raman spectroscopy has been used successfully in archaeology to demonstrate the molecular deterioration suffered by biomaterials and skeletal remains under a variety of different environmental conditions at different [21-25].

The aim of this study was to understand the extent of degradation of the tissue and their state of preservation in order to advise the Rwandan government about the long-term conservation of the Murambi human remains.

2. Materials

Four different human remains, displayed at the Murambi Technical School, were sampled in 2010, sixteen years after death, following a specific sampling strategy adhering to the following criteria: respect for the remains, as little destruction accomplished as possible and of sufficient quantity to be scientifically meaningful. An ethics application was approved in July 2011 by the Biomedical, Natural and Physical Sciences Research Ethics panel of the University of Bradford for the analysis of the samples.

Samples RW5 to RW10 were collected from two human remains in room J2-14, and samples RW11 to RW16 from another two human remains in room H2-02. All four individuals were sampled three times: (1) from an area where the soft tissue was flaking, (2) from a region of the body that did not have a disrupted surface and therefore was more intact (3) a phalanx with adherent tissue (Table 1). In addition, four lime samples were collected (RW1-RW4).

All samples were examined with light and electron microscopy and analysed using Raman spectroscopy. Eleven soft tissue samples (RW5 to RW15) were subjected to histological analysis. RW16 could not be analysed histologically because of the absence of soft tissue.

3. Methods

Light and electron microscopy enabled initial examination of the outside of the samples. Imaging using an FEI Quanta 400 Scanning Electron Microscope (SEM) with secondary electron detector offered detail on the surface morphology of the samples in low vacuum mode. Specimen were not coated and positioned on a stub.

Soft tissue samples of roughly 1x1cm in dimensions were processed for histological analysis (Table 1). First, a blue glycerine marker was applied to the outside of the samples to identify the macroscopic external aspect of the tissue, as the outside is not always recognisable in degraded tissue. Mummified tissues are often dry and crisp. Therefore the samples have to be rehydrated prior to any histological processing. A comprehensive overview of the various rehydration techniques is summarised by Aufderheide [26] and Mekota & Vermehren [27]. In this study a rehydration recipe, developed by the KNH Centre for Biomedical Egyptology in the Faculty of Life Sciences at the University of Manchester, was used for rehydration. It consisted of 10% formalin in 0.9% saline and 1% sodium lauryl sulphate [20]. The tissue was next processed with a carousel tissue processor (Shandon Citadel) in which it was first dehydrated with numerous changes of alcohol, then cleared with HistoClear II (Agar Scientific) and impregnated with modified paraffin wax (Paraplast Plus – Sigma). Following embedding, sections were cut at 5-6µm thickness with a Leitz 1512 rotary

microtome. Once cut, they were mounted onto pre-coated glass slides (SuperFrost Plus from Menzel-Gläser).

Prior to staining, the wax was removed from the sections by reversing the sequence of the processing reagents; removal of the wax with xylene followed by alcohol and water.

The most appropriate stains were selected in order to collect as much information as possible based on the aim of assessing tissue structure and preservation and detecting the presence of micro-organisms. To demonstrate the overall structure of the tissue a common Hematoxylin and Eosin (H&E) technique was selected based on Luna [28] and the H&E protocol at the University of Manchester used in a previous study [29]. The second stain was an aqueous solution of toluidine blue based on Kiernan [30], Clayden [31] and the toluidine blue protocol from the University of Manchester [29]. This stain is used often in mummified tissue to bring out the contrast between the background and bacteria, staining bacteria dark blue [20]. The third stain consisted of the Gram-technique based on the gram stain protocol at the University of Manchester [29] and the specialist literature [30, 31] in order to distinguish Gram-positive and Gram-negative bacteria.

The slides were visualised using either a Leica RMDB research microscope (Leica Biosystems Peterborough Ltd, Peterborough, UK) with images captured by a Deltapix Infinity digital camera, and an Olympus System Microscope BX51 (Olympus Optical, London) with AnalySIS software (Olympic Soft Imaging System, Munster).

All slides were assessed and scored by two individuals, using a scoring protocol developed for this study. The overall structure of the tissue was rated on a 4 point scale, where a zero score meant no observable changes (tissue is intact; muscle, epithelial, connective or nervous tissues are identifiable and might still contain nuclei). A one score indicated limited changes, with different tissue types still identifiable but showing the onset of degradation. Scoring two meant that significant changes were observed with mainly collagenous fibres and/or connective tissue structures left. Finally, a three score indicated extensive changes in the tissue with almost no structures being identifiable anymore. In addition, other observations were made such as the colour of the stain, the presence of bacteria or artefacts. The results were discussed between the two observers, resulting in a 95% absolute agreement.

FT-Raman spectra were recorded using a Bruker IFS 66 infrared spectrometer with an FRA 106 module attachment and dedicated Raman microscope with Nd³⁺/YAG excitation at 1064 nm. The sample spot size was 100 µm diameter. Spectra were obtained with 500-1000 scans of data accumulation. In addition, spectra were recorded using a Renishaw InVia confocal Raman microscope operating with 785 nm excitation in the near infrared. The spectral data were scanned

for the acquisition of up to 10 accumulations and 10 seconds laser exposure time with a spectral footprint of about 2 μ m (x50 objective lens). Significant peaks were determined using Grams 8.0 software, ThermoFisher Scientific.

4. Results

4.1 Environmental conditions

Rwanda's location just south of the equator brings a humid climate with frequent rain over two rainy seasons. Gikongoro, the closest city to Murambi, has moderate, stable temperatures due to its high elevation with daily temperatures between 14°C and 25°C. Precipitation is present through the year with variation within the months. The most rainfall occurs around April and the least rainfall in around June-July. The average relative humidity varies between 68-85% [32].

At the time the specimen were collected, the school was laid out in blocks of classrooms. Samples were obtained from two rooms with human remains placed on raised wooden platforms. In both rooms living insects were observed due to easy access. Bird's nests and bird droppings were noticed inside the room and on the remains. Water was dripping through the ceiling at several locations and white mould was observed on the plywood ceiling panels. The rooms did not have air-conditioning. The present humidity conditions and ambient temperatures had not favoured the preservation of the displayed human remains.

4.2 Microscopic assessment

SEM observations showed no defined structure of the limed tissue surfaces. Fungal fruiting bodies were detected on one sample (RW5) (Fig. 2).

As often seen with mummified tissue, histological processing appeared to be challenging. The samples were dry, crisp and embrittled. The repeated application of lime had resulted in alteration to the tissue which had become so solid such that the rehydration and fixation solution did not infiltrate well. The tissue samples were hard to cut with the thickness of the section having to be increased to 6 μ m for some of the samples. The most common problem encountered during the staining process was that the stain did not successfully react with the tissue components because they were heavily degraded, leaving it colourless, over or under stained depending on the extent of tissue degradation and collapse of the cells (see for example figures 5a and 6 where the H&E stain is not well differentiated due to degradation, leaving the intrinsic basic brown colour of the tissue). Eight of the eleven samples were processed twice, but this did not yield a better result.

In general, most tissue was in a bad state of preservation (8 out of 11 samples) (Table 1). Although all derived from the same remains, the tissue samples that had been taken from non-disrupted areas

were slightly better preserved than tissue from disrupted, flaked areas or tissue from bone samples (Table 1). The only remaining tissue was connective tissue known to be more resistant to degradation than any other tissue [20, 26, 33]. Muscle or adipose tissue were no longer distinguishable. The most identifiable tissue was the collagen of the dermis (Fig. 3 & 4). The collagen fibres were recognisable but not well preserved. Surviving epidermis could not be noted, as often seen in mummified tissue. The lime layer, which stained darker, had started to infiltrate (Fig. 3). Longitudinal sections of collagen showed vertical lines, which could be mistaken for muscle striations (Fig. 4). However, it was realised that these were cutting artefacts (knife chatter) resulting from the fact that the tissue was hard to cut

Some fascia were still identifiable within degraded tissue (Fig. 5 & 6). Fascia consists of connective tissue that surrounds muscles, bones, nerves, blood vessels and other structures. Fascia are solid and responsible for maintaining structural integrity and providing support and protection. The fascia in the studied tissue still had birefringence properties when viewed using crossed polarising microscopy, but was observed to be degrading (Fig. 5). Figure 6 shows a degrading blood vessel surrounded by degraded tissue with part of the blood vessel showing destruction, with its wall broken.

Bacteria were more often observed in the tissue (Fig. 4, 7 & 8) than at the surface of the tissue (Fig. 3 & 9). Since the bodies from Murambi had been limed several times, it can be argued that the bacterial colony numbers might have been reduced after every liming session. Deeper into the tissue, where the lime had not reached, the tissue was degrading with the presence of mainly Gram-negative bacteria detected (Fig. 10). Bacteria were generally observed in between the collagen fibres, preferring the path of least resistance (Fig. 4 & 8).

Whilst mould was evident in some of the rooms where the human remains were displayed and fungal fruiting bodies were detected on one sample (Fig. 2), no fungal structures were observed in the histological sections. This confirms that a limed environment is not favourable for fungi as shown in previous research with fungal cultures of limed pig experiments [19]. However, this does not mean that fungi were completely absent in the Murambi remains. Fungi could grow on unlimed parts of the bodies or inside the body cavity, with sufficient moisture and oxygen available.

Three samples contained crystalline structures (RW6, RW8 & RW10) (Fig. 11). It is assumed that these are a form of calcium oxalate (CaC_2O_4). Calcium oxalates occur widely in nature and can result from microbial activity. Whewellite and weddellite, the mono- and dihydrates of calcium oxalate respectively, are minerals reported to be present in soils and sediments [34]. The Murambi human remains were covered with lime and left for sixteen years in a warm, humid environment. Those conditions favour calcium oxalate formation. Watchman et al [35] report that oxalic acid is produced

by algae, bacteria and fungi in damp, shaded micro-environments. According to Del Monte et al [34] weddellite and whewellite can be formed easily in natural systems. These researchers state that the secretion of oxalic acid from any micro-organism interacts with calcium carbonate present, forming calcium oxalate monohydrate and dihydrate.

4.3 Molecular assessment

White scrapings, sampled from the upper surface of the skin, were analysed using Raman spectroscopy together with the skin's upper surface and the under surface of the sample. The spectra were compared to the spectra of fresh pig skin. The spectra of samples RW10 and RW12 are presented in Table 2. FT-Raman spectroscopy and dispersive Raman spectroscopy provided information about the environmental deterioration of the samples, the presence of organics and the conversion of the lime from calcium hydroxide to calcium carbonate.

Overall, the skin's integrity was found to be best preserved deeper within the tissues. Decay products were observed in the white scrapings rather than at the upper surface of the skin itself. The skin's upper surface showed Raman bands of generally low intensity. Water OH stretching bands are normally observed in the region above 3100 cm^{-1} . Water bands were very weak in fresh skin and in the skin's under surface. They were absent in the upper surface and in the white scrapings. Bands from tissue proteins (collagen) were found in the regions $1640\text{-}1680\text{ cm}^{-1}$ (amide I), $1220\text{-}1300\text{ cm}^{-1}$ (amide III) and $1440\text{-}1460\text{ cm}^{-1}$ ($\delta\text{CH}_2/\text{CH}_3$). The last band overlaps with the $\delta(\text{CH}_2)$ scissoring vibrations of the skin lipids. These bands were greatly reduced in intensity at the upper surface of the skin and in the white scrapings. The amide I and amide III bands were also absent at the skin's upper surface and in the white scrapings. The above findings indicate changes in both the protein content and secondary protein structures due to decay. Collagen loss was in accordance with reduced intensity of the amide I and amide III bands. The peak at $2900\text{-}2950\text{ cm}^{-1}$ represents CH stretching. Changes in CH stretching bands further support protein structural alternation. A diminution in the intensity of the bands around 1300 cm^{-1} refers to changes in the skin lipid structure. Additionally, a peak at 960 cm^{-1} was observed in Murambi sample RW10, showing the presence of bone (hydroxyapatite $\nu(\text{PO})$) as this can be assigned to phosphate symmetric stretching. With dispersive Raman spectroscopy calcite was identified through its characteristic bands at 280 , 710 and 1084 cm^{-1} with some of the lipids were also visible (Table 2). However, using FT-Raman spectroscopy, calcium carbonate could not be identified in the white scrapings or on the upper surface of the skin (band around 1084 cm^{-1} or subsidiary features), despite the white appearance and the several liming sessions. This is caused by the incomplete or absent conversion of calcium hydroxide into calcium carbonate. Hydrated lime has the property to harden on reaction with carbon

dioxide, a process called carbonation. On exposure to air, re-absorption of carbon dioxide by hydrated lime occurs and water is driven off, resulting once again in the formation of calcium carbonate. Carbonation is often observed in lime burials by the formation of a lime cast [17, 36]. Hydrated lime exhibits a broad asymmetric Raman band centred near 790 cm^{-1} , assigned to an electronic Raman transition. This band is excited with a 1064 nm laser wavelength and not seen with a lower wavelength excitation [37]. This means that the conversion from calcium hydroxide to calcium carbonate can be measured by the relative Raman intensities of the bands at 790 cm^{-1} and 1086 cm^{-1} [36, 38, 39]. In the Murambi samples, very little conversion from calcium hydroxide to calcium carbonate by reaction with atmospheric carbon dioxide had occurred. After sixteen years of display there was still evidence of a significant presence of hydrated lime. This insignificant conversion is likely related to the humid environment and the difficulty for atmospheric carbon dioxide to penetrate the lime layers.

5. Discussion

Decomposition is a complex process whereby the soft tissues break down and eventually disintegrate, resulting in skeletonisation. However, under specific artificial or natural conditions decomposition processes can be slowed down and the body preserved [26, 40, 41]. Attempts to produce soft tissue preservation might therefore not always succeed. For example, Brooks Garcia et al. [42] tried to understand pre-Inca mummification by imitating pre-Inca tombs in an experiment with dead pigs, but mummification did not occur.

Mummies often show high degree of variability in soft tissue preservation due to the environmental conditions and the micro-environment associated with the remains. In many cases significant variation in preservation within the body can be observed [26, 40, 43]. This is clearly the case in the displayed human remains from the Murambi Technical School. Within one individual macroscopic observations showed that some areas of soft tissue were intact, others were flaking, and some were skeletonised. On a microscale, this study revealed deterioration and tissue damage on a histological as well as a molecular level.

Intentional preservation of human remains is challenging. Desiccation requires an air current and, ideally, a dry environment and absence of humidity [26, 40, 41]. Whilst it has been demonstrated that human remains can desiccate in humid environments when positioned in an airflow [26], the environmental conditions at Murambi were not in favour of desiccation. In addition, dehydration of an entire carcass, complete with internal organs and its bacterial community is complex. This study confirms previous studies by Schotsmans et al. [17, 19] that applying lime to the outside of the human bodies, even if carried out regularly, does not prevent decomposition from the inside

outwards by micro-organisms derived by the body itself. It is, therefore, not surprising that visceral organs were removed during artificial mummification in certain cultures such as in the Andes [44] or in ancient Egypt [45, 46].

Another difference between artificial mummification in ancient cultures and at the Murambi Technical School is the reason for conservation. In ancient cultures, artificial mummification was mainly developed based upon the notion that the body of the deceased should be rendered incorruptible and that the physical appearance should be maintained as closely as possible [26, 43]. At the Murambi Technical School the aim for conservation and display was educational: retaining the site frozen at a point in time in order to present a certain moment of the genocide [4, 5]. This activity of conservation is closely related to memorialisation and commemoration and located at the intersection of heritage conservation, forensic science, archaeology and biological anthropology. As with every strategy for the long-term preservation of human remains in collections, a protocol for well-planned storage and display is necessary in addition to the maintenance of stable environmental conditions. This study shows that the preservation technique, conservation strategy and maintenance conditions at the Murambi Genocide Memorial Centre were not ideal and might have lacked scientific support at the time the samples were collected.

6. Conclusion

To date, human remains from the Rwandan Genocide are still displayed at the Murambi Technical School, although conservation strategies are currently being applied. In the past, repeated efforts were made to desiccate the bodies with lime. While visual degradation of the bodies in the Murambi Genocide Memorial Centre was reported, this study confirms deterioration on a microscopic and molecular level causing tissue damage. Both histological and Raman spectroscopic analysis revealed that the limed bodies from Murambi were deteriorating sixteen years after the genocide. On a microscopic level only collagen survived. However, even the collagen component demonstrated molecular deterioration when compared to fresh pig skin, indicated by the weak peak intensities shown by FT-Raman spectroscopy. The presence of active bacteria in the tissue showed that several liming sessions had not stopped micro-organisms colonisation and biodeterioration. In addition, the hydrated lime was only partially converted at best into calcium carbonate.

The observations of this study might have an impact on the future decisions taken by the Rwandan Government and the Genocide Memorial Commission regarding the long-term preservation of the Murambi human remains.

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Figure captions

Table 1: Description of the Murambi samples and histology results. (0 – no changes observed; 1 – limited changes; 2 – significant changes; 3 – extensive changes).

Table 2: Raman spectral wavenumbers and vibrational assignments of fresh pig skin and specimens RW10 and RW12.

Fig. 1: Individuals who died during the 21 April 1994 massacre in Murambi Technical School are displayed on raised wooden platforms, preserved by several layers of lime (Photo: W.A. McClue).

Fig. 2: Fungal fruiting bodies observed by SEM on the limed surface of sample RW5.

Fig. 3 (Tol): Well preserved dermis with lime layer (stained in dark blue) and low presence of bacteria. The lime started to infiltrate in the tissue (arrows).

Fig. 4 (H&E): Longitudinal section of collagen fibres. The vertical lines are cutting artefacts from the knife. Bacteria can be observed in between the fibres shown as blue dots (arrows).

Fig. 5 (H&E): Fascia surrounded by degraded tissue shown with standard bright field light (a), with differential interference contrast light (b) and with polarised light (c). The fascia is better preserved than the tissue, leaving the more putrefaction resistant collagen, as visualised by the birefringence. H&E staining is not well differentiated due to degradation, leaving the intrinsic basic brown colour of the tissue (5a).

Fig. 6 (H&E): Degrading blood vessel with broken wall surrounded by degraded tissue. The H&E stain is not well differentiated due to decay, leaving the intrinsic basic brown colour of the tissue.

Fig. 7 (Tol): More bacteria (arrows and blue dots) were observed in the tissue than on the surface of the tissue. Bacteria are present between the collagen fibres, preferring the path of least resistance.

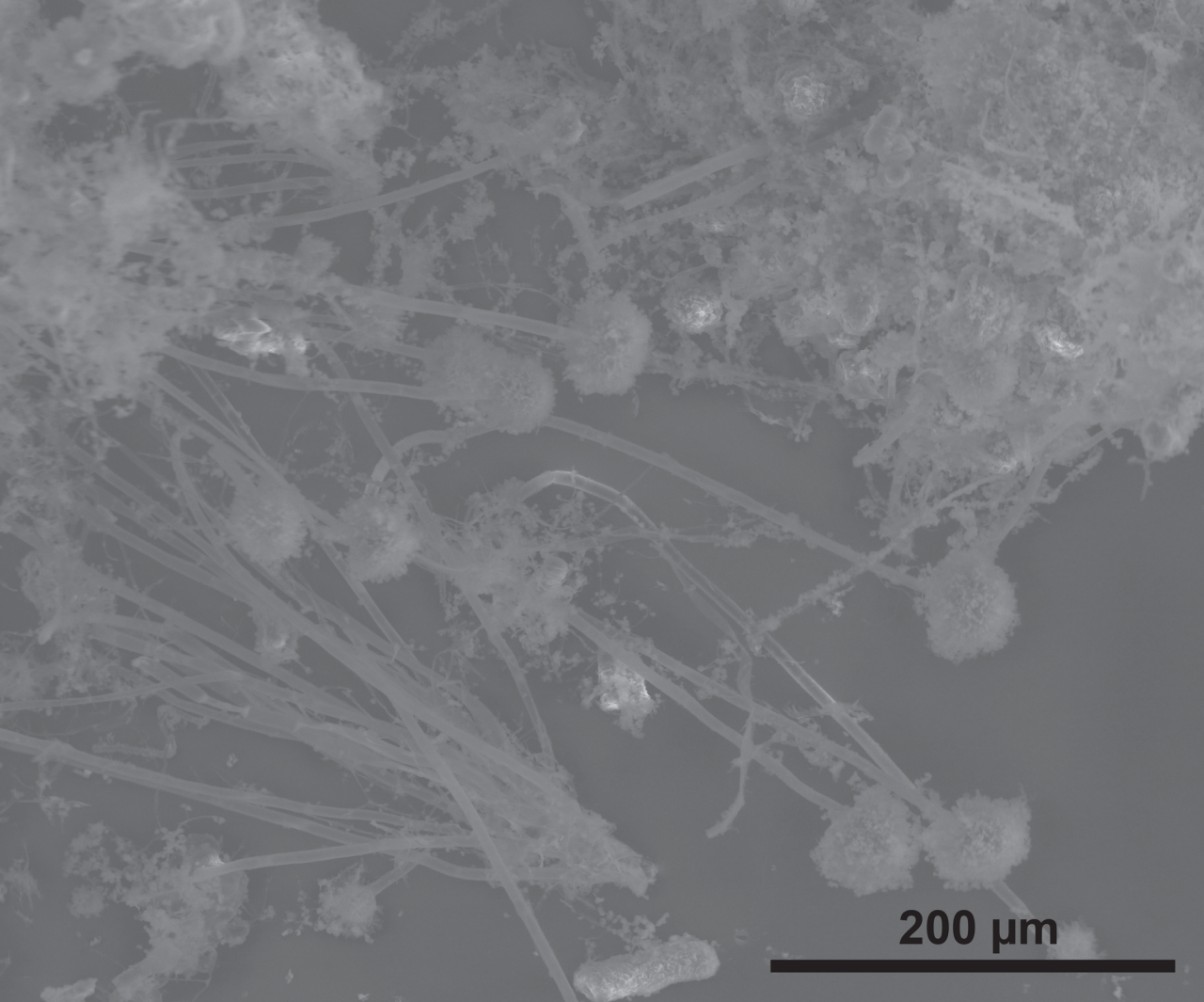
Fig. 8 (H&E): Collagen with a proliferation of bacteria shown as collections of blue dots.

Fig. 9 (Gram): Collagen tissue with few bacteria.

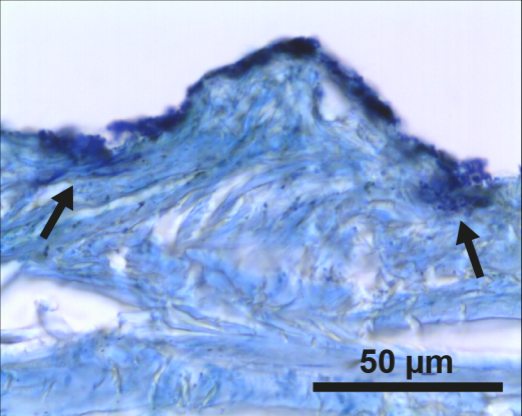
Fig. 10 (Gram): Highly degraded tissue mainly populated with Gram-negative bacteria shown as red dots.

Fig. 11: Crystalline structures typical of calcium oxalate, observed in three of the Rwandan specimen (RW6, RW8 & RW10). Figure 11a shows sample RW6 with standard bright field light; figure 11b displays the same section with differential interference contrast light (DIC); figure 11c shows polarised light (Pol) and figure 11d displays quarter wave polarised light which uses a quartz compensating filter (QWpol).

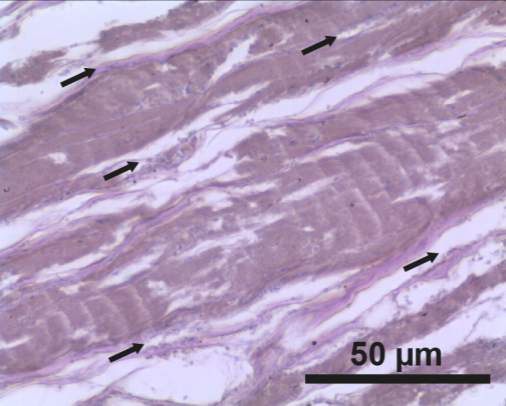


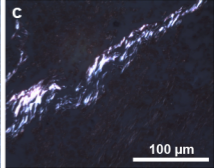
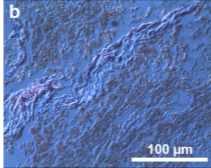
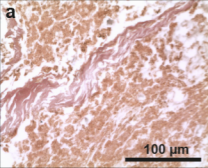


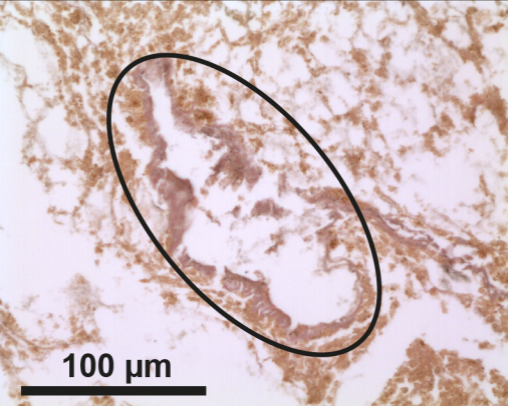
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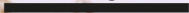
50 μm

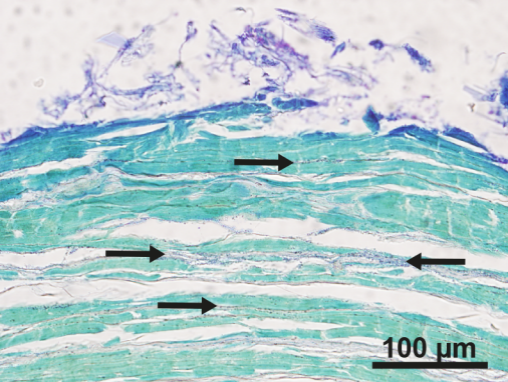




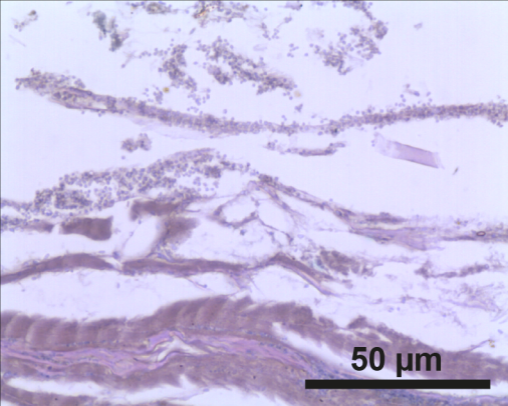


100 μm

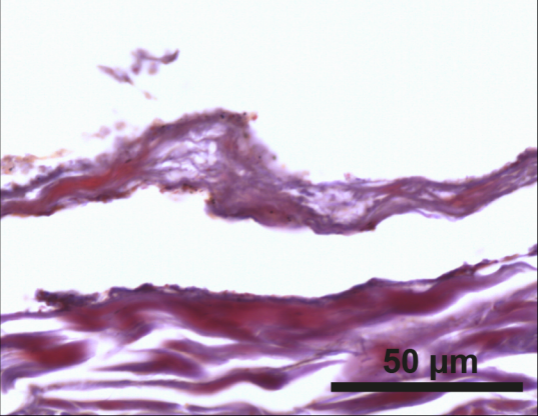




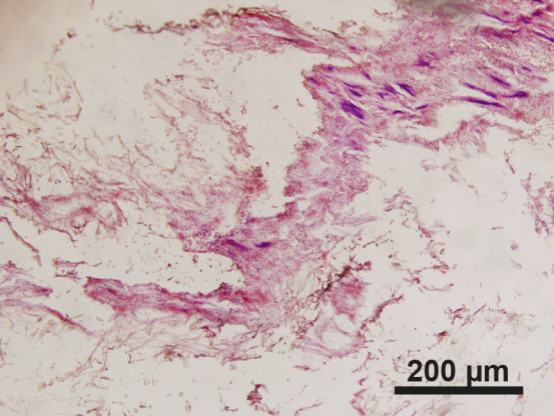
100 μm



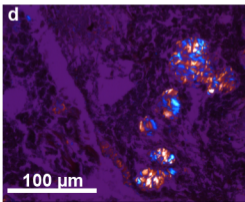
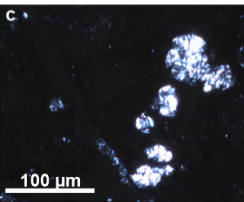
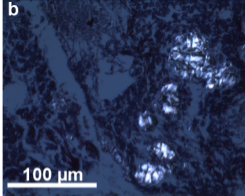
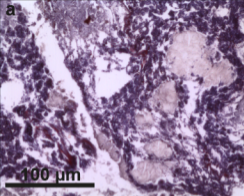
50 μm



50 μm



200 μm



Sample no	Sample description	Sample location	Preservation (general structure)	Presence of bacteria	Presence of crystal. struct.
RW1-4	Lime scrapings from unidentified individuals				
RW5	Tissue sample from area where skin/muscle is flaked	Proximal end right forearm	2-3		
RW6	Individual 1 Tissue from non-disrupted area	Right side thorax	3	✓	✓
RW7	Tissue from bone sample	3rd distal phalanx of right hand	3		
RW8	Tissue sample from area where skin/muscle is flaked	Proximal end of right leg	3	✓	✓
RW9	Individual 2 Tissue from non-disrupted area	Right side abdomen	2	✓	
RW10	Tissue from bone sample	3rd distal phalanx of left hand	3	✓	✓
RW11	Tissue sample from area where skin/muscle is flaked	Distal end of left arm	3	✓	
RW12	Individual 3 Tissue from non-disrupted area	Left side thorax	2	✓	
RW13	Tissue from bone sample	2nd medial phalanx of left hand	3	✓	
RW14	Tissue sample from area where skin/muscle is flaked	Distal end of left leg	2-3		
RW15	Individual 4 Tissue sample cut from pristine area	Left side thorax	2	✓	
RW16	Bone sample	4th distal phalanx of left hand	na		

SKIN	FT-Raman (1064nm)						Dispersive Raman (785nm)	Approximate assignment of vibrational mode (ν = stretching / δ = deformation)
	RW10	RW10	RW10	RW12	RW12	RW12	RW12	
Fresh pig skin	Inferior	Superior (upper surface)	Posterior (bone part)	Inner side tissue (under side of sample)	Limed upper surface	White scrapings surface	White scrapings surface	
2930m	2932m		2931m	2930m		2936m		v(CH3) asymmetric
2885s	2880s	2880m	2880m	2881s	2885w	2881m		v(CH2) asymmetric
2851s	2855m	2855w	2855m	2852m	2852w	2852m		v(CH2) asymmetric
1660m				1662m				v(C=O) helix; amide I
1497	1495		1495	1495				Instrument artefact FT
1440s	1442m		1446m	1442m	1438w	1440w	1438m	δ(CH ₂ /CH ₃)
1295m	1292m	1295w		1295w			1290m	δ(CH ₂) lipid
1260m								δ(NH); amide III
	1130w	1130w		1131m			1124w	v(CC) skeletal; trans conformation
							1084s	v(CO ₃ ²⁻) symmetric
1065w	1060w	1061w	1065m	1065m		1062w	1058m	v(CC) skeletal; trans conformation
	960w		960m					v(PO ₄ ³⁻) symmetric
							709w	in plane bending mode carbonate
	670-780m	670-780s	670-780s		670-780s	670-780s		electronic transition hydrated lime
							278w	lattice mode carbonate ion

s=strong, m=medium, w=weak