

NATURALLY MUMMIFIED REMAINS FROM
HISTORIC CAVE, LIMPOPO, SOUTH AFRICA

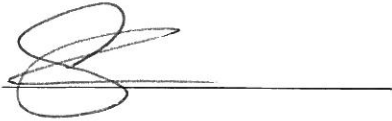
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A dissertation submitted to the Faculty of Science, University of the
Witwatersrand, Johannesburg, in fulfilment of the requirements for the
degree of Master of Science.

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DECLARATION

I declare that this dissertation is my own, unaided work. It is being submitted for the Degree of Master of Science in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other university.



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ABSTRACT

The ‘Makapan Mummy’ (A1081), the Makapan Child and human and animal remains with desiccated tissue attached indicate that the environment inside Historic Cave was dry enough to cause mummification. Environmental studies conducted in 1992 and 2011 suggest that dry air in the well-ventilated cave preserved the soft tissue and mummified the corpse. The aim of this study is to examine desiccated tissue sampled from the ‘Makapan Mummy’ (A1081) and from the human remains excavated at Historic Cave using scanning electron microscopy (SEM), energy-dispersive spectroscopy (EDS) and light microscopy. The morphological features of the naturally desiccated tissue from Historic Cave were recorded and described using the microscopic analyses and were then compared to normal and mummified human tissue. Mummified tissue is hard and dry and often embedded with dust and debris. In its hard and dry state, mummified tissue needs to be prepared differently to normal human tissue. In this study, various methods used to clean, rehydrate and stain the desiccated tissue were investigated and compared. Through this comparative analysis it was possible to determine the most suitable method for examining desiccated tissue from Historic Cave. In the SEM analysis, epidermal keratinocytes and vellus hairs were observed on the surface of the skin tissue. Histological analyses demonstrated the exclusive preservation of collagen fibres in the muscle tissue, the connective tissue and the skin tissue. This suggests that the collagen fibres play an integral part in preserving the structure of desiccated tissue that is devoid of cellular elements. The results are consistent with the histology of desiccated tissue remains from the Republic of Korea, Egypt and the Americas.

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The ‘Makapan Mummy’ (A1081) is housed in Raymond Dart Collection at the School of Anatomical Sciences, University of the Witwatersrand. I would like to thank the School of Anatomical Sciences for providing access to the ‘Makapan Mummy’ (A1081).

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CONTENTS

DECLARATION.....	i
ABSTRACT	ii
ACKNOWLEDGEMENTS	iii
CONTENTS	iv
LIST OF FIGURES	vii
LIST OF TABLES.....	xiv
1 INTRODUCTION.....	1
1.1 General Introduction.....	1
1.2 Aims and Objectives.....	3
1.3 Outline of Chapters.....	4
2 HISTORIC CAVE.....	5
2.1 Introduction	5
2.2 The History of Discoveries	5
2.3 Historic Cave.....	6
2.4 The Mugombane Siege	7
2.5 Archaeological Excavations at Historic Cave.....	10
2.6 Conclusion	13
3 NATURALLY MUMMIFIED REMAINS FROM HISTORIC CAVE	14
3.1 Naturally Mummified Material	14
3.2 Description of the ‘Makapan Mummy’ (A1081)	15
3.3 Description of the Makapan Child	16
3.4 Description of Desiccated Human Remains	17
3.5 Conclusion	17

4	STUDIES OF MUMMIFIED TISSUE	19
4.1	Introduction	19
4.2	Stages of Decomposition	19
4.3	The Process of Mummification	20
4.4	Environmental Factors Contributing to Mummification	22
4.4.1	Desiccation.....	22
4.4.2	Freeze-drying	23
4.4.3	Chemical mummification.....	23
4.5	Mummification at Historic Cave.....	25
4.6	The Structure of Normal Human Skin and Muscle Tissue.....	29
4.7	The Natural Mummification of Soft Tissue.....	31
4.7.1	Korean mummies	32
4.7.2	Native American mummies	34
4.7.3	Iceman's mummification	35
4.8	The Artificial Mummification of Soft Tissue	35
4.9	Conclusion	36
5	MICROSCOPY OF MUMMIFIED TISSUE	38
5.1	Introduction	38
5.2	Imaging	38
5.3	Light Microscopy	40
5.4	Materials and Methods.....	40
5.4.1	Materials	40
5.4.2	Photography	41
5.4.3	Scanning electron microscopy (SEM).....	41
5.4.4	Light microscopy.....	43

6	RESULTS	49
6.1	Introduction	49
6.2	SEM of A1081-5 from the ‘Makapan Mummy’ (A1081)	49
6.3	SEM of Specimen 69 from the Palate (69-M1 RBL)	50
6.4	SEM of Specimen 74 from the Mandible (74-Dg1 M1 RBL)	50
6.5	SEM of Normal Human Hair	51
6.6	Light Microscopy Results of Specimen 50 from the Rib (50-M1 RBL)	52
6.7	Light Microscopy Results of Specimen 69 from the Palate (69-M1 RBL) ..	54
6.8	Light Microscopy Results of Specimen 74 from the Mandible (74-Dg1 M1 RBL) ..	55
6.9	Light Microscopy Results of the Specimens from the ‘Makapan Mummy’ (A1081)	57
7	DISCUSSION	58
7.1	Introduction	58
7.2	The Morphology of the Surface of the Skin	58
7.3	General Tissue Histology	61
7.4	Dust and Debris Coating the Surface of the Skin	64
7.5	Comparing the Sonication Procedures	65
7.6	Comparing the Rehydration Solutions	67
7.7	Comparing the Staining Procedures	68
8	CONCLUSION	70
	APPENDICES	73
	APPENDIX A	73
	APPENDIX B	96
	APPENDIX C	110
	REFERENCES	152
	BIBLIOGRAPHY	163

LIST OF FIGURES

Figure A.1 Location of Makapans Valley in southern Africa	73
Figure A.2 Map showing Historic Cave, Limeworks Cave, Cave of Hearths, Rainbow Cave and Buffalo Cave (after Latham & Herries 2004).....	74
Figure A.3 Map showing the eastern and western chambers at Historic Cave. The grids labelled Dg1 to Dg8 are the excavated areas in the eastern chamber (after Esterhuysen <i>et al.</i> 2009)	75
Figure A.4 The 'Makapan Mummy' A1081 (after Esterhuysen <i>et al.</i> 2009).....	76
Figure A.5 The Makapan Child (after Cresswell 1993)	76
Figure A.6 Figure illustrating the decomposition process.....	78
Figure A.7 The Ginger mummy was naturally mummified by the hot Egyptian sand (Pemberton 2001; image provided by the British Museum).....	79
Figure A.8 Layout of the coffins from the Republic of Korea (after Shin <i>et al.</i> 2003b). (A) and (B) are the inner wooden coffins and (C) is the enclosure of lime and soil	79
Figure A.9 The structure of normal human skin (Histological section provided by Drs Gritzman & Thatcher Laboratories)	94
Figure A.10 Hematoxylin-eosin stain of normal human striated muscle tissue. (1) Muscle fibres; (2) fascicle or bundle of muscle fibres; (3) epimysium; (4) perimysium; and (5) nuclei (Histological section provided by Drs Gritzman & Thatcher Laboratories)	95
Figure B.1 The scanning electron microscope	96
Figure B.2 Diagram illustrating how a scanning electron microscope equipped with an energy-dispersive spectrometer (EDS) works	96
Figure B.3 The light microscope	97
Figure B.4 The mandible excavated at Historic Cave (74-Dg1 M1 RBL). The desiccated tissue was sampled from the outer surface of the right side of the mandible (arrow)	97

Figure B.5 The maxilla excavated at Historic Cave (69-M1 RBL). The desiccated tissue was sampled from the inferior surface of the hard palate of the superior maxilla (arrow)	98
Figure B.6 The rib excavated at Historic Cave (50-M1 RBL). The desiccated tissue was sampled from the inner surface of the rib (arrow).....	98
Figure B.7 (A) The right arm of the 'Makapan Mummy' (A1081); (B) the desiccated tissue specimen was sampled from the anterior surface of the right arm (A1081-5).....	99
Figure B.8 (A) Three desiccated tissue specimens (A1081-1 to A1081-3) were sampled from the exposed upper anterior femoral region of the 'Makapan Mummy' (A1081); (B) magnified view of the area from which the desiccated tissue specimens were sampled as shown in (A)	100
Figure B.9 SAHRA permits for the 'Makapan Mummy' (A1081) and for the human remains excavated at Historic Cave	101
Figure B.10 The stereo microscope	102
Figure C.1 Stereo microscope observation of the dorsal surface of the desiccated skin (sample 5-2). The sample was not cleaned. Dust is observed on the surface of the sample	111
Figure C.2 SEM observation showing dust and debris coating the surface of the skin (sample 5-2). This sample was not cleaned.....	112
Figure C.3 SEM observation of flattened epidermal keratinocytes (asterix) on the surface of the skin (sample 5-2). This sample was not cleaned	112
Figure C.4 Stereo microscope observation of the ventral surface of the desiccated skin (sample 5-3). The sampled was cleaned in absolute alcohol. A layer of dust and debris was observed on the surface of the tissue.....	113
Figure C.5 SEM observation of flattened epidermal keratinocytes (asterix) on the surface of the skin (sample 5-3).....	114
Figure C.6 SEM observation of a hair shaft hole (arrow) on the surface of the skin (sample 5-3)	114

Figure C.7 A foreign particle (asterix) on the surface of the skin (sample 5-3). The particle showed a high calcium count when analysed with EDS	115
Figure C.8 SEM observation of the surface of the skin (sample 74-2). The sample was cleaned absolute alcohol. Most of the dust and debris was removed.....	115
Figure C.9 SEM observation of flattened epidermal keratinocytes (arrows) on the skin (sample 74-2)	116
Figure C.10 SEM observation of hair shaft holes (arrows) on the skin (Sample 74-2).....	116
Figure C.11 SEM observation of a minute structure (arrow) resembling a hair on the skin (sample 74-2).....	117
Figure C.12 Stereo microscope observation showing minute structures (arrows) resembling hairs on the surface of the skin (sample 75-3) prior to the sonication treatment.....	118
Figure C.13 Magnified image of the minute structures (arrows) shown in Figure C.12	118
Figure C.14 SEM observation of dust on the surface of the skin (sample 74-3). The sample was cleaned in acetone	119
Figure C.15 SEM observation of dust on the surface of the skin (sample 74-3). The sample was cleaned in acetone	119
Figure C.16 Hair on the surface of the skin (sample 74-3).....	120
Figure C.17 Hair on the surface of the skin (sample 74-3).....	120
Figure C.18 (A) Hair on the surface of the skin (sample 74-3); (B) Magnified image of the hair in (A).....	121
Figure C.19 (A) Hair on the surface of the skin (sample 74-3); (B) Magnified image showing keratinised scales on the hair shown in (A)	122
Figure C.20 Hair on the surface of the skin (sample 74-3).....	123
Figure C.21 Keratin scales on the cuticle of a normal human hair from the scalp	124
Figure C.22 SEM observation of the hair from the arm	124

Figure C.23 (A) Hematoxylin-eosin stain of the desiccated muscle tissue from the rib (sample 50-1) with dust is embedded in the tissue (1). (B) Muscle fibres with blood vessels (2). ×20 objective lens.....	134
Figure C.24 Stereo microscope observation of the dorsal (A) and the ventral (B) views of the desiccated tissue from the rib (sample 50-2). A layer of dust and debris was observed on the surface of the tissue.....	135
Figure C.25 Hematoxylin-eosin stain of the desiccated tissue from the rib (sample 50-2). Dust is embedded in the muscle tissue (1) which is composed of fascicles (2) and the perimysium (3). ×10 objective lens.....	136
Figure C.26 Masson trichrome stain of the desiccated tissue from the rib (sample 50-2). The muscle tissue is composed of fascicles (1), the epimysium (2) and the perimysium (3). ×20 objective lens.....	136
Figure C.27 Stereo microscope observation of the surface of the desiccated tissue from the rib (sample 50-4). The stereo microscope observation showed that much of the dust was removed	137
Figure C.28 Aldehyde hematoxylin-eosin stain of the desiccated tissue from the rib (sample 50-4). The muscle tissue is arranged as a series of fascicles (1). ×20 objective lens.....	137
Figure C.29 Stereo microscope observation of the surface of the desiccate tissue from the rib (sample 50-5). Much of the dust was removed after cleaning	138
Figure C.30 Aldehyde hematoxylin-eosin stain of the desiccated tissue from the rib (sample 50-5). Fascicles are identified (1). ×20 objective lens	138
Figure C.31 Verhoeff's stain of desiccated muscle tissue from the rib (sample 50-5). The muscle tissue is arranged as a series of fascicles (1); connective tissue is stained yellow (2). ×10 objective lens	139
Figure C.32 Stereo microscope observation of the desiccated tissue from the palate (sample 69-3). Dust is observed on the surface of the sample	139
Figure C.33 Masson trichrome stain of the desiccated tissue from the palate (sample 69-3). Muscle fibres (1) and collagen fibres (2) are identified. ×20 objective lens.....	140

Figure C.34 Hematoxylin-eosin stain of the desiccated muscle tissue from the palate (69-3). Fascicles (1) and the perimysium (2) are identified. ×10 objective lens.....	140
Figure C.35 Stereo microscope observation of the desiccated tissue from the palate. (A) Sample 69-4 and (B) sample 69-5. Dust was observed on the surfaces of the samples.....	141
Figure C.36 Masson trichrome stain of the desiccated tissue from the palate (sample 69-4). Muscle tissue is stained red (1); connective tissue is stained green (2); the epithelium is stained red (3). ×10 objective lens.....	141
Figure C.37 Hematoxylin-eosin stain of the desiccated tissue from the palate (sample 69-4). Connective tissue composed of muscle fibres (1) and collagen fibres (2). The connective tissue lies beneath the epithelium (3). ×10 objective lens.....	142
Figure C.38 Masson trichrome stain of the desiccated tissue from the palate (sample 69-5). Dust is embedded in the tissue (1); identified as connective tissue with muscle fibres (2) and collagen fibres (3). ×10 objective lens	143
Figure C.39 Stereo microscope observation of the surface of the desiccated tissue from the mandible (74–Dg1 M1 RBL). SEM confirmed that the tissue is skin.	144
Figure C.40 Stereo microscope observation of the desiccated skin from the mandible (sample 74-1). The stereo microscope observation showed the sample was covered by a layer of dust.....	144
Figure C.41 Masson trichrome stain of desiccated skin from the mandible (sample 74-1). The tissue is striated muscle tissue with fascicles (1) and connective tissue known as the perimysium (2). ×20 objective lens.....	145
Figure C.42 Hematoxylin-eosin stain of the desiccated skin from the mandible (sample 74-1). The tissue is striated muscle tissue with fascicles (1) and connective tissue known as the perimysium (2). ×20 objective lens.....	145
Figure C.43 Stereo microscope observation of the desiccated skin from the mandible (sample 74-4). Dust was observed on the surface of the sample	146

Figure C.44 Masson trichrome stain of the desiccated skin from the mandible (sample 74-4). Dust is embedded in the tissue (1). The tissue is striated muscle tissue with fascicles (2) and the epimysium (3) and perimysium (4). ×20 objective lens.....	146
Figure C.45 Hematoxylin-eosin stain of desiccated skin from the mandible (sample 74-4). Dust is embedded in the tissue (1). The striated muscle tissue has fascicles (2) and connective tissue known as the perimysium (3). ×10 objective lens.....	147
Figure C.46 Stereo microscope observation of the desiccated skin from the mandible (sample 74-5). The stereo microscope observation showed that a layer of dust covered the surface of the sample	147
Figure C.47 Masson trichrome stain of desiccated skin from the mandible (sample 74-5). Dust is embedded in the tissue (1). The dermis comprises muscle fibres (2) and collagen fibres (3); the skin tissue is well-preserved with an epidermis (4). ×10 objective lens	148
Figure C.48 Hematoxylin-eosin stain of desiccated skin from the mandible (sample 74-5). The muscle fibres and collagen fibres of the remaining dermis were stained pink. ×10 objective lens.....	148
Figure C.49 Stereo microscope observation of the surface of the desiccated tissue from the mummy (A1081-1). The surface of the specimen is covered by a layer of dust and debris.....	149
Figure C.50 Stereo microscope observation of the surface of the desiccated tissue from the mummy (A1081-2). The surface of the specimen is covered by a layer of dust and debris.....	149
Figure C.51 Stereo microscope observation of the surface of the desiccated tissue from the mummy (A1081-3). The surface of the specimen is covered by a layer of dust and debris.....	150

Figure C.52 Masson trichrome stain of the desiccated muscle tissue from the mummy (A1081-3). The striated muscle tissue has fascicles (1); there is connective tissue known as the perimysium in between the fascicles (2). ×40 objective lens.....	150
Figure C.53 Hematoxylin-eosin stain of the desiccated muscle tissue from the mummy (A1081-3). The striated muscle tissue can be observed (1). ×40 objective lens.....	151
Figure C.54 Stereo microscope observation of the desiccated tissue from the palate (69 – M1 RBL) showing dust and debris on the surface of the tissue	151

LIST OF TABLES

Table A.1 Table illustrating the location of human remains with desiccated skin attached in the excavation site.....	77
Table A.2 Average minimum and maximum temperatures in Mokopane in February 2011 (http://rp5.co.za consulted January 2012)	80
Table A.3 Average minimum and maximum temperatures in Mokopane in March 2011 (http://rp5.co.za consulted January 2012).....	82
Table A.4 Average daily temperature, humidity and water vapour pressure for Humi 2 in February 2011	84
Table A.5 Average daily temperature, humidity and water vapour pressure for Humi 2 in March 2011.....	85
Table A.6 Average daily temperature, humidity and water vapour pressure for Humi 3 in February 2011	87
Table A.7 Average daily temperature, humidity and water vapour pressure for Humi 3 in March 2011.....	88
Table A.8 Average daily temperature, humidity and water vapour pressure for Humi 4 in February 2011	90
Table A.9 Average daily temperature, humidity and water vapour pressure for Humi 4 in March 2011.....	91
Table A.10 Average temperature in degrees Celsius (°C), relative humidity percentage (RH %) and water vapour pressure (VP) measured in kilopascals (Kpa) for Humi 2, Humi 3 and Humi 4 in February and March 2011.....	93
Table B.1 SEM procedures used in the examination of desiccated tissue from Historic Cave.....	103
Table B.2 Histological procedures used in the examination of desiccated tissue from Historic Cave	104
Table C.1 Table illustrating the cleaning procedure, the SEM results, and the EDS results for the desiccated tissue from Historic Cave	110
Table C.2 Table illustrating the histological procedures and the results for the desiccated tissue from Historic Cave	125

Table C.2 Table illustrating the histological procedures and the results for the desiccated tissue from Historic Cave	125
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1 INTRODUCTION

1.1 General Introduction

Historic Cave in the Makapans Valley¹, South Africa, has been known at various points in history as Makapans Cave, Makapansgat Cave and the Cave of Gwaša. Historic Cave is famous for an event that took place there in 1854 wherein a number of Kekana Ndebele who took refuge in the cave were placed under siege by the Boers. The siege at Historic Cave was extensively written about by archaeologists and historians during the 20th and 21st century (Preller 1920 – 1938; Tobias 1945; Tobias 1947; Dart 1959; Tobias 1972; De Waal 1978; Naidoo 1987; Hofmeyr 1988; Cresswell 1993; Hofmeyr 1993; Esterhuysen 2006; Esterhuysen 2008a; Esterhuysen 2008b; Esterhuysen *et al.* 2009).

The only documents written in the mid-19th century comprise a report by Commandant General M.W. Pretorius, which was written a month after the siege, and a number of comments and recollections preserved in diaries and newspapers. Kekana Ndebele oral accounts of the siege were recorded in the late 1970s (Hofmeyr 1993) and appeared to have been heavily influenced by Afrikaner propaganda produced in the 20th century. The influence and bias of Afrikaner propaganda raised questions about what really happened at the site and how many Kekana were killed during the event (Naidoo 1987).

From 2001 to 2007 archaeological excavations were carried out at Historic Cave (Esterhuysen 2008a; Esterhuysen *et al.* 2009; Esterhuysen 2010). The excavations yielded numerous cultural artefacts as well as human and animal skeletal remains some of which had mummified tissue attached. Prior to these excavations, the cave

¹ ‘Makapans Valley’ and not ‘Makapan’s Valley is the official name of the valley in which Historic Cave is situated.

was visited by tourists and archaeologists and it was on these occasions that several human remains were removed from the cave. One of these, the Makapan Child was described by M. Cresswell (1993) and the other, the 'Makapan Mummy' (A1081) was described by Esterhuysen *et al.* (2009).

Natural mummification often occurs in environments in which the surrounding matrix preserves the soft tissue and mummifies the corpse. Environmental factors that contribute to mummification include ventilation and low humidity, extreme temperatures, and chemicals such as lime and salt. Under these conditions, the surrounding matrix slows and halts the process of decomposition and preserves soft tissue. There are several studies that explore mummification in different parts of the world. These studies investigate and examine how it occurs and the degree of preservation of the soft tissue. Examples of studies of natural and artificial mummification come from the Republic of Korea, Egypt and the Americas (Lewin 1968; Lausarot *et al.* 1972; Riddle *et al.* 1976; Lewin & Cutz 1976; Reyman *et al.* 1976; Baracco 1977; Barraco *et al.* 1977; El-Najjar *et al.* 1980; Hino *et al.* 1982; Montes *et al.* 1985; Klys *et al.* 1999; Jansen *et al.* 2002; Shin *et al.* 2003a; Shin *et al.* 2003b; Carod-Artal & Vazquez-Cabrera 2006; Chang *et al.* 2006a; Chang *et al.* 2006b; Prikhodko *et al.* 2007; Chang *et al.* 2008; Lim *et al.* 2008; Hoesen & Arriaza 2011).

Studies of natural mummification have examined the histological structure of the mummified tissue. These studies have documented a reduction in the thickness of the layers of the skin. The epidermis undergoes the most deterioration and can be reduced in thickness to a point where it may disappear altogether. The dermis is well preserved and composed of collagen fibres. Generally, collagen fibres form the bulk of the remaining structure of the tissue that is devoid of any cellular elements.

Microscopic studies on mummified tissue also show that there are differences in the histologic structure between naturally mummified skin tissue and artificially mummified skin tissue. In comparison to natural mummification, the artificial mummification process ensures the integrity of the skin's structure with epidermal cells so well preserved that organelles such as mitochondria can be identified within the cells. Collagen and elastic fibres are easily identified in dermal layer of artificially mummified skin tissue.

1.2 Aims and Objectives

The aim of this study is to examine and describe the morphology of the naturally mummified tissue from Historic Cave. Mummified tissue is hard and dry and has to be processed differently to fresh human tissue in order to be studied microscopically. Mummified tissue requires rehydration and due to contamination with soil or sand, the tissue may require cleaning. In order to examine the morphology of the desiccated soft tissue, a number of different techniques will be applied to clean, rehydrated, stain and analyse the tissue. The desiccated tissue samples will be examined using scanning electron microscopy (SEM), energy-dispersive spectroscopy (EDS) and light microscopy. Prior to examination the desiccated tissue samples will be photographed and examined under a stereo microscope. Each technique and protocol used in this study will be described and the results compared to other studies of mummification in which similar techniques and protocols have been used. An outcome of this study is to describe a technique that can be used to analyse and examine the morphology of naturally desiccated human tissue from Historic Cave.

1.3 Outline of Chapters

Chapter Two will discuss Historic Cave and the Mugombane² siege. The archaeological excavations carried out from 2001 to 2007 at Historic Cave will also be discussed in this chapter.

The naturally mummified human remains and the excavated human skeletal remains with desiccated tissue attached are the focus of the study. Descriptions from M. Cresswell (1993) on the Makapan Child and from Esterhuysen *et al.* (2009) on the 'Makapan Mummy' (A1081) as well as descriptions of the desiccated human remains excavated from the cave will be presented in Chapter Three.

This study deals with mummification and it is important to understand how mummification occurs. Chapter Four will review the decomposition process and the environmental factors that impede decomposition and assist in mummification. Environmental studies conducted in 1992 and most recently in 2011 will present a description of the climate in Historic Cave. Chapter Four will then review normal human skin and muscle tissue as well as five other examples of naturally and artificially mummified human tissue. The analytical techniques used in this study will be discussed in Chapter Five followed by the results, discussion and conclusion in Chapters Six, Chapter Seven and Chapter 8. All figures and tables are presented in Appendices A to C.

² Mugombane is also known as Makapan, Makapaan and Mokopane. 'Mugombane' is the Sindebele iteration of his name.

2 HISTORIC CAVE

2.1 Introduction

Makapans Valley is situated about 20 kilometres from Mokopane which was previously known as Potgietersrus (Figure A.1 in Appendix A). The valley is an important world heritage site that forms part of the Cradle of Humankind and its heritage status has been awarded because of its three million year record of human evolution. Makapans Valley comprises an extensive pre-Cambrian dolomite cave system and is famous for the palaeoanthropological, archaeological and historical material it has produced (Tobias 1972). There are various caves within the cave system such as Limeworks Cave, Buffalo Cave, Cave of Hearths, Rainbow Cave, Hyena Cave and Historic Cave (Figure A.2 in Appendix A).

2.2 The History of Discoveries

In 1925, the White Lime Ltd. Company began lime mining operations within Makapans Valley. It was at this time that the palaeoanthropological significance of the valley was recognised when Mr W.I. Eitzman visited the valley and collected several fossils from an extensively mined site. These fossils were sent to Professor Raymond A. Dart at the School of Anatomical Sciences, University of the Witwatersrand, who then visited the valley and collected numerous fossils from Limeworks Cave. In 1937, Dr Robert Broom began investigating Buffalo Cave and subsequently made a number of archaeological discoveries there as well.

In 1936, Professor C. van Riet Lowe was sent to the valley by the National Monuments Council which is now called the South African Heritage Agency. Professor van Riet Lowe surveyed the area and discovered ash in the Early Stone Age (ESA) layers in a cave adjacent to Historic Cave (Mason 1988; Latham & Herries 2004). The ash was thought to indicate the construction of hearths at the cave (van

Riet Lowe 1938; Barbour 1949; Mason 1988). As a result, the cave was named the Cave of Hearths. In 1937, R. J. Mason visited Makapans Valley and discovered a fifth cave that had been uncovered by the miners and which became known as Rainbow Cave (Mason 1988). Excavations at Rainbow Cave produced several Middle Stone Age (MSA) artefacts.

Limeworks Cave, Buffalo Cave, the Cave of Hearths and Rainbow Cave have produced important archaeological artefacts dating back to the ESA, MSA and Late Stone Age (LSA). Fossils such as *Australopithecus africanus* and early *Homo* have also been excavated at these sites. In 1938, Makapans Valley was declared a national monument by the National Monuments Council because of these important archaeological and palaeoanthropological finds (Mason 1988).

2.3 Historic Cave

Historic Cave lies adjacent to the famous Cave of Hearths. It is a large cave formed within the dolomite limestone that characterises Makapans Valley. Historic Cave has two large chambers and numerous smaller caverns which are connected by shafts. The two large chambers, the eastern and western chambers, each have their own entrances. Excavations took place from 2001 to 2007, primarily in the eastern chamber as the western chamber was deemed unsafe (Esterhuysen *et al.* 2009; Esterhuysen 2010).

Following the discovery of the mummified child in 1992, M. Cresswell (1993) conducted a study of the climate within Historic Cave. This study was done in September 1992, at a time of regular droughts and when the general climate was warm and dry. A second study was carried out in 2011 and the results are presented and discussed in Chapter Four, Section 4.3.2.

2.4 The Mugombane Siege

In the 1830s, the Boers began the Great Trek from the Cape colony to the interior of South Africa. During the 1850s, the Boers settled in an area that is now known as Mokopane. This land was occupied by the Kekana and Langa Ndebele and the Boers arrival in the area deepened tensions between them and the local Ndebele. These tensions were not new and according to many historians, conflict had been building between the Boers and the Ndebele regarding the slave and ivory trade (Tobias 1945; Bonner 1983; Delius & Trapido 1983; Hofmeyr 1988; Esterhuysen *et al.* 2009). Once they settled in the interior, the Boers assumed control of the slave and ivory trade. The local African communities would provide the Boers with labourers, particularly women and children from the Kekana Ndebele, Khoe, Venda, Kwenya and Ganawa (Bonner 1983; Delius & Trapido 1983; Morton 2005; Esterhuysen *et al.* 2009). The Boers would then trade with Portuguese merchants. The most famous slave and ivory trader amongst the Boers was Hermanus Potgieter who was ruthless in his encounters with the Ndebele (Orpen 1964).

In 1854, two Ndebele leaders, the Kekana Ndebele chief Mugombane and the Langa Ndebele chief Mankopane, planned to prevent the Boers from settling in their lands and stop the Boer raids on their chiefdoms. It is widely accepted that three particular incidents involving these two chiefdoms led to the siege of Mugombane in 1854 (Tobias 1945). Hermanus Potgieter visited the Ndebele chiefdoms regularly to demand ivory and labour. On one particular occasion, whilst visiting the Langa Ndebele, Hermanus Potgieter and 13 others were captured and killed (Tobias 1945; Dart 1959; Hofmeyr 1988). In a second incident, two Boer hunters, M.A. Venter and his son Willem, were killed at chief Mugombane's capital (Tobias 1945; Dart 1959; Hofmeyr 1988). In a third incident, 12 Boers were killed on Kekana Ndebele territory at Moordrift by the Kekana Ndebele. The Boers, angered by these killings and fearing a growing rebellion among the Ndebele, planned to take armed action against chief Mugombane (De Waal 1978).

Under the threat of an armed action against them, chief Mugombane and his people fled to Historic Cave with provisions of food, water, livestock and guns. Commandant General M.W. Pretorius and Piet Potgieter formed a commando that tracked chief Mugombane and his people to Historic Cave. The sequence of events that took place during the siege was reported on by Commandant General M.W. Pretorius and summarised by De Waal (1978: 106 – 115). Many archaeologists and historians have referred to De Waal's synopsis of the Mugombane siege (Tobias 1945; Tobias 1947; Dart 1959; Tobias 1972; Naidoo 1987; Hofmeyr 1988; Hofmeyr 1993; Esterhuysen 2006; Esterhuysen 2008b; Esterhuysen *et al.* 2009).

According to De Waal (1978) the Boers arrived at Historic Cave on the 25th of October 1854 and proceeded to attack the Kekana Ndebele. They attempted to blast off the roof of the cave and when this did not work they lit fires at the entrances to the cave to smoke the group out. The Kekana Ndebele resisted the Boer attacks because they were protected by the cave and because they were well provisioned. In early November 1854, the Boers realised that the only way to force the Kekana Ndebele out was to 'starve' them out (Bredell & Grobler 1902: 29). The Boers cleared the entrances to the cave to prevent people escaping from the cave at night.

As the siege progressed, the Kekana Ndebele's provisions began to diminish. On the 6th of November, Piet Potgieter was shot dead outside the entrance to the cave. On the same day, a group of women and children fled the cave allegedly driven by thirst because they ran down to a stream and many of them died from drinking water too quickly (De Waal 1978: 111). On the 8th of November, a group of men, women and children fled the cave and were either shot or taken captive by the Boers. On the 17th of November, a group of 364 women and children surrendered (De Waal 1978: 111).

Allegedly, as the Kekana Ndebele's water supply dwindled, resistance from the cave weakened (Esterhuysen *et al.* 2009). With very little resistance coming from the cave, the Boers ventured in and they were met with many dead and decomposing bodies. At the end of the siege on the 21st of November 1854, the Boers collected about 700 prisoners and 5000 sheep, cattle and goats while Pretorius estimated that 900 bodies lay outside the cave and 3000 bodies lay inside (De Waal 1978).

The main problem concerning the Mugombane siege is the unreliability of the historical records. The historical account that does exist is Commandant General M.W. Pretorius' report which represents the Boer perspective. This Afrikaner account was captured in the 1890s, 40 years after the siege, and provided biased interpretations of the Mugombane siege. The Kekana oral tradition, on the other hand, does not focus on the siege but rather on the installation of the next chief about 15 years after the siege. Hofmeyr (1993) studied the Kekana Ndebele oral narratives and found that because the Kekana Ndebele were two or three generations removed, they no longer had first-hand knowledge of the siege. The Kekana Ndebele oral accounts that do exist seem to have been heavily influenced by the popular version of the event formulated by the Afrikaners in the early 1900s (Hofmeyr 1993).

Recent archaeological work at the cave was prompted by this contradiction in the written and oral records of the siege (Esterhuysen 2008). Pretorius' written report provides the only contemporary description of the siege but some scholars have queried the reliability of his description, particularly his account of the dead. Some have argued that the number of decomposing bodies, poor visibility and the threat of armed survivors in the cave may have affected the Boers calculation of the total number of bodies and that Pretorius escalated the numbers to justify the siege (Naidoo 1987).

There are also conflicting interpretations about what exactly led to the demise of the Kekana Ndebele at the end of the Mugombane siege. Pretorius' account suggests dehydration as the cause of death and Tobias (1947) alleges that the Kekana Ndebele were weakened by thirst and were eventually defeated by the Boers. Others suggest that the Kekana Ndebele died from smoke inhalation after the Boers fanned smoke into the cave, while another interpretation, that is supported by the oral record (Hofmeyr 1993: 111) and some archaeologists (Mason 1988), suggests disease as the cause of death.

2.5 Archaeological Excavations at Historic Cave

Prior to excavations, Historic Cave served as a tourist site with many professional and amateur archaeologists and tourists visiting the cave. Unfortunately, during these visits many skeletal remains and other archaeological material were removed from the cave without being recorded (Tobias 1947; Dart 1959). The archaeological excavations were conducted from 2001 to 2007. These excavations provided information about the preparation of the cave prior to the siege, the conditions of the cave during the siege and insight into the variety of interpretations about the siege.

The archaeological excavations were conducted in the eastern chamber of Historic Cave (Esterhuysen 2006; Esterhuysen 2008a; Esterhuysen *et al.* 2009; Esterhuysen 2010). A survey of the cave led the archaeologists to excavate in eight separate areas of the site (Dg1 to Dg8 in Figure A.3). Archaeological material excavated from each area showed that 'households', defined by grass fences and low walls, were arranged by the occupants of the cave (Esterhuysen 2008a: 465). The households were equipped with separate sleeping and food preparation areas (Esterhuysen 2006; Esterhuysen 2010). Each household, communal area, storage area and livestock pen had either a dung floor or a compressed layer that separated the occupation level from the levels on top and below which indicated that all the archaeological material

belonged to a single occupation (Esterhuysen 2008a; Esterhuysen 2008b; Esterhuysen *et al.* 2009; Esterhuysen 2010).

In addition to the preparation of households, stone walls were constructed at the cave's entrances. The historical accounts suggest that the stone walls were specifically constructed at the cave's entrances to create a defensive system during the siege (Dart 1959; Esterhuysen *et al.* 2009). Both the excavations and historical accounts suggest that the cave was well prepared prior to the siege (Esterhuysen *et al.* 2009).

Human remains were recovered from the archaeological excavations. The number of human skeletal remains at Historic Cave is approximately 250. According to Esterhuysen *et al.* (2009), roughly 25 to 30 individuals were recovered from the excavated area which was approximately 38m². Based on this, there is an average of just under one person per 1m² and when this is multiplied by the total area of the cave (2300m²) the projected number of individuals is roughly between 1380 and 1794 (Esterhuysen *et al.* 2009: 1048; Esterhuysen 2010).

Prior to the archaeological excavations, six human remains, including two naturally mummified individuals, were collected by archaeologists and tourists visiting Historic Cave. These six individuals are housed in the Raymond Dart Collection at the School of Anatomical Sciences, University of the Witwatersrand. In general, the archaeological excavations at Historic Cave showed that the organic material was well preserved which suggests that the conditions in the cave were conducive to the preservation of human remains and cultural artefacts dating back to the time of the Mugombane siege (Esterhuysen *et al.* 2009; Esterhuysen 2010).

The archaeological excavations provided some clarification about the nature and scale of the event. The projected number of individuals is between 1380 and 1794 and in spite of the disappearance of numerous skeletal remains, the archaeological excavations have for the first time provided an indication that the siege took a large number of lives.

Esterhuysen *et al.* (2009) described the two mummified individuals from Historic Cave. The 'Makapan Mummy' (A1081) is in the upright sitting position with both hands clasped in the lap, a position that is argued by Esterhuysen *et al.* (2009: 1046) to be consistent with dehydration. As discussed previously, Pretorius' claim of dehydration is largely contested by archaeologists and historians. Esterhuysen *et al.* (2009) argued that dehydration may have been caused by low water provisions, a change in diet from carbohydrates to protein, and hot and dry conditions inside the cave. Dehydration causes an imbalance of electrolytes (sodium and potassium) in the body and severe dehydration results in a deadly increase of sodium which causes cellular dehydration (Adrogué & Madias 2000: 1493; Esterhuysen *et al.* 2009: 1047). Severe dehydration causes confusion, weakness, hypotension and shock (Wedro 2008). Organ failure, coma and eventually death may result if dehydration is not treated. Extreme dehydration can lead to hypernatremia which occurs when a dehydrated body is rehydrated too quickly. In chronic hypernatremia, if rehydration follows too quickly, cerebral oedema can occur leading to brain damage and resulting in convulsions, coma and death (Wedro 2008). Pretorius' account of the siege suggests that a number of women and children who fled the cave had done so allegedly because they were driven by thirst and that many of them died upon drinking water. This is consistent with hypernatremia, a condition induced by dehydration (Esterhuysen *et al.* 2009).

2.6 Conclusion

Historic Cave is situated within Makapans Valley which is famous for its palaeoanthropological and archaeological record. Because of the incredible preservation quality of the dolomite limestone caves, the archaeological material uncovered through excavations at Historic Cave have been used to reconstruct the events that took place during a siege that occurred in 1854.

Prior to the excavations, the main problem concerning the Mugombane siege was the lack of unbiased historical accounts. The Boer perspective on the siege promoted Afrikaner propaganda and the Kekana Ndebele accounts did not provide a first-hand interpretation of the siege. The two questions often asked about the siege are how many Kekana Ndebele died in the cave and what caused their deaths.

The archaeological excavations at Historic Cave have shown that the construction of households and stone walls created a defensive system that protected the occupants of the cave during the siege. The human skeletal remains excavated from the cave gave an indication of the number of people present in the cave at the end of the siege and the naturally mummified material provided evidence to support Pretorius' claim that the primary cause of death was dehydration. The abundant material recovered from the archaeological excavation, particularly the human remains, has been used to re-evaluate the siege and provide some clarification. Further studies of the naturally mummified human remains from Historic Cave may contribute to a greater understanding of the cave and the siege.

Although the primary aim of this study is not to understand the reasons for mummification, the outcome of the study may be used as a reference for further examinations into the natural mummification of human remains at Historic Cave.

3 NATURALLY MUMMIFIED REMAINS FROM HISTORIC CAVE

3.1 Naturally Mummified Material

Two near-complete mummified human skeletons, mummified rodents and desiccated human and animal remains were collected and excavated at Historic Cave by archaeologists and tourists. In 1948, the 'Makapan Mummy' (A1081) was brought to the Raymond Dart Collection at the School of Anatomical Sciences, University of the Witwatersrand, where it remains today. Unfortunately, the details of its discovery were not recorded but according to Esterhuysen *et al.* (2009), the degree of mummification is consistent with other desiccated material excavated at Historic Cave. The second near-complete mummified individual, known as the Makapan Child, was removed from the cave by Dr Wium in March 1991 and placed in the Raymond Dart Collection. In 1993, M. Cresswell conducted a study of the Makapan Child and upon completion of the study the mummy was misplaced. Fortunately, in 2010 the specimen was found and is currently housed in the Raymond Dart Collection.

Many other bones excavated at Historic Cave have desiccated tissue attached and possibly represent naturally mummified human remains that have not remained intact. This material can be found in the Archaeology Collection at the School of Geography, Archaeology and Environmental Sciences, University of the Witwatersrand. This chapter will focus on the mummified material from Historic Cave by providing descriptions of the mummified humans and desiccated human remains.

3.2 Description of the ‘Makapan Mummy’ (A1081)

The ‘Makapan Mummy’ (A1081) was brought to the Raymond Dart Collection in 1948. A detailed description of the mummy was provided by Esterhuysen *et al.* (2009: 1044 - 1047) and will be discussed here (Figure A.4 in Appendix A).

The mummy is in the upright position with both legs flexed under the body so that it assumes the kneeling position. This position indicates that the mummy was not buried at the time of death. At some point either before or after the mummy was discovered, the right arm had separated from the body and was reattached by means of a rope. The right hand folded neatly around the left hand indicating that the individual died, while kneeling, with both hands clasped in the lap (Esterhuysen *et al.* 2009: 1044). The skull had become separated from the body postmortem and with the skull absent, it is possible to view the thoracic cavity internally. In the thoracic cavity, blowfly pupa casings are visible but no internal organs can be identified without endoscopic probing.

Esterhuysen *et al.* (2009) describes the skin as leathery and hard. There are numerous perforations in the skin which were probably made by blowfly larvae (maggots), beetles and other insects that penetrated the skin. Skin has preserved well in the areas of the forearms, hands, thorax, abdomen and legs with occasional tears in the skin in these areas. These ‘tears’ were probably caused by skin shrinkage which often occurs during mummification (Esterhuysen *et al.* 2009: 1044). The general structure of the skin is so well preserved on the upper left thigh that the shape of a well-formed muscle is observed here (Esterhuysen *et al.* 2009). The areas where the skin has worn away are the upper left and right humeri, the perineum and the inner thighs.

Usually the cranium and dentition are used to determine the age of an individual. Aging can also be done by examining fusions at the ossification centres. In the absence of the cranium and dentition in the case of the 'Makapan Mummy' (A1081), the age was determined through digital X-rays of the body which showed the ossification centres. The digital X-rays showed that the epiphyseal fusions were recent unions and based on this, the individual appears to be older than 14 years but younger than 19 years (Esterhuysen *et al.* 2009: 1046). The digital X-rays also show that the feet overlap with the pelvis making it difficult to determine the sex of the individual. However, based on the wide sciatic notch and the sub-pubic angle, Esterhuysen *et al.* (2009) suggest that the individual is female.

3.3 Description of the Makapan Child

A second mummy from the cave is the Makapan Child (Figure A.5 in Appendix A). The Makapan Child was removed from the cave and described by Dr Wium in March 1992 and by M. Cresswell in 1993. These descriptions will be presented here.

At the time of death, the Makapan Child was placed in the customary foetal position and partially buried (Wium 1992). The mandible, cervical and thoracic vertebrae and a few ribs and rib fragments were found in the soil near the body (Wium 1992). In 1992, the right forearm of the child was discovered by a group of school children visiting the cave.

According to Cresswell (1993), the skin is well preserved and has only worn away in some areas. These areas include the anterior neck, the anterior superior thorax, the facial regions and the dorsum which was resting on the cave floor (Cresswell 1993: 12). The skin of the legs and arms are well preserved to the extent that ridges and fingerprints can be observed on the hand (Cresswell 1993). Various holes are present

in the skin and numerous casings were found in the body and in the surrounding soil (Cresswell 1993). As in the case of the 'Makapan Mummy' (A1081), the holes in the skin were a result of blowfly larvae and beetles penetrating the skin. No viscera were recorded in Cresswell's (1993) study but desiccated fibrous material in the thorax and abdomen were observed and interpreted as intercostal muscles and muscle tissue of the left lobe of the liver, the spleen and the kidney.

The estimated length of the body is 78mm which is the estimated length of a one year old. However, dental analysis and CT-scans of the Makapan Child indicate that the child was three or four years old at the time of death. The sex of the Makapan Child is unknown because the child was pre-pubescent and because no external genitalia are visible (Cresswell 1993).

3.4 Description of Desiccated Human Remains

Archaeological excavations were conducted between 2001 and 2007 at Historic Cave. A survey of the cave led to the excavation of the site in eight separate areas with Dg1 being at the base of the cave, followed by Dg2 to Dg7 and Dg8 at the top (Esterhuysen 2008a). Other than the archaeological artefacts, human and animal remains were excavated from these areas. From the excavated human remains, 27 bone specimens with desiccated tissue attached were identified. These bone specimens include a cranium, maxilla, mandible, an ulna and humerus, ribs and vertebrae, and a tibia and femur. Table A.1 in Appendix A shows the area of the cave excavated and the associated bone specimens with desiccated tissue attached.

3.5 Conclusion

Through the excavations, archaeologists have been able to document the preparation of the cave prior to the siege. The excavation of naturally desiccated human and

animal remains suggests that at the time of the siege and during the postmortem interval, specific environmental conditions were conducive to mummification. Because the location of the Makapan Child was unknown at the time, permits and samples could only be acquired for the 'Makapan Mummy' (A1081) and for the desiccated human remains excavated at Historic Cave.

4 STUDIES OF MUMMIFIED TISSUE

4.1 Introduction

Under normal circumstances the remains of animals and humans decompose after death and do not mummify. The decomposition process follows a number of stages which result in the skeletonisation of the body. Several factors are needed to slow and halt the decomposition process so that soft tissue is preserved and skeletonisation is prevented. In this chapter, the stages of decomposition and the process of mummification will be discussed. Because the aim of this study is to examine and describe the morphology of naturally desiccated human tissue from Historic Cave, five different cases of mummification will be examined. In each study, the methods used to analyse the desiccated tissue will be discussed and the general structure of mummified tissue will be presented.

4.2 Stages of Decomposition

Decomposition begins soon after death with the process of autolysis (Figure A.6 in Appendix A). During this stage the body's enzymes breakdown the cells. Once the cells die, aerobic respiration stops and as the body begins to lose oxygen it also loses the ability to fight off bacteria. The next stage after autolysis is putrefaction. During this stage, anaerobic bacteria, which do not require oxygen to function, begin to digest the body by breaking down soft tissue and large molecules such as proteins, lipids and carbohydrates (Dent *et al.* 2004; Bereuter *et al.* 1997). As the soft tissue is broken down, chemicals and gases are released and this attracts insects (Gill-King 1997). Putrefaction is followed by liquefaction and skeletonisation. In these later stages of decomposition, the breakdown of organs, cells and protein renders the decomposed body a structureless mass of tissue which is eventually lost to the environment leaving only bone and teeth.

Various insects such as the Diptera (flies) and the Coleoptera (beetles) play an important role in the decomposition process. Of the Diptera, the Calliphorids or blow flies are first to occupy the decomposing body (Haskell *et al.* 1997). The female blow fly will lay eggs in fairly protected areas of the body where the larvae can access food easily. These areas include the body's cavities such as the nasal passages, mouth, and hair, along the boundary line where the body meets the ground and in the folds of clothing (Haskell *et al.* 1997). Other species of Diptera that colonise the body are the Sarcophagae or flesh flies. In contrast to the blow fly, the flesh fly leaves living larvae in the decomposing tissue (Haskell *et al.* 1997: 428).

The blow fly occupation of the body comprises three stages commonly known as instars. During the first stage, the blow fly eggs hatch and the larvae begin feeding on the decomposing tissue. The second stage follows with heavier feeding on the decomposing tissue and the third stage is known as the stage of the 'maggot mass' (Haskell *et al.* 1997: 427). After the third stage, the larvae migrate away from the body to begin the pupal stage. A hard outer casing known as the puparium, forms around the larvae which then undergo metamorphism (Haskell *et al.* 1997). The Coleoptera (beetles) follow the Dipterans in the decomposition process. Some beetles feed on the eggs and larvae of the flies and in doing so the beetles regulate the Dipteran population (Haskell *et al.* 1997). Other species of Coleoptera feed directly on tissue. These species include silphids, dermestids, nitidulids and clerids (Haskell *et al.* 1997).

4.3 The Process of Mummification

Decomposition relies on the adequate availability of water, appropriate temperature and humidity levels as well as a particular solute (salt) concentration. Changes to these environmental parameters could affect the roles played by micro-organisms,

bacteria and insects in the decomposition process and if the conditions are right, a change in the decomposition process could result in mummification.

The body is an important source of water and the metabolic action of micro-organisms and bacteria during decomposition requires water and oxygen (Berkeley & Campbell 1985: 76). Because enzymes such as hydrolase enzymes utilize water to breakdown large molecules, the rapid dehydration of tissue prevents this from taking place. A slow decay rate, caused by dehydration, prevents decomposition and results in the preservation and mummification of the soft tissue.

For the successful development of the Dipteran eggs and larvae, moisture must be available and the site should be protected from sunlight (Haskell *et al.* 1997). The Diptera will only lay eggs during the initial stages of decomposition when moisture is still available because in the later stages of decomposition the body becomes dehydrated as moisture is lost through bacterial activity (Galloway 1997). In contrast, the beetles do not depend on moisture and they often colonize mummified remains and desiccated tissue (Galloway 1997).

According to Mann *et al.* (1990), temperature and humidity affect the rate of decomposition because insect activity is also governed by these factors. The rapid decomposition of a body occurs in environments where temperature and humidity are high. The slow decomposition of a body occurs in environments where humidity is low because moisture from the body evaporates causing rapid desiccation of the body. In this environment, insect activity is minimized as the female blowfly will not lay eggs in desiccated tissue. The rapid desiccation may also minimize odour and prevent insects and carnivores from being attracted to the decomposing body (Galloway 1997).

The solute concentration of tissue also affects the rate of decomposition by preventing micro-organisms and bacteria from functioning. When placed in an environment with a high solute concentration, water moves out of the bacterial cell because of the process of osmosis (Berkeley & Campbell 1985). This causes the cell to shrink as there is a contraction of the plasma membrane from the cell wall. This condition is known as plasmolysis. Bacteria and fungi will not function under this condition and this can preserve the soft tissue.

Because decomposition relies on water, heat and high humidity, changes to these environmental conditions may promote the desiccation of the body which could affect the roles played by micro-organisms, bacteria and insects in the decomposition process and result in mummification.

4.4 Environmental Factors Contributing to Mummification

Under certain conditions, instead of decomposing, bodies may become mummified. Mummification is a process of preservation in which the tissue survives decomposition and resembles its once-living morphology (Aufderheide 2003; Vass 2001). The mummification of tissue is achieved through several mechanisms such as desiccation (drying), freeze-drying and chemical mummification.

4.4.1 Desiccation

Desiccation or dehydration is a loss of water from an organism and often occurs in environments that are extremely hot and dry. Naturally mummified human remains have been found buried in semi-arid deserts which are extremely hot and dry (Figure A.7 in Appendix A). In these climates, the hot sun and the hot sand surrounding a body causes a loss of fluid and preserves the soft tissue (Peck 1983; Jansen *et al.* 2002). There have been some cases suggesting that a dry micro-climate in a humid

environment leads to desiccation and mummification of corpses (Cresswell 1993; Aufderheide 2003). Air drying has also been reported to cause mummification (Aufderheide 2003). Numerous corpses have been found in well-ventilated environments which caused the desiccation of corpses and the mummification of soft tissue (Kaufmann 1996; Kauffmann-Doig 1998).

4.4.2 Freeze-drying

Spontaneous mummification has been reported to occur in extremely cold environments. The extreme cold prevents enzyme activity and results in a slow decay rate and in the preservation of the soft tissue (Aufderheide 2003). Freeze-drying is a process that involves freezing and desiccating the soft tissue which leads to mummification. Ötzi the Iceman, for example, was surrounded by ice layers and freeze-dried (Bahn 1996; Murphy *et al.* 2003; Spindler 2004).

4.4.3 Chemical mummification

Natural mummification often occurs in dry environments that are rich in lime or salt. For example, in the dry salt pans of the Tarim Basin in China where human remains were excavated, the salt in the soil absorbed the body's fluids and dehydrated the soft tissue (Wang 1996). Under these conditions, the lime or salt affected the decomposition process and induced mummification.

During decomposition, enzymes and bacteria break down large molecules such as proteins, lipids and carbohydrates. These enzymes function in an acidic environment that has a high water-content (Aufderheide 2003: 288). Salt is often used in food preservation because it prevents enzyme activity by dehydrating the food. Lime, which is salt-based, has a similar effect on soft tissue. Thew (2000) investigated the role of lime in the decomposition process and aimed to understand how lime

contributes to the preservation of soft tissue. According to Thew (2000), the results showed that lime does not slow down decomposition but there is a difference in preservation between shallow lime burials and shallow unlimed burials. Esterhuysen *et al.* (2009) suggests that lime-enriched soil has a drainage quality that may aid in preservation as fluid is absorbed from the soft tissue. Enzymes will not be able to breakdown the soft tissue because of the lack of fluid and so the body will preserve.

There are a few examples of mummification in environments where lime is present. In the Republic of Korea, mummified human remains were found dating back to the Chosun Dynasty. The human corpses were placed in wooden coffins (**A** and **B** in Figure A.8 in Appendix A) which were then placed in an enclosure of lime and soil (**C** in Figure A.8). According to Shin *et al.* (2003a), because of the absorbing qualities of lime, the inner wooden coffins became completely sealed off and the humidity in the coffins decreased over time. The lack of humidity in the coffins caused the body to become desiccated and the soft tissue to preserve and mummify (Shin *et al.* 2003a: 382).

Similarly, Barraco (1978) investigated the sodium, potassium, magnesium and calcium concentrations in various Ancient Egyptian mummies and the St. Lawrence Eskimo mummy. The muscle tissue of the St. Lawrence Eskimo demonstrated a high clay content which was believed to have resulted from the high lime content of the soil at the burial site of the mummy (Baracco 1978: 490). As in the Korean cases of mummification, the preservation of the St Lawrence Eskimo is attributed to lime at the burial site. In another study, Owsley *et al.* (1985; 1987) argued that soil with a high clay content contributed to the preservation of human remains in cemeteries in New Orleans, Louisiana. Lime therefore has significant preservation qualities which can contribute to the mummification of soft tissue.

4.5 Mummification at Historic Cave

As previously mentioned, mummified human remains are commonly found in environments that are either extremely hot and dry (Egyptian mummies) or extremely cold and dry (Ötzi the Iceman). The environment plays an important role in the decomposition process and specific environmental conditions can account for the desiccation and mummification of human corpses.

Cresswell (1993) argued that the atmosphere in Historic Cave contributed towards the mummification of the Makapan Child. He conducted a study from the 11th of September 1992 to the 13th of September 1993 in Historic Cave and took both wet and dry bulb temperature readings over the 72 hour period. Temperature and water vapour pressure readings were taken at the entrance of the cave, at the site where the Makapan Child was found, and half way between these points. The interior had a lower vapour pressure of 0.875 Kpa compared to the entrance of the cave which had a vapour pressure of 0.975 Kpa. These results show that a gradient, which remains constant, exists between the points measured (Cresswell 1993). Cresswell (1993: 24) determined that despite the outside fluctuations, the temperature in the cave remained stable while humidity was low. Cresswell (1993) also noted that even though the cave was well lit particularly at sunrise, the back regions of the cave received little or no sunlight and no draft of air. Cresswell (1993: 25) argued that because it was claimed that 3000 to 4000 people died in the cave and there were only three mummified individuals discovered at the time, the micro-climate and micro-environment immediately surrounding the child was dry enough to prevent the actions of bacteria and other micro-organisms. This dry micro-environment prevented decomposition by slowing bacterial activity and allowing soft tissue to preserve and mummify.

A recent climate study was carried out during the summer wet months of February and March 2011 at Historic Cave by the author of this paper. February and March are the wettest months and therefore provide an indication of the climate in the cave during the wet period. In February 2011, the average temperatures in Mokopane were a minimum of 19.09°C and a maximum of 30.58°C. These average temperatures in Mokopane increased slightly in March 2011 with minimum and maximum temperatures of 19.32°C and 31.26°C respectively (average temperatures were calculated based on the temperature readings for February and March 2011 provided by <http://rp5.co.za> consulted January 2012, see Table A.2 and Table A.3 in Appendix A). Three data loggers (Extech Instruments RHT10, Humidity and Temperature Dataloggers) were placed in Historic Cave to measure temperature and humidity. The first data logger (Humi 2) was placed at the base of the cave in the Dg1 excavation site (Figure A.3 in Appendix A). The second data logger (Humi 3) was placed in the middle of the cave. The third data logger (Humi 4) was placed outside at the entrance to the cave. Temperature, humidity and dew point readings were taken every six hours starting at 12:53am from the 17th of February 2011 to the 27th of March 2011. The average temperature, humidity and water vapour pressure in February and March 2011 were calculated for all three data loggers (Table A.4 to Table A.9 in Appendix A).

The results showed that the base of the cave had a temperature of 18.71°C which was slightly lower than the middle of the cave (19.26°C) and the entrance to the cave (20.43°C) in February 2011. In March 2011, there was a slight increase in temperature with the base of the cave still maintaining a lower temperature of 19.35°C than the middle of the cave (19.98°C) and the entrance to the cave (21.64°C). In February 2011, the base of the cave had a higher relative humidity of 72.77% compared to the middle of the cave (71.48%) and the entrance to the cave (66.69%). In March 2011, there was a decrease in relative humidity with the base of the cave still maintaining a higher relative humidity of 68.40% than the middle of the

cave (66.69%) and the entrance to the cave (61.78%). The temperature and relative humidity remained stable with no extreme fluctuations.

In order to compare the 2011 readings with Cresswell's (1993) results, it was necessary to calculate the average water vapour pressure for February and March 2011. The Arden Buck equation (Buck 1981), which uses ambient temperature and relative humidity, was used to calculate the average water vapour temperature at Historic Cave during February and March 2011. In February 2011, the average water vapour pressure inside the cave was 1.57 Kpa (at both Humi 2 and Humi 3) and 1.60 Kpa at the entrance to the cave. In March 2011, the average water vapour pressure decreased slightly. The base of the cave had an average water vapour pressure of 1.54 Kpa, the middle of the cave where the Makapan Child was found had an average water vapour pressure of 1.56 Kpa and the entrance to the cave had an average water vapour pressure of 1.57 Kpa. The average water vapour pressure at all three points was stable and the results confirm Cresswell's (1993) study that a gradient exists with lower water vapour pressure inside the cave than outside the cave (Table A.10 in Appendix A).

Relative humidity is the maximum amount of water that can be absorbed by the air (known as saturation) before condensation occurs (Ahrens 2007). Warm air can hold much more water than cool air. In cooler temperatures, condensation occurs quicker because the maximum amount of water that can be absorbed (relative humidity) is reached far more rapidly than in warm air. The general rule is that if temperature goes down, relative humidity goes up and if temperature goes up, relative humidity goes down. Vapour pressure is the actual amount of water molecules in the air. At Historic Cave, temperature and relative humidity data show that in cooler temperatures, relative humidity and water vapour pressure increases because more water molecules

are required for saturation and this causes the air to become moist. As the temperature increases, the relative humidity and water vapour pressure decreases because less water molecules are required for saturation and this causes the air to become dry.

The average relative humidity and water vapour pressure inside the cave was significantly higher in February and March 2011 than in September 1992. Between February and March 2011, the cooler temperature was maintained inside the cave and during visits to the cave at this time, the cave was well ventilated and damp and water was observed seeping through the cave roof. This may explain the relative coolness inside the cave as well as the higher water vapour pressure. Cresswell's (1993) study was conducted during the hot and dry month of September and represents the minimum humidity at Historic Cave. Given that the 2011 study was carried out during a particularly wet period, arguably these results capture the higher end of the humidity levels at Historic Cave. The siege occurred in October and during a drought so the climate in the cave would have been hotter and drier at this time. Furthermore, during the siege fires were lit inside the cave. The smoke and heat caused by the fires may have increased the temperature in the cave which would have resulted in a decrease in water vapour pressure causing the air to become dry as any humidity inside the cave decreased during and immediately after the siege. It is likely that a combination of dry air and ventilation at the time of the siege contributed to the natural mummification of humans and animals at Historic Cave during and after the siege.

The 'Makapan Mummy' (A1081) was not buried in the sand but was in the upright kneeling position. Limestone or lime-rich sand could not have directly caused mummification as in the Korean mummies. Instead, the constant flow of dry air inside the cave desiccated the corpse and preserved the soft tissue. Cresswell (1993)

thought that the mummification of the Makapan Child was a unique occurrence and the product of a dry micro-climate surrounding the child, however the results from the February and March 2011 study indicate that the climate inside the cave is stable with minute differences between the base of the cave and the entrance to the cave. The 2001 to 2007 excavations yielded numerous desiccated human and animal remains which suggest that a broader environment in the cave, not just the micro-environment surrounding the Makapan Child, was dry enough to cause the desiccation of numerous human and animal remains.

Now that the factors and mechanisms of mummification have been discussed, it is necessary to review the structure of normal human skin and muscle tissue and compare this to the structure of naturally and artificially mummified human skin and muscle tissue.

4.6 The Structure of Normal Human Skin and Muscle Tissue

In the human body there are four types of tissue: connective tissue, epithelial tissue, muscular tissue and nervous tissue. Connective tissue is the support structure of the body and its tissue. Examples of connective tissue include, blood, cartilage, adipose tissue (fat) and fibrous and loose connective tissue. Connective tissue is made up of protein-rich collagen fibres that are extremely durable (Kanitakis 2002).

The human body is covered by two types of skin (Kanitakis 2002). The first type is hairless skin that is only found on palms and soles and has a thick epidermis. The second type is hairy skin that covers the rest of the body and has a thin epidermis. The structure of human skin is arranged in three layers: the epidermis, the dermis and the hypodermis (Figure A.9).

The epidermis is composed of stratified squamous epithelium and makes up the outer layer of the skin (Kanitakis 2002). In addition to the squamous cells (keratinocytes), the epidermis also contains melanocytes which are found in the basal layer of the epidermis, Langerhans cells and Merkel cells. The structure of the epidermis is defined by an upward development of squamous cells to the surface (Kanitakis 2002). This upward development of squamous cells forms four well-defined layers in the epidermis which are known as the basal layer at the bottom, followed by the spinous layer (prickle cell layer), the granular layer and the corneal layer (horny layer) at the surface. The corneal layer is characterised by flattened epidermal keratinocytes which are squamous cells that have lost their nuclei and cytoplasm during the transition to the surface. The main component of these flattened epidermal keratinocytes is keratin. Other morphological features of the epidermis are the exposed surface areas of eccrine sweat glands and hair follicles which are also made of epidermal cells (Kanitakis 2002: 1).

At the dermo-epidermal junction beneath the epidermis, the basement membrane of the basal layer separates the epidermis from the dermis. The dermis is a dense network of connective tissue that consists of two kinds of protein fibres: collagen fibres and elastin fibres. There are two layers within the dermis: the papillary (surface) layer and the reticular (basal) layer. The papillary layer is composed of loose collagen bundles and thin elastic fibres whereas the reticular layer is composed of coarse collagen bundles and elastic fibres (McGrath *et al.* 2010). Fibroblasts, mast cells and histiocytes are also found in the dermis. Below the dermis is the subcutaneous fatty layer known as the hypodermis. The hypodermis is composed of adipose or fatty tissue. The cells which form the fatty tissue are adipocytes (Kanitakis 2002: 1).

Environmental factors such as temperature, humidity and insect activity affect the rate of decomposition and results in gross morphological changes in the skin such as skin slippage, skin discolouration, blisters, dehydration and mummification (Bardale *et al.* 2011). Studies in skin histology have also shown that structural changes in the skin occur at the microscopic level during the postmortem period (Kovarik *et al.* 2005; Bardale *et al.* 2011). These changes include the appearance of vacuoles in the basal layer of the epidermis; the separation of the basal layer of the epidermis from the basement membrane at the dermo-epidermal junction; the degeneration of the dermis and the degeneration of the sweat glands through the appearance of vacuoles in the cytoplasm, a condition known as eccrine duct necrosis (Kovarik *et al.* 2005; Bardale *et al.* 2011). These histological changes occur in cool to warm temperatures.

Skeletal or striated muscle tissue is attached to the bones in most cases and the contraction of the muscle tissue allows for movement. The skeletal muscle is made up of muscle fibres (cells) which are arranged in parallel-running lines (Figure A.10). The muscle fibres are packed together in series of fascicles which are enclosed within a layer of connective tissue known as the epimysium. The epimysium prevents friction between the muscle and the bone (Martini 2005). Between each fascicle, or bundle of muscle fibres, is connective tissue known as the perimysium (Martini 2005). Within each fascicle, the individual muscle fibres are enclosed within connective tissue known as the endomysium. Nuclei are observed in the muscle cells. The skeletal muscle fibres are soft and fragile and are supported by the connective tissue (Martini 2005).

4.7 The Natural Mummification of Soft Tissue

The archaeological study of mummies originated in the 19th century with the investigation of Ancient Egyptian culture. In the 20th century, mummy studies evolved to include the examination of mummified skin and tissue through

histological and radiological techniques. This evolution was brought about by three major events: Zimmerman's (1972) comparison of mummified skin to modern human skin; the Palaeopathology Club; and the Manchester Museum Mummy Project (Aufderheide 2003). Today, the archaeological study of mummies involves an assessment of the preservation through various analytical and histological techniques. The findings of five studies and the methods used in each study are presented below.

4.7.1 Korean mummies

Two naturally mummified individuals were discovered buried in traditional Korean lime-enclosed wooden coffins (see Section 4.3.2). The first individual was a mummified male child from Yangju and was described by Shin *et al.* (2003b). The second individual was from Daejeon and was described by Chang *et al.* (2006a).

Light microscopy, electron microscopy and energy-dispersive spectroscopy (EDS) were performed on skin and muscle tissue samples from the naturally mummified Korean human remains. The analyses and results described by Shin *et al.* (2003b: 173 - 177) will be discussed here. For the light microscopy examination, the mummified tissue was rehydrated and fixed overnight in 4% paraformaldehyde. The mummified tissue samples were then dehydrated in a graded series of ethanol. After embedding in paraffin wax, sections were cut using a microtome, mounted onto slides and deparaffinised in xylene. The sections were stained with haematoxylin and eosin. The results of the light microscopy and electron microscopy showed that the structure of the skin remained intact with all three layers present (the epidermis, dermis and hypodermis).

In comparison to normal human skin, the mummified skin demonstrated a significant difference in the thickness of the layers. The epidermis, for example, showed a

reduction in thickness compared to the dermis. Within the epidermis, the only visible layer was the corneal layer as the granular, spinous and basal layers of the epidermis did not preserve (Shin *et al.* 2003b). Within the dermis only collagen fibres were identified by their characteristic cross striations when viewed by transmission electron microscopy (TEM) (Shin *et al.* 2003b). Images of the mummified skin tissue, keratinocytes and vessels were also observed using TEM (Shin *et al.* 2003b).

In the light microscopy examination of the muscle tissue, striated muscle tissue with parallel-running muscle fibres were stained red by the hematoxylin and eosin stains and nuclei were identified in the muscle cells (Shin *et al.* 2003b). The relatively good preservation of the muscle tissue was attributed to the localization of the muscle deep within the body. The skin, which had deteriorated, was exposed to harsh environmental elements (Shin *et al.* 2003b). The preservation of the collagen fibres was suggested to have been an important factor in maintaining the structure of the tissue (Shin *et al.* 2003b).

In the study conducted by Chang *et al.* (2006a), tissue samples were taken from the back muscle tissue of the mummy. For light microscopy, the sections were stained with haematoxylin and eosin, Masson's trichrome, Fontana-Masson, orcein and silver staining. Samples were cleaned with an ultrasonic cleaner and prepared according to Hayat (1970) and Bozzola & Russell (1992) for transmission electron microscopy (TEM) and scanning electron microscopy (SEM).

The light microscopy and electron microscopy results showed that the mummified tissue differed from normal human skin tissue in the reduction in thickness of the epidermis. The basal layer of the epidermis in the mummified skin was identified through Fontana-Masson staining and TEM which showed the presence of melanin

pigments (Chang *et al.* 2006a: 673). In normal human skin, melanin is usually found in melanocytes in the basal layer of the epidermis. Although the epidermis has decreased in thickness and is difficult to identify, the melanin pigments confirm the presence of the epidermis. In the dermis, collagen fibres were identified but reticular fibres and elastic fibres were absent. SEM images of the mummified skin showed hair shaft holes and a white substance on the surface of the skin. Using SEM and EDS, the white substance was found to be rich in calcium (Ca), a component of lime in which the corpses were sealed in (Chang *et al.* 2006a).

4.7.2 Native American mummies

Many naturally mummified human remains have been discovered in parts of North America, particularly in the southern and south western areas. In these areas, natural mummification occurred in rock shelters and caves. Two naturally mummified individuals, an adult male and an infant, were excavated at Canyon de Chelly and Canyon de Muerto in Arizona. Here, ancient burial practices involved the wrapping the corpse in a fur blanket or a deerskin and placing the corpse in a cave (El-Najjar *et al.* 1980: 198).

Skin tissue samples were collected from the adult male and the infant and analysed through light microscopy. The methods of analysis and the results were described by El-Najjar *et al.* (1980: 200 – 201). The desiccated skin samples were rehydrated in Ruffer's solution, sectioned according to normal surgical practices, and stained using Masson trichrome, periodic acid-Schiff, van Gieson and phosphotungstic acid haematoxylin staining. The results showed that no nuclei preserved. On the surface of the skin, hair shaft holes and erector muscles were present but no epidermal keratinocytes could be identified. El-Najjar *et al.* (1980: 198) suggests that the epidermal keratinocytes may have been compromised during the histological processing. In the dermis, connective tissue had preserved well.

4.7.3 Iceman's mummification

Ötzi the Iceman was a late Neolithic, approximately 45 year old male shepherd or hunter who may have died from a wound to the shoulder. His body became trapped by the ice layers and was only discovered in 1991, 5000 years after his death. Bereuter *et al.* (1997: 1034) investigated the circumstances leading to the mummification of Ötzi the Iceman by examining the mummified tissue through light microscopy and infrared spectroscopy (IR spectroscopy). The tissue samples were not fixed in formalin (Bereuter *et al.* 1997: 1034). Histological sections of the desiccated skin samples were stained with Goldner-Masson trichrome staining. Immunohistochemistry staining was used to identify squamous epithelial cells by using anti-cytokeratin AE1/AE3 antigens (Bereuter *et al.* 1997: 1034).

The results were described by Bereuter *et al.* (1997: 1034 – 1036). The Goldner-Masson trichrome staining identified the presence of collagen bundles in the dermis but no nuclei or other cellular structures had preserved. The immunohistochemistry staining demonstrated a loss of epidermis as squamous epithelial cells could not be identified (Bereuter *et al.* 1997: 1036). The loss of the epidermis as well as other keratin-based structures (hair and nails) suggests that the iceman was immersed in water first, which resulted in the disintegration of the epidermis, and then desiccated (Bereuter *et al.* 1997: 1036). This process of freeze-drying resulted in the dehydration and mummification of the corpse (Murphy *et al.* 2003: 615).

4.8 The Artificial Mummification of Soft Tissue

In cases of natural mummification, the desiccated skin is exposed to harsh environmental and chemical conditions which may break down the structure of the skin (Chapel *et al.* 1981). These conditions include extreme hot and cold temperatures, chemicals from the soil and clothing and animal wrappings. Artificial

mummification, however, maintains the integrity of the skin as it prevents fungal growth and bacterial activity (Chapel *et al.* 1981).

The skin from an embalmed Egyptian mummy of a 25 year old female was examined by transmission electron microscopy (TEM). The skin samples from the right shoulder of the mummy were rehydrated in a solution of cacodylate and sucrose. After preparation, the sections were examined in the transmission electron microscope and the results were described by Hino *et al.* (1982: 27 – 30). In the epidermis, epidermal cells and their organelles (mitochondria) were identified. Desmosomes are the intercellular bridges that help hold cells together and in the mummified tissue, desmosomes were observed in between the epidermal cells which were defined by their cell membranes. The basal membrane of the epidermis did not preserve. Collagen fibres and elastic fibres were identified in the dermis. Below the dermis, the hypodermis was present but had deteriorated. In both the dermis and hypodermis, bacterial spores were identified.

4.9 Conclusion

For mummification to occur, several environmental factors (extreme heat or cold, low humidity, and chemicals such as lime) are needed to preserve the soft tissue. Microscopic studies of desiccated tissue from naturally mummified human remains demonstrate that in cold or warm and dry conditions, particularly in caves and in lime-enriched environments, the mummification process results in distinct morphological changes in the soft tissue. These changes include a shrunken epidermis that is devoid of cells and the preservation of a dermis with the exclusive presence of collagen fibres. The microscopic studies further suggest that there are differences in the histologic structure of naturally mummified tissue and artificially mummified tissue. In comparison to natural mummification, the artificial mummification process ensures the integrity of the skin's structure with epidermal cells preserved along with

their organelles such as mitochondria. Collagen fibres and elastic fibres are also easily identified. Several techniques have been used to study desiccated tissue from the Republic of Korea, Egypt and the Americas but there is no standard technique for studying desiccated tissue from South Africa, particularly from Historic Cave.

5 MICROSCOPY OF MUMMIFIED TISSUE

5.1 Introduction

The aim of this study is to investigate, examine and compare the naturally desiccated tissue from Historic Cave using two microscopy techniques: scanning electron microscopy (SEM) and light microscopy. This chapter will describe, compare and discuss the methods used to prepare the desiccated tissue for scanning electron microscopy and light microscopy. All figures and images are presented in Appendix B.

5.2 Imaging

Scanning electron microscopy (SEM) is a non-destructive imaging technique that is used to observe and examine the surfaces of organic and inorganic material (Goldstein *et al* 1981). Energy-dispersive spectrometry (EDS) is an analytical technique that examines the chemical composition of specimens. A scanning electron microscope (Figure B.1) equipped with an energy-dispersive spectrometer is used to conduct an elemental analysis on a specimen to determine its chemical composition and to ascertain the origin of the material.

Atomic absorption spectrometry (AAS) is another analytical technique used to analyse the composition of a specimen. When using AAS to analyse mummified tissue, the specimen must either be digested or ashed and then made into a solution for the analysis. The specimen could also be ground into a fine powder but this method of preparation could contaminate the sample. For SEM, the specimen can be coated with a conductive material such as carbon, gold or platinum prior to analysis. The sample preparation for AAS is too destructive for examining the structure of mummified tissue. SEM is better suited for mummified tissue because specimens to be examined should be hard and dry and the outer integrity of the tissue is preserved.

The principle of using electrons to increase resolution is similar in transmission electron microscopy (TEM) and SEM. However, there are several differences between SEM and TEM. Using SEM, large areas of the tissue's surface can be observed at high magnification and resolution levels (Hayat 1978). Using TEM, the tissue must be sectioned but for SEM the tissue does not need to be sectioned and this makes the sample preparation of hard, dry and mummified tissue easier.

Because SEM is better suited for analysing mummified tissue, it is important to understand how it works (Figure B.2). At one end of the scanning electron microscope there is an electron gun that produces a beam of electrons which travels in a vacuum in the form of a beam. The electron beam is focused on the surface of the specimen using electromagnetic lenses. When the electron gun is focused on the specimen, it scans the specimen's surface in a raster pattern (Hayat 1978). As the beam scans the surface, secondary electrons are produced which are collected to form an image of the specimen's surface. In addition to the secondary electrons, X-rays are produced. These X-rays have an energy characteristic of the element struck by the electron beam. These X-rays can be collected and their energy measured and the chemical elements present in the specimen can be identified (Hayat 1978).

There are numerous studies using SEM as a method of analysis for mummified tissue. For example, the preserved skin structure from the fifteenth-century mummy from Daejeon in the Republic of Korea was examined using SEM, TEM and EDS (Chang *et al.* 2006a). SEM and EDS was also used in a study by Hoesen & Arriaza (2011) who used these imaging and analytical techniques to investigate the use of clay in the mummification of the Chinchorro mummies from northern Chile. In this study of mummified human remains from Historic Cave, desiccated tissue samples will be examined through SEM and the results of the SEM observations will be compared to other cases of natural and artificial mummification.

5.3 Light Microscopy

Histology is the study of tissues. In histology, sections of tissue are stained with a variety of dyes which stain each structure of tissue such as nuclei, muscle, fibrin, collagen fibres and elastic fibres. After staining, one or more of these tissue structures can be identified when the section of tissue is observed under the light microscope (Figure B.3). For connective tissue, trichrome staining techniques are used to demonstrate muscle, nuclei, fibres, fibrin, erythrocytes, collagen fibres and elastic fibres. There are two particular trichrome staining techniques used for connective tissue and they are the Masson trichrome technique and the Verhoeff's elastic tissue stain. Other stains include the hematoxylin eosin stain which demonstrates muscle fibres and collagen fibres and the aldehyde fuchsin stain which demonstrates elastic tissue fibres (Jones 2002).

5.4 Materials and Methods

The microscopic analyses will record and describe the morphological features of the desiccated tissue. The morphology and histological structure of the naturally desiccated tissue from Historic Cave will be compared to normal and mummified human tissue. Through this comparative analysis it may be possible to determine the most suitable method for examining desiccated tissue from Historic Cave.

5.4.1 Materials

Desiccated tissue specimens were sampled from human remains excavated at Historic Cave from 2001 to 2007. Three bone specimens, a mandible, a maxilla and a rib were selected for analyses (Figure B.4 to Figure B.6). Desiccated tissue specimens were sampled from the outer surface of the right side of the mandible (74–Dg1 M1 RBL), the inferior surface of the hard palate of the superior maxilla (69–M1 RBL) and from the inner surface of the rib (50–M1 RBL). Each desiccated tissue specimen was cut

into five samples: the samples from the mandible were labelled 74-1 to 74-5; the samples from the palate were labelled 69-1 to 69-5; and the samples from the rib were labelled 50-1, 50-2, 50-4, 50-5 and 50-7.

Desiccated tissue specimens were also sampled from the 'Makapan Mummy' (A1081). One desiccated tissue specimen (A1081-5) was sampled from the anterior surface of the right humerus of the mummy (Figure B.7). This specimen was cut into three samples labelled 5-1 to 5-3. Three desiccated tissue specimens, labelled A1081-1 to A1081-3, were sampled from the exposed upper anterior femoral region (inner thigh/groin) of the mummy (Figure B.8). The permits for the examination of human remains were obtained from the School of Anatomical Sciences, University of the Witwatersrand and from the South African Heritage Resource Agency (SAHRA) (Figure B.9).

5.4.2 Photography

The specimens used in this study were photographed prior to the analyses. The desiccated tissue samples from the mummy, the mandible, the palate and the rib were photographed under the stereo microscope (Figure B.10). A Nikon Coolpix 5400 (Tokyo, Japan) digital camera equipped with a Nikon Coolpix Lenz was used together with the stereo microscope (Kyowa, Tokyo) to photograph the tissue specimens. The ventral and dorsal surfaces of the tissue specimens were photographed.

5.4.3 Scanning electron microscopy (SEM)

The desiccated tissue samples from right arm of the mummy (5-2 and 5-3), from the mandible (74-2 and 74-3) and from the palate (69-1 and 69-2) were selected for SEM. All tissue samples were mounted onto a carbon disk and gold coated in an Emitech

K550V sputtercoater. The gold coated tissue samples were then scanned in a JEOL JSM-5600 scanning electron microscope. Energy dispersive spectroscopy (EDS) was used to analyse the chemical composition of the tissue samples. Table B.1 summarises the SEM procedures used in this study.

The desiccated tissue sample from the right arm of the mummy (5-2) was examined in the scanning electron microscope. The microscopic image produced by the scanning electron microscope showed that the surface of tissue was covered by dust and debris which obscured the surface detail of the tissue. The desiccated tissue samples from the palate (69-1 and 69-2) were cleaned (sonicated) in acetone for 10 minutes in an ultrasonic cleaner. Once the samples were dry, they were gold coated for four minutes and examined in the scanning electron microscope.

Following Chang *et al.* (2006a; 2006b), another cleaning method was used. Sample 5-3 from the right arm of the mummy was fixed in absolute alcohol for 24 hours. The sample was cleaned in absolute alcohol for 5 minutes in the ultrasonic cleaner. The absolute alcohol was replaced and the sample was cleaned for another five minutes. The sample was then dried for two hours in an oven at 60°C. Once dry, the sample was gold coated for four minutes and examined in the scanning electron microscope.

Acetone evaporates quickly and its use in the cleaning method reduces the time it takes to dry the tissue. The desiccated tissue sample from the mandible (74-2) was fixed in absolute alcohol for 24 hours and cleaned in absolute alcohol for five minutes in the ultrasonic cleaner. The absolute alcohol was replaced and the sample was cleaned for another five minutes. After the sonication procedure, the sample was placed in acetone for five minutes and left to dry for approximately ten minutes. The

sample was then gold coated for six minutes to prevent the sample from charging in the electron beam. It was then examined in the scanning electron microscope.

A desiccated tissue sample from the mandible (74-3) was photographed and examined under the stereo microscope. During the stereo examination of the sample, minute structures were observed on the ventral surface of the tissue. These minute structures resembled hairs. The sample was cleaned in acetone for five minutes in the ultrasonic cleaner. The acetone was replaced and the sample was cleaned for another five minutes. The sample was left to dry for ten minutes after which it was photographed under the stereo microscope. The sample was mounted onto a carbon disk, with the ventral surface facing up and gold coated for six minutes prior to SEM.

Two hair samples were taken from the author of this study for comparison with the hairs found on the desiccated tissue. One hair sample was taken from the scalp and another hair sample was taken from the arm of the author.

5.4.4 Light microscopy

Desiccated tissue samples from the right arm (5-1) and the exposed upper anterior femoral region of the mummy (A1081-1 to A1081-3), and from the mandible (74-1, 74-4 and 74-5), the palate (69-3 to 69-5) and the rib (50-1 to 50-5 and 50-7) were selected for histological analyses. All the samples were sectioned after embedding in paraffin wax with a microtome at 5µm and floated in a water bath at 50°C. Once dry, the sections were mounted onto adhesive slides. The Masson trichrome technique, the hematoxylin-eosin stain, Verhoeff's elastic tissue stain and the aldehyde fuchsin hematoxylin-eosin stain were used to stain the sections. The histological procedures used to prepare the desiccate tissue from Historic cave for light microscopy are presented in Table B.2.

The Masson trichrome technique uses three dyes for the demonstration of connective tissue. This technique stains nuclei blue or black, muscle fibres, fibrin and erythrocytes red and collagen fibres green (Jones 2002). In normal human skin, the epidermis is stained red and the connective tissue is stained green by the Masson trichrome technique. After embedding in paraffin wax, the sections were deparaffinised in xylene and a graded series of alcohol and then washed in water. The mercury pigment was removed by an iodine and sodium thiosulfate sequence. The sections were washed in tap water and stained with the celestine blue-hematoxylin method which stains nuclei (Jones 2002). Next, the sections were differentiated with 1% acid alcohol and washed in tap water. The sections were then stained for five minutes in an acid fuchsin solution which comprises 0.5g acid fuchsin, 0.5ml glacial acetic acid and 100ml distilled water. After the sections were rinsed in tap water, they were stained for five minutes in a phosphomolybdic solution which comprises 1g phosphomolybdic acid and 100ml distilled water. The sections were drained and stained for two to five minutes in a methyl blue solution which consists of 2g methyl blue, 2.5ml glacial acetic acid and 100ml distilled water. The sections were then treated with 1% acetic acid for two minutes. Lastly, the sections were dehydrated through a graded series of alcohol, cleared in xylene and the coverslip was mounted on the glass slide using entellan.

The Verhoeff's method is used to demonstrate elastic fibres. The Verhoeff's method stains elastic fibres dark red or black, nuclei grey-black and muscle fibres red. The van Gieson solution, which is used as a counterstain, stains connective tissue yellow. After embedding in paraffin wax, the sections were deparaffinised in xylene and a graded series of alcohol and then washed in tap water. The sections were stained for 20 minutes with Verhoeff's solution which comprises 5ml of 5% alcoholic hematoxylin, 2ml of 10% Ferric chloride and 2ml Lugol's iodine. The sections were washed gently in tap water and differentiated in 10% ferric chloride. This differentiation of the sections was repeated until the elastic fibres were black. After

the sections were washed in tap water, they were counterstained for two to three minutes with the van Gieson solution which comprises 50ml of 1% acid fuchsin, 450ml saturated aqueous picric acid and 500ml distilled water. The sections were blot dried and the coverslip was mounted on the glass slide using entellan.

The hematoxylin-eosin stain is used to demonstrate muscle striations and collagen fibres in tissue sections (Jones 2002). This technique stains muscle fibres and collagen fibres pink, fibrin pink and cartilage purple. After embedding in paraffin wax, the sections were deparaffinised in xylene and a graded series of alcohol and then rinsed gently in tap water. The sections were immersed in hematoxylin for 15 minutes, rinsed in tap water and differentiated in 1% acid alcohol. The sections were counterstained in eosin for three minutes, rinsed in tap water and differentiated in 1% acid alcohol. The sections were dehydrated in a graded series of alcohol and cleared in xylene. Lastly, the coverslip was mounted on the glass slide using entellan.

Hematoxylin-eosin can also be used with aldehyde fuchsin to stain elastic tissue blue-purple. After embedding in paraffin wax, the sections were deparaffinised in xylene and a graded series of alcohol and then washed in tap water. The sections were immersed in aldehyde fuchsin for 15 minutes and rinsed in tap water. The sections were then differentiated in 1% acid alcohol briefly and washed in tap water. The hematoxylin stain was used to stain the sections for 15 minutes. After this, the sections were rinsed in tap water, differentiated in 1% acid alcohol and then counterstained in eosin for three minutes. Lastly, the sections were washed and the coverslip was mounted on the glass slide using entellan.

The desiccated tissue sample from the right arm of the mummy (5-1) was fixed in 10% buffered formalin for 24 hours. The desiccate rib tissue (50-1) was fixed in 10%

buffered formalin for seven days. After embedding in paraffin wax, transverse sections of the tissue were cut. Sample 5-1 was stained with the Masson trichrome stain and sample 50-1 was stained with the hematoxylin-eosin stain.

Tissue samples from the mandible (74-1), the palate (69-3) and the rib (50-2) were photographed and examined under the stereo microscope. The photographs showed that the surfaces of the desiccated tissue samples were covered by dust. The samples were cleaned in absolute alcohol for five minutes in the ultrasonic cleaner. The absolute alcohol was replaced and the samples were cleaned for another five minutes. The samples were oven dried for two hours and then fixed in 10% phenol for seven hours. Sample 69-3 was fixed in 10% buffered formalin for 24 hours prior to paraffin embedding. Transverse sections of the tissue were cut from samples 74-1 and 69-3 and longitudinal sections of the tissue were cut from sample 50-2. The sections were stained with the Masson trichrome stain and the hematoxylin-eosin stain.

A number of histological studies on mummified tissue have experimented with rehydration techniques. In this study, four rehydration solutions were used on the desiccated tissue specimens. The solutions used were the Sandison's rehydration solution (Sandison 1955; Sandison 1970), the Ruffer's rehydration solution (Zimmerman *et al.* 1971; Zimmerman & Smith 1975), glycerine (Evans 1962) and 10% buffered formalin.

For the Sandison's method, the rehydration solution was made up of three parts 96% ethyl alcohol, five parts 1% aqueous formalin, two parts 5% sodium carbonate and saline to make 0.85% (Sandison 1955; Sandison 1970). The sample was suspended in the rehydration solution, one to 25 parts, for 24 hours at room temperature. The sample was then fixed in 10% buffered formalin prior to paraffin embedding

(Sandison, 1955; Sandison, 1970). For the Ruffer's method, the rehydration solution was made up of five parts water, three parts absolute ethyl alcohol, two parts 5% sodium carbonate and saline to make 0.85% (Zimmerman *et al.* 1971; Zimmerman & Smith 1975). The sample was suspended in the rehydration solution, one to 25 parts, for 24 hours at room temperature. The sample was then fixed in 10% buffered formalin prior to paraffin embedding (Zimmerman *et al.* 1971; Zimmerman & Smith 1975). For the Evans (1962) rehydration method, the samples were soaked in glycerine for seven days and fixed in 10% buffered formalin prior to embedding in paraffin wax. Lastly, 10% buffered formalin was used to rehydrate mummified tissue.

Samples from the desiccated tissue of the mandible (74-4 and 74-5) and the palate (69-4 and 69-5) were photographed and examined under the stereo microscope prior to histological analyses. Under the stereo microscope, dust and structures that resembled minute hairs were observed on the ventral surfaces of the samples from the mandible. The samples were cleaned in acetone for ten minutes in the ultrasonic cleaner. The acetone was replaced and the samples were cleaned for another ten minutes. Samples 74-4 and 69-4 were rehydrated in the Sandison's solution, sample 74-5 was rehydrated in glycerine and sample 69-5 was rehydrated in the Ruffer's solution. After embedding in paraffin wax, transverse sections of the tissue were cut. The sections were stained with the Masson trichrome stain and the haematoxylin-eosin stain.

Three desiccated samples from the rib (50-4, 50-5 and 50-7) were photographed and examined under the stereo microscope prior to histological analysis. The samples were cleaned in absolute alcohol for five minutes in the ultrasonic cleaner. The absolute alcohol solution was replaced and the samples were cleaned for another five minutes. Sample 50-4 was rehydrated in the Ruffer's solution, sample 50-5 was rehydrated in the Sandison's solution and sample 50-7 was rehydrated in 10%

buffered formalin. After embedding in paraffin wax, longitudinal sections of the tissue were cut. The sections were stained with the aldehyde haematoxylin-eosin stain and the Verhoeff's elastic tissue stain.

The three desiccate tissue specimens from the exposed upper anterior femoral region of the mummy (A1081-1 to A1081-3) were photographed and examined under the stereo microscope. The stereo microscope observation showed that the surfaces of the specimens were covered by a layer of dust and debris. The desiccated tissue specimens were cleaned in absolute alcohol for five minutes in the ultrasonic cleaner. The absolute alcohol was replaced and the specimens were cleaned for another five minutes. Sample A1081-1 was rehydrated in the Ruffer's solution, sample A1081-2 was rehydrated in glycerine and sample A1081-3 was rehydrated in the Sandison's solution. After embedding in paraffin wax, longitudinal sections of the tissue were cut. The sections were stained with the Masson trichrome stain and the haematoxylin-eosin stain.

6 RESULTS

6.1 Introduction

Scanning electron microscopy (SEM) examines the surface morphology of the desiccated tissue and energy-dispersive spectrometry (EDS) analyses the chemical component of the tissue. Light microscopy investigates the histological structure of the desiccated tissue. This chapter will present the results if the SEM, EDS and light microscopy analyses of the desiccated tissue from Historic Cave. Table C.1 presents the SEM and EDS results and Table C.2 presents the light microscopy results. All figures and tables are presented in Appendix C.

6.2 SEM of A1081-5 from the ‘Makapan Mummy’ (A1081)

The desiccated tissue sample 5-2 was photographed under the stereo microscope (Figure C.1). The stereo microscope observation showed that the surface of the sample was covered by dust. The sample was examined in the scanning electron microscope without undergoing sonication. The microscopic images from the SEM examination showed that the surface of the desiccated tissue was coated with a layer of dust and debris (Figure C.2). Energy-dispersive spectrometry (EDS) was used to analyse the coating on the surface of the tissue and the results showed that the coating contained carbon (C), oxygen (O), sodium (Na), magnesium (Mg), potassium (K), calcium (Ca) and very high count of silicon (Si). Even though dust and debris coated the surface of the tissue, flattened epidermal keratinocytes were observed in patches (Figure C.3). No other morphological features of the skin (hair and hair shaft holes) were identified.

During the examination of sample 5-3 under the stereo microscope, a layer of dust and debris was observed on the surface of the desiccated tissue (Figure C.4). The desiccated tissue was cleaned in absolute alcohol and scanned in the scanning

electron microscope. Flattened epidermal keratinocytes (Figure C.5) and hair shaft holes (Figure C.6) were observed on the surface of the desiccated tissue and these morphological features confirm that the tissue is skin tissue. EDS of the sonicated surface showed that the presence of carbon (C), oxygen (O), sodium (Na), magnesium (Mg), potassium (K) and calcium (Ca) were lower than in the unsonicated sample from the mummy (5-2). Even though much of the dust and debris were removed during the sonication treatment, some foreign particles were left behind and when using EDS, a high calcium count was detected (Figure C.7). In addition, although the presence of silicon (Si) had decreased, in some areas the silicon (Si) count remained high.

6.3 SEM of Specimen 69 from the Palate (69-M1 RBL)

Desiccated tissue samples from the inferior surface of the hard palate of the maxilla were photographed and examined under the stereo microscope. During the examination under the stereo microscope, a layer of dust and debris was observed coating the surface of the desiccated tissue. The tissue samples were cleaned in acetone which removed some of the dust and debris. EDS showed higher counts of carbon (C), oxygen (O), sodium (Na), magnesium (Mg), silicon (Si), potassium (K) and calcium (Ca).

6.4 SEM of Specimen 74 from the Mandible (74-Dg1 M1 RBL)

The desiccated tissue sample 74-2 was photographed and examined under the stereo microscope. The sample was cleaned in absolute alcohol before SEM. The SEM observation showed that not much dust could be seen as in the SEM observation of sample 5-2 (Figure C.8). EDS of the sonicated surface of the tissue showed the presence of magnesium (Mg), phosphorus (P), potassium (K) and calcium (Ca). The presence of carbon (C), sodium (Na) and silicon (Si) were reduced in some areas of

the surface and absent in others. Flattened epidermal keratinocytes, which were observed in patches (Figure C.9), and numerous hair shaft holes (Figure C.10) were identified on the surface of the tissue. The presence of these morphological structures confirms that the tissue is skin. The most significant find in this analysis was the structure resembling a hair (Figure C.11).

Minute structures were observed on the surface of sample 74-3 during the stereo microscope examination (Figure C.12 and Figure C.13). The minute structures were protruding outward and appeared to be embedded in the skin. Based on this as well as on their superficial morphology, the minute structures were believed to resemble hair. Acetone was used to clean the tissue. The SEM observation showed that the surface of the skin was coated in a layer of dust and debris (Figure C.14 and Figure C.15). EDS of the sonicated layer showed the presence of potassium (K) and in some places calcium (Ca). Carbon (C), magnesium (Mg) and silicon (Si) either had decreased counts or were absent. Even though there was a layer of dust coating the surface of the tissue, the structures resembling hairs could clearly be seen. Approximately six of these structures were observed on the surface of the skin tissue (Figures C.14 and Figure C.16 to Figure C.20).

6.5 SEM of Normal Human Hair

Two hair samples were taken for analysis in the scanning electron microscope. One hair was taken from the scalp (Figure C.21) and the second hair was taken from the arm (Figure C.22) of the author of this paper. The hair control from the scalp measured 91.6 μ m in diameter whereas the hair control from the arm measured 34.2 μ m in diameter.

6.6 Light Microscopy Results of Specimen 50 from the Rib (50-M1 RBL)

The desiccated tissue sample 50-1 was prepared for histological analysis without undergoing sonication. As stated previously, the hematoxylin-eosin stain is used to demonstrate muscle striations by staining muscle fibres pink. The section from sample 50-1 was stained pink by the hematoxylin-eosin stain which indicates that the section is a cross-section of muscle tissue (Figure C.23). Dust was embedded in the tissue (Figure C.23A). Fascicles were observed but the individual muscle fibres could not be observed. The nuclei were absent but blood vessels were identified between the fascicles (Figure C.23B).

The desiccated tissue sample 50-2 was examined under the stereo microscope and a layer of dust and debris was observed on the surface of the tissue (Figure C.24). The sample was cleaned in absolute alcohol prior to sectioning. The hematoxylin-eosin stained the section pink which indicates that the section is a longitudinal section of striated muscle tissue (Figure C.25). The muscle tissue was arranged as a series of fascicles but the individual muscle fibres and their nuclei were not discernible. The perimysium was identified between the fascicles. The Masson trichrome stained the section red and green (Figure C.26). The muscle tissue was stained red and arranged as a series of fascicles. The individual muscle fibres and their nuclei could not be identified. The collagen fibres were stained green and were identified as the connective tissue encompassing the fascicles (epimysium) and the connective tissue found between the fascicles (perimysium). The hematoxylin-eosin stain and the Masson trichrome stain identified the sample from the rib as striated muscle tissue with parallel-running muscle fibres arranged in a series of fascicles and with connective tissue between the fascicles.

The desiccated tissue sample 50-4 was photographed and examined under the stereo microscope (Figure C.27). The sample was cleaned in absolute alcohol and

rehydrated in the Ruffer's solution prior to sectioning. Prior to analysis, the sample was photographed under the stereo microscope. The stereo microscope observation showed that surface of the sample was not covered by as much dust as the samples that were not cleaned in absolute alcohol. Under the light microscope, the section was stained deep pink by the aldehyde hematoxylin-eosin stain (Figure C.28). The tissue was identified as a longitudinal section of striated muscle tissue that is arranged as a series of fascicles. No nuclei or connective tissue were identified. The sections stained by the Verhoeff's method had deteriorated to a point where the structure could not be identified.

The desiccated tissue sample 50-5 was photographed and examined under the stereo microscope (Figure C.29). The sample was cleaned in absolute alcohol and rehydrated in the Sandison's solution prior to sectioning. After cleaning, the sample was photographed under the stereo microscope. The stereo microscope observation showed that surface of the sample was not covered by as much dust as the samples that were not cleaned in absolute alcohol. The section was stained deep pink by the aldehyde hematoxylin-eosin stain (Figure C.30). The section was identified as a longitudinal section of striated muscle tissue that is formed by a series of fascicles which are bundles of parallel-running muscle fibres. Nuclei were not identified in the muscle cells and connective tissue was not observed. With the Verhoeff's stain, the connective tissue was stained yellow and the tissue was identified as a longitudinal section of muscle tissue (Figure C.31). Fascicles were identified but the individual muscle fibres and their nuclei were not observed. The aldehyde hematoxylin-eosin stain and the Verhoeff's stain identify the sample from the rib as striated muscle tissue with connective tissue.

Sample 50-7 was cleaned in absolute alcohol and rehydrated in 10% buffered formalin for 24 hours prior to sectioning. The sections were stained with aldehyde

hematoxylin-eosin and Verhoeff's elastic tissue stain. During the sectioning and staining, the tissue was very fragile and washed away when sectioned. The sections stained the aldehyde hematoxylin-eosin washed away during the staining procedure and the sections stained with the Verhoeff's method were poorly preserved as the structure of the tissue did not remain intact.

6.7 Light Microscopy Results of Specimen 69 from the Palate (69-M1 RBL)

The desiccated tissue sample 69-3 was photographed and examined under the stereo microscope (Figure C.32). The stereo microscope observation showed that the surface of the sample was covered by dust. The sample was cleaned in absolute alcohol prior to sectioning. The sections were identified as muscle tissue because the Masson trichrome stained the tissue red (Figure C.33). Collagen fibres were stained green by the Masson trichrome stain but were in a state of deterioration. The muscle tissue was stained pink with the hematoxylin-eosin stain and the tissue was identified as a cross-section of muscle tissue (Figure C.34). Fascicles were identified but unlike cross-sections of normal human muscle tissue, the individual muscle fibres were not discernible in the desiccated tissue sections. Between the fascicles, although not easily identified, is the connective tissue known as the perimysium.

The desiccated tissue sample 69-4 was photographed and examined under the stereo microscope (Figure C.35A). Dust was observed on the surface of the tissue. The sample was cleaned in acetone and rehydrated in the Sandison's solution prior to sectioning. The section was stained red and green by the Masson trichrome stain (Figure C.36). The tissue was identified as a dense network of connective tissue with muscle fibres. The epithelium was identified but it had separated from the underlying connective tissue. The section was stained pink by the hematoxylin-eosin stain and identified as connective tissue with collagen and muscle fibres (Figure C.37). The epithelium was identified overlying the dense network of connective tissue.

The desiccated tissue sample 69-5 was photographed and examined under the stereo microscope (Figure C.35B). Dust was observed on the surface of the tissue. The sample was cleaned in absolute alcohol and rehydrated in the Ruffer's solution prior to sectioning. The sections stained by the hematoxylin-eosin stain were poorly preserved and washed away during sectioning. The sections stained by the Masson trichrome stain were slightly better preserved although dust was embedded in the tissue (Figure C.38). The tissue was identified as connective tissue with muscle fibres and collagen fibres. Epithelial tissue could not be identified.

6.8 Light Microscopy Results of Specimen 74 from the Mandible (74-Dg1 M1 RBL)

The desiccated tissue specimen from the outer surface of the right side of the mandible excavated at Historic Cave was examined through histological analyses (Figure C.39). The stereo microscope observation showed that the surface of the sample was covered by a layer of dust. During the SEM analysis, epidermal keratinocytes, hairs and hair shaft holes were observed on the surface of the tissue which identified the tissue as skin.

The desiccated tissue sample (74-1) was photographed and examined under the stereo microscope (Figure C.40). The stereo microscope observation showed that a layer of dust covered the surface of the sample. The sample was cleaned in absolute alcohol prior to sectioning. The section was stained intensely red by the Masson trichrome stain and identified as striated muscle tissue composed of bundles of parallel-running muscle fibres known as fascicles (Figure C.41). Nuclei were not observed. Collagen fibres were stained green but they were not easily discernible. These collagen fibres form the connective tissue known as the perimysium. The hematoxylin-eosin stained the section pink and identified the tissue as striated muscle tissue composed of

fascicles (Figure C.42). Nuclei were not identified. The connective tissue known as the perimysium was observed between the fascicles.

The desiccated tissue sample 74-4 was photographed and examined under the stereo microscope (Figure C.43). Dust was observed on the surface of the sample. The sample was cleaned in acetone and rehydrated in the Sandison's solution prior to sectioning. The Masson trichrome stained the section red and green (Figure C.44). Dust was embedded in the tissue. The section was identified as striated muscle tissue with parallel-running muscle fibres packed into bundles known as fascicles. The nuclei were not observed but collagen fibres were stained green and were identified as the connective tissue known as the epimysium and the perimysium. The striated muscle tissue was stained pink by the hematoxylin-eosin stain. The muscle fibres were identified and connective tissue known as the perimysium was observed in between the fascicles (Figure C.45). Dust was embedded in the tissue. No epidermis or hair follicles were identified in either the sections stained by the Masson trichrome stain or the hematoxylin-eosin stain.

The desiccated tissue sample 74-5 was photographed and examined under the stereo microscope (Figure C.46). The stereo microscope observation showed that a layer of dust covered the surface of the sample. The sample was cleaned in absolute alcohol and rehydrated in glycerine prior to sectioning. The section was stained red and green by the Masson trichrome stain (Figure C.47). Although the structure of the tissue is well preserved, dust was embedded in the tissue. The tissue was identified as skin tissue and the general architecture of the skin was well preserved. The epidermis was identified as it was stained red by the Masson trichrome stain. However, the corneal layer and basal layer could not be identified which indicates that the epidermis had decreased in thickness. The underlying dermis is connective tissue that is composed of collagen fibres and muscle fibres. The section was stained pink with the

hematoxylin-eosin stain (Figure C.48). Dust was embedded in the tissue. The structure of the tissue was not as well-preserved as seen in the section stained with the Masson trichrome technique because the epidermis could not be identified. However, the muscle fibres and collagen fibres that were stained pink form the remaining dermis.

6.9 Light Microscopy Results of the Specimens from the ‘Makapan Mummy’ (A1081)

The desiccated tissue specimens A1081-1 (Figure C.49) and A1081-2 (Figure C.50) were photographed and examined under the stereo microscope. The surfaces of the specimens were covered by a layer of dust and debris. The specimens were cleaned in absolute alcohol. Specimen A1081-1 was rehydrated in the Ruffer’s solution and A1081-2 was rehydrated in glycerine prior to sectioning. The light microscopy examination showed that tissue was deeply embedded with dust. The tissue was fractured and poorly preserved.

The desiccated tissue sample A1081-3 (Figure C.51) was photographed and examined under the stereo microscope. The surface of the specimen was covered by a layer of dust and debris. The sample was rehydrated in Sandison’s solution prior to sectioning. Under the light microscope, the sample showed a better preservation of structure compared to samples A1081-1 and A1081-2. The location of the sample in the upper femoral region suggests that the tissue is muscle tissue and this was confirmed by the Masson trichrome staining which demonstrated striated muscle tissue with parallel-running muscle fibres and connective tissue known as the perimysium (Figure C.52). The striated muscle tissue was stained pink by the hematoxylin-eosin stain but compared to the section stained by the Masson trichrome stain, the section stained by hematoxylin-eosin was in a poorer state of preservation (Figure C.53).

7 DISCUSSION

7.1 Introduction

The desiccated tissue was sampled from the 'Makapan Mummy' (A1081) and from the human remains excavated at Historic Cave. Three techniques, scanning electron microscopy (SEM), energy-dispersive spectroscopy (EDS) and light microscopy were used to examine the desiccated tissue. A number of different protocols were employed to clean, rehydrate, stain and analyse the desiccated tissue. In this chapter, the morphology of the desiccated skin tissue from Historic Cave will be described. The general histology of the skin and muscle will be discussed and compared to the histology of normal and mummified human skin. Lastly, the methods of tissue preparation and examination will be compared.

7.2 The Morphology of the Surface of the Skin

During the SEM observations of the skin, epidermal keratinocytes, hair shaft holes and hairs were identified on the surface of the skin. Although the epidermal keratinocytes were identified, the epidermal layer demonstrated a poor state of preservation as the epidermal keratinocytes appeared in patches along the skin's surface. Hairs and hair shaft holes were also identified during the SEM observation of the desiccated skin. However, like the epidermal keratinocytes, the hairs were only identified on two out of the four samples analysed. It is possible that because the structure of the desiccated skin is so fragile, the overlying epidermal layer may have been lost during the sample preparation for SEM and histology. It is also possible that over the years, the constant handling of the mummy and desiccated human remains may have compromised the integrity of the epidermal layer of the desiccated skin.

In this study, two normal human hairs, one from the scalp and one from the arm, were scanned in the scanning electron microscope and the diameters of both hair samples

were measured in the electron microscope. These hair samples were taken from the author of this paper and used as control samples to be compared to hair found on the desiccated tissue from the mandible. The hair control from the scalp measured 91.6µm in diameter whereas the hair control from the arm measured 34.2µm in diameter. So considering that there is difference in diameter between hair from the scalp and hair from the arm, there must be an even bigger difference in diameter between the hair from the mandible, which is possibly much thinner, and the hair from the scalp and the arm.

The human hair is a great source of information about an individual's life and diet and if the person died, the human hair may also provide information about the manner of death (Hashimoto 1988; Saukko & Knight 2004). Changes in the body that are caused by diet, disease, illness and exposure to harsh environmental conditions result in morphological changes to the human hair (Saukko & Knight 2004; Kempson *et al.* 2006). Some poisons, such as arsenic and other heavy metals, can be detected by analysis of the hair (Daniel *et al.* 2004; Kempson *et al.* 2006). So by examining human hair, biological, archaeological and forensic information about the individual can be gathered. The hair found on the naturally desiccated human tissue from Historic Cave was examined using SEM and EDS. The EDS results showed that the hairs were negative for arsenic or any other heavy metals.

Normal human hair is structured into three main layers: the cuticle (outermost layer), the cortex (middle layer) and the medulla (innermost layer) (McCrone & Delly. 1973). The outermost layer, the cuticle, is composed of several overlapping keratin scales. There are two types of human hair: terminal hair and vellus hair. The majority of hairy regions of the human body are covered by terminal hair, except for the face and the back of the neck which, particularly in women and children, are covered by vellus hair. Vellus hair is much thinner than terminal hair and is usually between one

and two centimetres long (Ahluwalia 2009). It is only found on the face and back of the neck, particularly in women and children as men have thicker body hair.

Analytical data on vellus hair are quite limited but a study done by Ahluwalia (2009) analysed vellus hair through SEM. Vellus hair samples were taken from the middle jaw line of nine individuals and the results showed that vellus hair is very similar to terminal hair in appearance except in the diameter values (Ahluwalia 2009). Based on the study, the vellus hair diameter ranges from 12 μm to just over 30 μm with an average of 22 μm (Ahluwalia 2009). The results also showed that the spacing of the keratin scales is larger on vellus hairs than on terminal hairs (Ahluwalia 2009). Terminal human hairs are between 50 μm and 150 μm in diameter (McCrone & Delly 1973).

The desiccated skin tissue (74-3) from the mandible excavated at Historic Cave was analysed through SEM. The SEM observation of the skin showed numerous minute structures resembling hairs on the skin's surface. The skin was taken from the outer surface of the left side of the mandible, an area known as the facial region. The mandible was identified as male because the chin is square and it has a prominent mental eminence. The hairs found in the facial regions are known as vellus hairs, however, in males terminal hairs are also found in the facial region. Six hairs on the surface of the desiccated skin from the mandible were measured and the approximate diameters of the hairs were between 9.16 μm and 15.6 μm . These results are consistent with the results from the study conducted by Ahluwalia (2009) which suggests that the hairs from the desiccated tissue are vellus hairs. It is also possible that the hairs from the desiccated tissue are terminal hairs that have been exposed to shrinkage caused by desiccation.

Studies on natural and artificial mummification have investigated the morphology of the desiccated tissue and have also compared mummified human hair to normal human hair (Conti-Fuhrman & Rabino-Massa 1972; Hrdy 1978; Chang *et al.* 2006b; Mansilla *et al.* 2011). These studies have largely focused on terminal hair that have preserved with the mummified tissue. The current study has presented the possibility of the preservation of vellus hair on desiccated skin. However, further analyses have to be done to determine whether the hairs are vellus hairs or terminal hairs that have shrunk.

7.3 General Tissue Histology

The desiccated tissue sections from the rib (50-M1 RBL) were stained with the Masson trichrome stain, the hematoxylin-eosin stain, the aldehyde hematoxylin-eosin stain and Verhoeff's elastic tissue stain. The sections were then examined under the light microscope. The light microscopy observation showed that the tissue was arranged in parallel fibres enclosed within a network of loosely arranged connective tissue. The parallel fibres were stained red and the network of loose tissue was stained green for collagen fibres by the Masson trichrome technique. This demonstrates that the tissue is striated muscle tissue with parallel-running muscle fibres packed within a bundle (fascicle) that is enclosed within the epimysium. The perimysium is found between the fascicles as the connective tissue extends into the muscle tissue.

Although the basic morphology of the muscle tissue can be identified, the only remaining structures are the muscle fibres and the connective tissue as the muscle cell nuclei have not preserved. The preservation of the muscle tissue is limited to its appearance as the muscle fibres and surrounding connective tissue, form the structure

of the tissue. Because of the exceptional durability of collagen fibres, the connective tissue played an integral part in the preservation of the muscle tissue.

In normal tissue histology, the tissue on the oral surface of the hard palate is covered by keratinised stratified squamous epithelium that is structured into four layers similar to the epidermis of the skin (Meyer & Schroeder 1975). There is a thin layer of connective tissue below the epithelium. Mucous glands are also found on the hard palate. The histological results of the desiccated tissue are consistent with the structure of normal human tissue on the hard palate. The histological results demonstrated that the desiccated tissue constituted a network of fibrous connective tissue composed of collagen fibres and some muscle fibres. The overlying stratified squamous epithelium was identified but appeared to have separated from the underlying connective tissue.

Even though the general histology of the desiccated tissue from the palate showed deterioration particularly in the epithelium and the connective tissue, the structure of the tissue was in a better state of preservation than the muscle tissue from the rib and from the exposed upper femoral region of the mummy. Although the epithelium had undergone severe deterioration, the epithelium could still be identified and appeared to have separated from the underlying connective tissue. The remaining structure of the desiccated tissue was composed of fibrous connective tissue containing collagen fibres and it is suggested here that the connective tissue played an important role in maintaining the structural appearance of the desiccated tissue from the palate.

The desiccated tissue from the mandible (74-Dg1 M1 RBL) was identified as skin in the SEM observations. The histology of the skin showed that the epidermis was not that well-preserved because it was only identified in some places in the skin.

Furthermore, where the epidermis had preserved, the corneal layer and the basal layer could not be identified. The remaining structure of the skin was an arrangement of parallel-running muscle and collagen fibres forming a network of connective tissue that is believed to be the remaining dermis. Hair follicles that were observed in the SEM observations were not identified in the histological analyses.

Previous studies on desiccated tissue have shown a type of structure that is characteristic to naturally mummified skin (El-Najjar *et al.* 1980; Bereuter *et al.* 1997; Stucker *et al.* 2001; Romakov *et al.* 2002; Shin *et al.* 2003b; Chang *et al.* 2006a). These studies show that there is a general decrease in the thickness of the epidermis to a point where it has almost disappeared. Furthermore, the histology of these naturally mummified bodies demonstrates the preservation of a dermis that is composed entirely of collagen fibres but lacking in elastic fibres and cellular structures. In comparison, studies on artificial mummification have shown that the structure of the skin is well-preserved with an identifiable epidermis and a dermis that has collagen fibres as well as elastic fibres.

During decomposition, the skin is reduced to a structureless mass with degeneration occurring in both the epidermis and the dermis. Studies have shown that during the postmortem period, there is a separation of the basal layer of the epidermis from the basement membrane at the dermo-epidermal junction (Kovarik *et al.* 2005). During the mummification of the corpse, the skin shrinks as it is dehydrated which causes the layers to compress and decrease in thickness. The epidermis is the thinnest and outermost layer and mummification and exposure to the elements may result in a decrease of this layer to a point where it may disappear altogether.

The desiccated tissue from Historic Cave resembles the histologic pattern of naturally mummified skin. On macroscopic level, the appearance of the skin has preserved well. On microscopic level, however, the structure of the skin is in a poor state of preservation. Even though the appearance of the skin has remained intact, its structure is largely composed of the surviving layer of the skin: the dermis and its collagen fibres. During the SEM observations, epidermal keratinocytes were identified in patches and the hairs observed were confined to two skin samples which demonstrate that the desiccated skin is poorly preserved. It is therefore reasonable to assume that because the skin was already in a state of poor preservation, during the histology preparation, hair follicles were lost and the fragile layers of the skin were further deteriorated to a point where the epidermis either had decreased in thickness or had not preserved.

The general histology of the muscle and skin tissue examined here is consistent with the histology of naturally mummified tissue. Previous studies have shown that there is a general deterioration in the morphology of the muscle and skin tissue. In both the muscle and the skin tissue, the best preserved tissue is connective tissue composed of collagen fibres.

7.4 Dust and Debris Coating the Surface of the Skin

During the stereo examinations and SEM observations, a layer of dust and debris was observed coating the surface of the desiccated tissue. When analysed with the EDS, the results showed counts of sodium (Na), magnesium (Mg) and potassium (K) with particularly high counts of silicon (Si) and calcium (Ca). When these samples were cleaned through a sonication treatment, the counts of silicone (Si) and calcium (Ca) decreased and in some cases silicone (Si) was not detected.

Historic Cave is a limestone cave and environments rich in limestone are suitable to mummification because lime promotes preservation. The calcium-rich debris coating the surface of the desiccated skin from Historic Cave it is suggested to have protected the surface of the skin from exposure to temperature and humidity which may have aided in decomposition. Similarly, in a study conducted by Chang *et al.* (2006a), a substance coating the surfaces of the desiccated tissue was analysed with EDS. The substance contained sodium (Na), potassium (K) and calcium (Ca). Chang *et al.* (2006a) suggested that the calcium came from the lime-soil mixture that was used in the burial of the corpse. This calcium-rich lime aided in the mummification process by protecting the skin from exposure and therefore aided in the preservation of the skin (Chang *et al.* 2006a: 679).

7.5 Comparing the Sonication Procedures

The desiccated skin tissue from the right arm of the mummy was analysed through SEM and light microscopy. During the SEM observation, the surface of the skin tissue was coated with a layer of dust and debris which obscured the surface morphology using SEM. During the histological preparation of the tissue, the dust in the tissue blunted the microtome knife which tore and scored the tissue. Desiccated tissue samples were cleaned with a sonication treatment before analysis in the scanning electron microscope and the microscopic images from the SEM examination showed that the underlying epidermal keratinocytes, hair shaft holes and hairs had become exposed.

The desiccated skin tissue from the right arm of the mummy, the desiccated tissue from the palate and the desiccated muscle tissue from the rib were prepared for histological analyses. Under the stereo microscope, dust and debris were observed on the surfaces of the tissue (Figure C.54). Because of the dust and debris, the preparation of the desiccated tissue for light microscopy proved to be a difficult task

as the samples were very fragile and difficult to section and extra care had to be taken to ensure the sections did not wash away. The light microscopy observations showed that dust was embedded in the tissue. The dust and dirt disrupted the integrity of the tissue's structure and caused the tissue to deteriorate in places. It was therefore necessary to clean the tissue with a sonication treatment prior to histological analysis. In this study two sonication procedures were used to clean the desiccated tissue. The first sonication procedure used absolute alcohol as the cleaning agent and the second sonication procedure used acetone as the cleaning agent. The two cleaning methods each yielded different results.

The sonication procedure utilizing absolute alcohol as the cleaning agent removed most of the surface dust and debris from the tissue and exposed the underlying flattened epidermal keratinocytes and hair shaft holes. Similar results were achieved in a study conducted by Chang *et al.* (2006a) that used absolute alcohol as the cleaning agent. In the SEM observations described by Chang *et al.* (2006a), flattened epidermal keratinocytes and hair shaft holes were identified on the surfaces of the desiccated skin tissue. In the current study, only one hair was identified on the surface of the tissue cleaned in absolute alcohol even though there were numerous hair shaft holes. It may be possible that the hairs associated with the hair shaft holes were lost during the cleaning procedure.

The sonication treatment using acetone did not remove as much of the surface dust and debris as the sonication treatment using absolute alcohol. In the SEM observation of the desiccated skin cleaned in acetone, the flattened epidermal keratinocytes were not easily identified because dust and debris remained on the surface of the skin. Even though dust and debris remained after cleaning, hairs were observed on the surface of the skin. It can therefore be concluded that acetone is best used for

analysing hairs and absolute alcohol can be used when observing epidermal keratinocytes and hair shaft holes.

7.6 Comparing the Rehydration Solutions

The desiccated tissue samples that were rehydrated in the Sandison's solution were the skin tissue from the mandible (74-4), the tissue from the palate (69-4), the muscle tissue from the rib (50-5) and the muscle tissue from the exposed upper anterior femoral region on the mummy (A1081-3). Under the light microscope, all the samples showed a good preservation of the general structure of the tissue. Samples from the rib and from the mummy were cleaned in absolute alcohol which removed the dust and debris. The skin sample from the mandible and the tissue sample from the palate were cleaned in acetone which did not remove all of the dust and debris.

The desiccated samples that were rehydrated in the Ruffer's solution were the tissue from the palate (69-5), the muscle tissue from the rib (50-4) and the muscle tissue from the exposed upper anterior femoral region on the mummy (A1081-1). During the histology preparation of the tissue, all samples were extremely fragile and the sections washed away during the staining procedures. The tissue from the palate was cleaned in acetone which did not remove the dust. Similarly, dust was found in the tissue from the mummy even though the tissue was cleaned in absolute alcohol. The general structure of the desiccated tissue rehydrated in the Ruffer's solution was not well-preserved. Epithelial tissue, for example, was not identified in the tissue from the palate and connective tissue had deteriorated in the muscle tissue from the rib. Compared to the samples rehydrated in Sandison's solution, these samples were in a poorer state of preservation.

Two desiccated skin and tissue samples were rehydrated with glycerine. The skin sample from mandible and the muscle tissue from the exposed upper femoral region of the mummy were rehydrated in glycerine for seven days. In comparison to the Sandison's solution, the skin sample rehydrated in glycerine demonstrated a better preservation of the tissue. Both the epidermis and the dermis had preserved. The muscle tissue however, was poorly preserved. As with the Ruffer's solution, neither the acetone nor the absolute alcohol had successfully removed the dust and debris from the tissue rehydrated in glycerine.

Of the rehydration solutions, the Sandison's solution proved to be the most suitable technique for analysing the desiccated tissue sections under the light microscope. The desiccated tissue sections remained intact and did not wash away during preparation. Even though both sonication procedures using absolute alcohol and acetone were used on the tissue samples rehydrated in Ruffer's solution and glycerine, dust was still embedded in the tissue. The tissue samples rehydrated in Sandison's solution were cleaned with the same sonication procedures but did not have dust. Here it is suggested that the Sandison's solution acted in addition to the sonication procedures to remove much of the dust and debris embedded in the tissue samples.

7.7 Comparing the Staining Procedures

In this study, the Masson trichrome stain and the hematoxylin-eosin stain were used to stain the desiccated tissue samples from right arm and the upper femoral region of the 'Makapan Mummy' (A1081) as well as from the mandible, maxilla and rib excavated at Historic Cave. The structure of the muscle tissue and skin tissue stained with these techniques was well-preserved and did not wash away during staining.

The Verhoeff's method and the aldehyde hematoxylin-eosin stain were used to stain the sections from the desiccated rib. The staining of the sections was not as successful as the Masson trichrome stain and the hematoxylin-eosin stain because the sections washed away during the histological preparation. The sections that were stained with the Verhoeff's stain did not demonstrate the different tissue types as well as the Masson trichrome stain and the hematoxylin-eosin stain.

The results of the histology analyses have demonstrated that the Masson trichrome stain and the hematoxylin-eosin stain are the best staining methods for desiccated tissue sections from Historic Cave. The Verhoeff's elastic tissue stain and the aldehyde hematoxylin-stain were not as useful in demonstrating the histological structure of the desiccated tissue.

8 CONCLUSION

The 'Makapan Mummy' (A1081) from Historic Cave was not buried in the sand but was in the upright kneeling position. Dust and debris coating the surface of the desiccated tissue may have protected the surface of the tissue from deterioration but the limestone and lime-rich sand could not have directly mummified the corpse. Environmental studies conducted in 1992 and 2011 indicate that the temperature in Historic Cave is not as extreme as in the case of the Egyptian mummies and Ötzi the Iceman. The climate inside the cave is stable with minute differences between the base of the cave and the entrance to the cave. The cave is also well ventilated with a constant flow of air. The 2001 to 2007 excavations yielded numerous desiccated human and animal remains which suggest that a broader environment in the cave was dry enough to cause the desiccation of numerous human and animal remains.

Cresswell's (1993) study was conducted during the hot and dry month of September and represents the minimum humidity at Historic Cave. Given that the 2011 study was carried out during a particularly wet period, these results arguably capture the higher end of the humidity levels at Historic Cave. The siege occurred in October and during a drought so the climate in the cave would have been hotter and drier at this time. Smoke and heat caused by the fires lit inside the cave may have increased the temperature in the cave causing the air to become dry during and immediately after the siege. It is likely that the dry air inside the well ventilated cave desiccated the corpse and preserved the soft tissue.

An aim of this study was to investigate, identify and compare the morphology of mummified tissue. The mummified tissue is hard and dry and contaminated with dust so it had to be prepared differently to normal human tissue. The mummified tissue was prepared for multiple microscopic analyses and a number of different techniques

and protocols were applied to clean, rehydrate, stain and analyse the tissue. Each technique and protocol used in this study was described and compared.

Scanning electron microscopy (SEM) was used to investigate the surface of tissue. The mummified tissue was contaminated by sand and soil which obscured the surface detail and methods for cleaning the surface of the tissues were investigated. Two sonication procedures were identified and each yielded different results. For desiccated tissue from Historic Cave, absolute alcohol is best used to remove the dust and debris from the surface of the tissue and expose the underlying epidermal keratinocytes and hair shaft holes. Acetone does not remove as much of the surface dust and debris but it is best used to examine hairs.

In order to examine the mummified tissue histologically with a light microscope, two problems need to be overcome. First, dust and debris in the tissue makes the histological tissue sectioning of the desiccated tissue difficult. The dust gets caught by the microtome knife and damages it causing the tissue to tear when sectioned. This disrupts the integrity of the tissue. To overcome this, methods for cleaning the tissue were investigated and the results show that the best cleaning method for histological sections of desiccated tissue is the cleaning method using absolute alcohol. As with SEM, the tissue sample must be fixed in absolute alcohol for 24 hours and then cleaned in absolute alcohol for two five minute periods using an ultrasonic cleaner.

Secondly, the mummified tissue is hard and brittle and cannot be processed for histology in this state. Several methods can be employed to soften and rehydrate the hard mummified tissue and these were investigated in this study. The desiccated tissue rehydrated in the Sandison's rehydration solution provided the best histological

results. The tissue was much easier to section and the integrity of the tissue was not interrupted.

Even though the acetone provided the best results for analysing hair in the scanning electron microscope, in histological sections the hairs could not be observed. SEM is therefore recommended as the best method for examining hairs from naturally desiccated tissue from Historic Cave.

For histological staining of the desiccated tissue, numerous staining procedures were employed to demonstrate the histological structure of striated muscle tissue and skin tissue. The Masson trichrome stain and the hematoxylin-eosin stain provided better results than the Verhoeff's stain and the aldehyde hematoxylin-eosin stain. The Masson trichrome stain is best used on skin to demonstrate the epidermis and the dermis. The hematoxylin-eosin stain is best used for striated muscle tissue and connective tissue.

Although numerous techniques and protocols were used to clean, rehydrate and stain the tissue, the tissue was already very fragile. For further research on the desiccated human remains from Historic Cave, extra care should be taken when handling the desiccated tissue.

APPENDICES

APPENDIX A

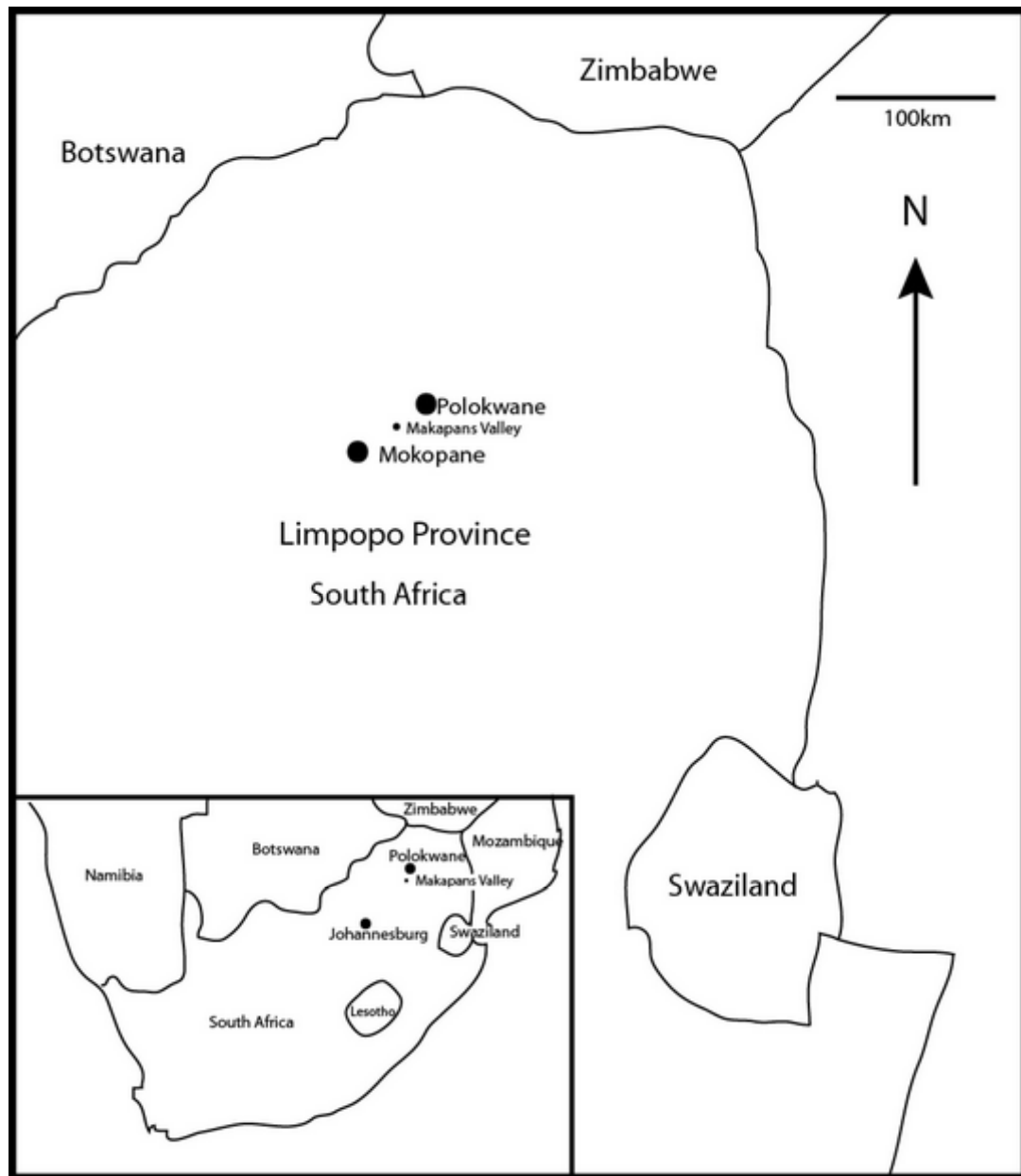


Figure A.1 Location of Makapans Valley in southern Africa

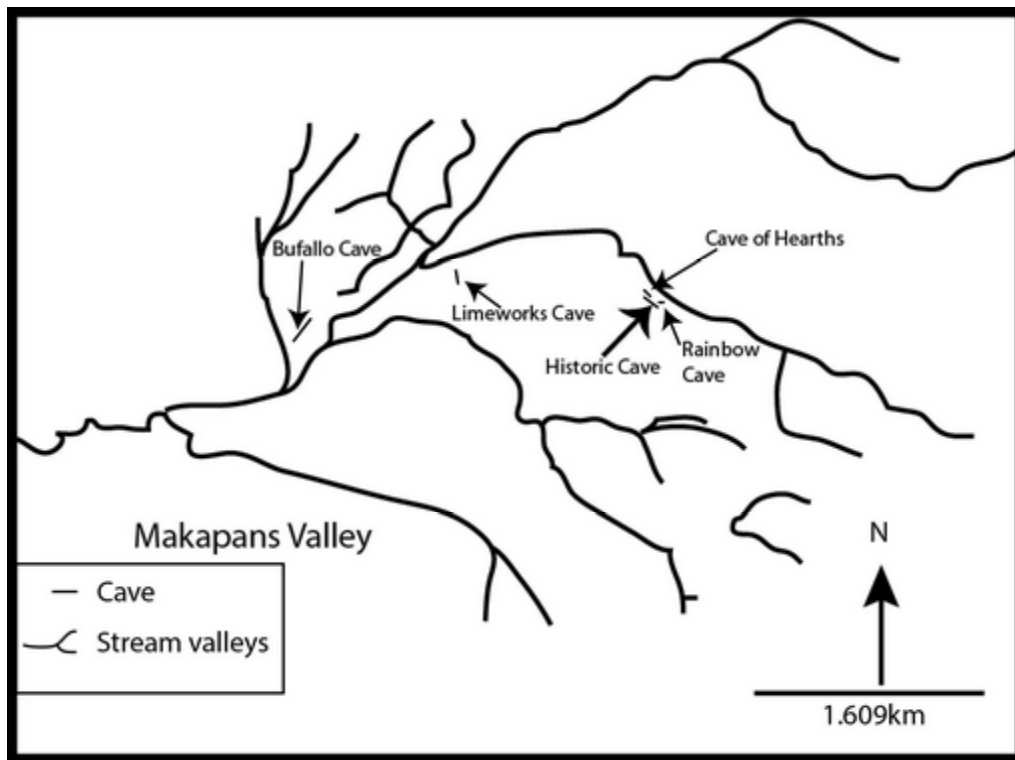


Figure A.2 Map showing Historic Cave, Limeworks Cave, Cave of Hearths, Rainbow Cave and Buffalo Cave (after Latham & Herries 2004)

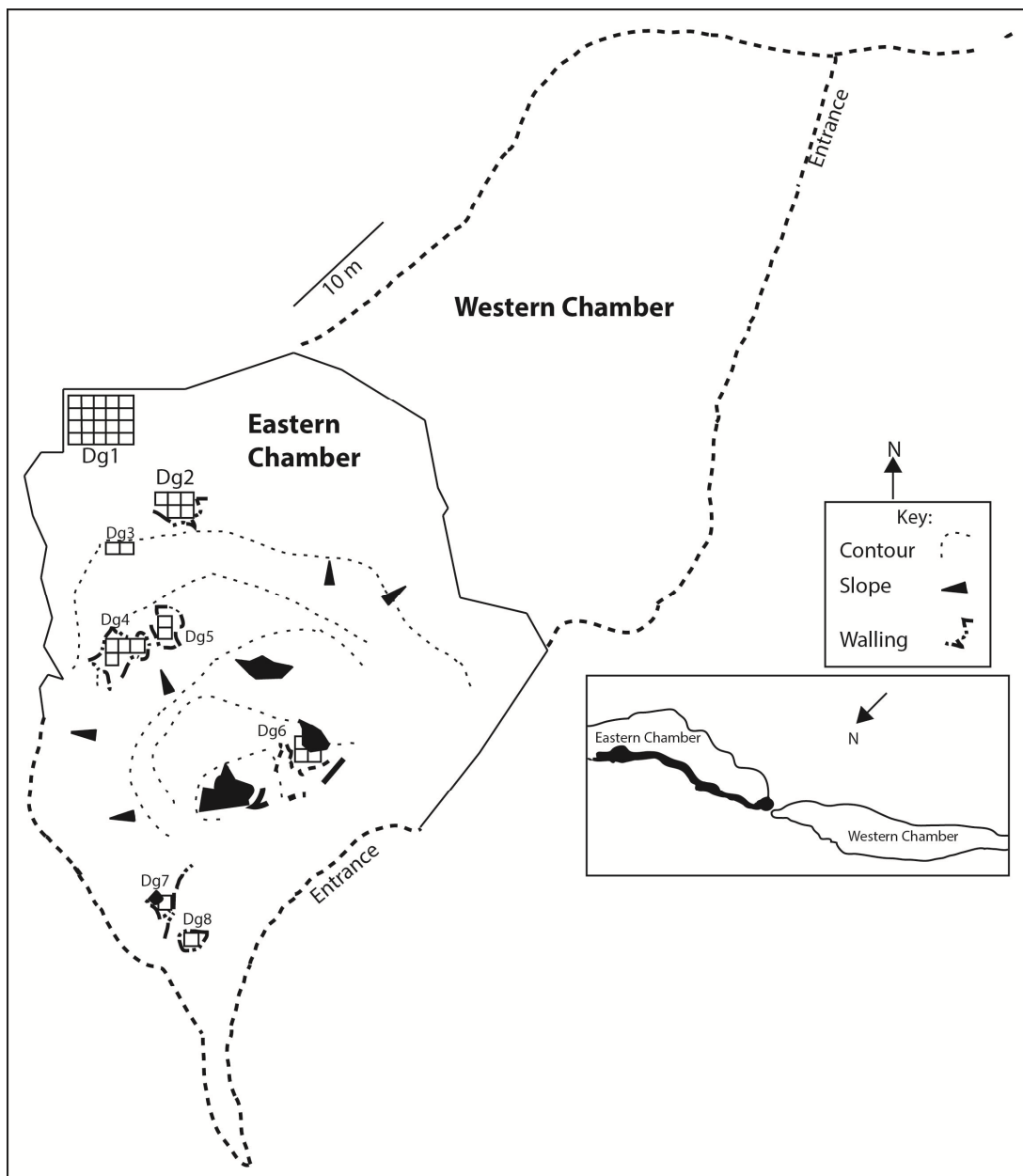


Figure A.3 Map showing the eastern and western chambers at Historic Cave. The grids labelled Dg1 to Dg8 are the excavated areas in the eastern chamber (after Esterhuysen *et al.* 2009)



Figure A.4 The 'Makapan Mummy' A1081 (after Esterhuysen *et al.* 2009)

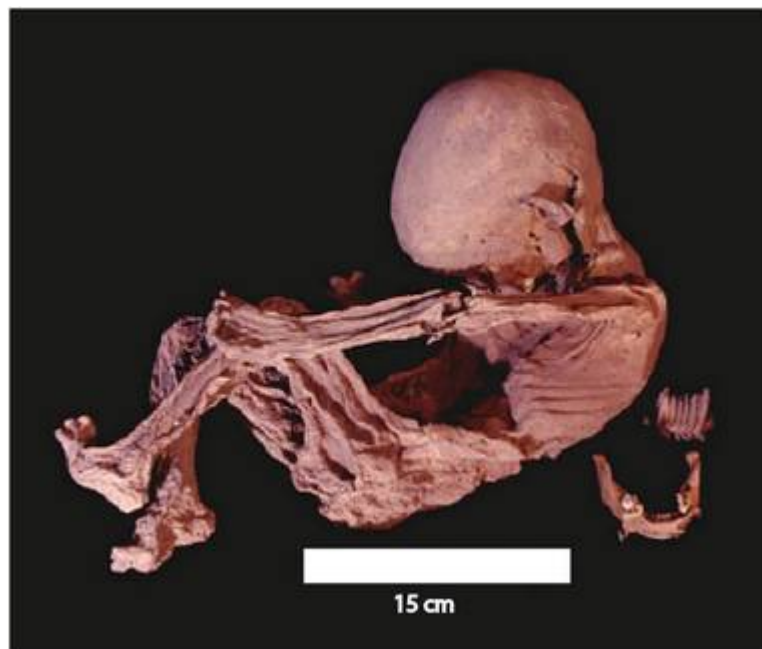


Figure A.5 The Makapan Child (after Cresswell 1993)

Table A.1 Table illustrating the location of human remains with desiccated skin attached in the excavation site

Area excavated	Stratigraphic unit	Skeletal element
Dg1	M1	One skull, one maxilla, one mandible, two ribs, and one vertebra
Dg1	M2	One mandible, one rib, one long bone, and two vertebrae
Dg1	M4	Three ribs
Dg1	N1	Six ribs
Dg1	O1	One radius
Dg1	O2	One maxilla and one mandible
Dg1	O3	Two ribs
Dg1	P2	Two bone fragments
Dg2	V9	Three bone fragments
Dg2	V10	One rib
Dg4	Q21	One bone fragment and one femur
Dg4	R21	Three vertebrae

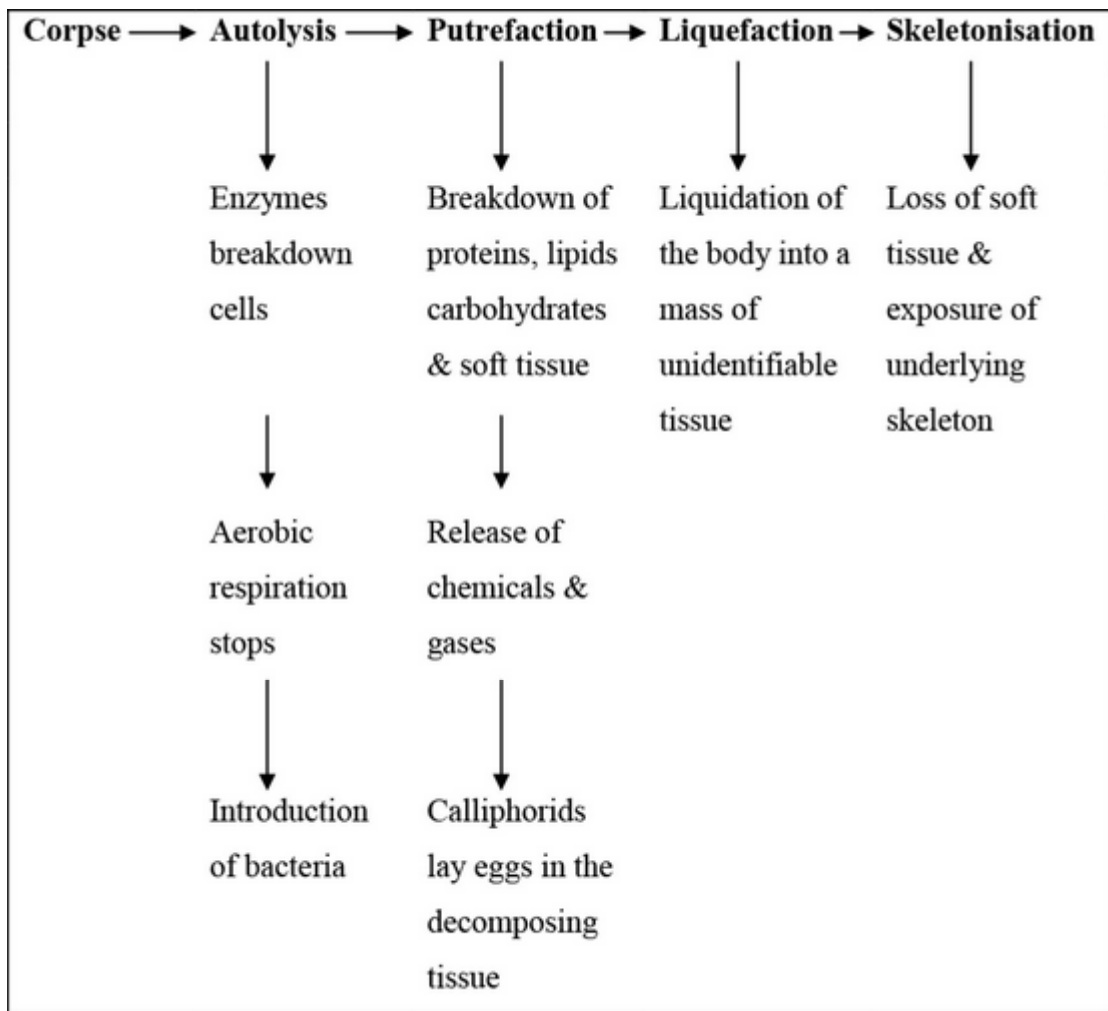


Figure A.6 Figure illustrating the decomposition process



Figure A.7 The Ginger mummy was naturally mummified by the hot Egyptian sand (Pemberton 2001; image provided by the British Museum)

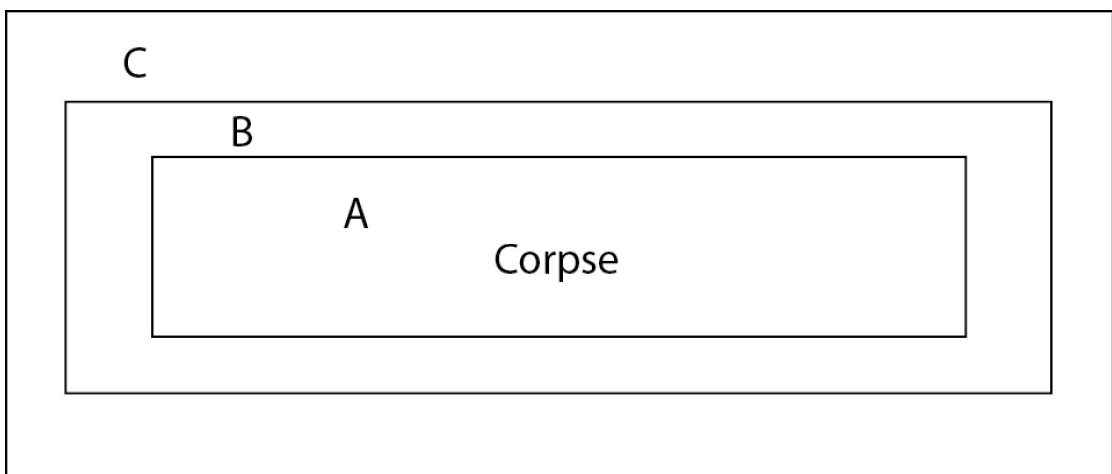


Figure A.8 Layout of the coffins from the Republic of Korea (after Shin *et al.* 2003b). (A) and (B) are the inner wooden coffins and (C) is the enclosure of lime and soil

Table A.2 Average minimum and maximum temperatures in Mokopane in February 2011 (<http://rp5.co.za> consulted January 2012)

Date	Average temperature in degrees Celsius¹	
	Minimum °C	Maximum °C
2011/02/01	19.30	30.67
2011/02/02	18.80	29.47
2011/02/03	17.40	29.33
2011/02/04	16.03	29.57
2011/02/05	18.50	29.10
2011/02/06	18.78	30.97
2011/02/07	20.15	32.77
2011/02/08	21.83	29.25
2011/02/09	20.90	31.67
2011/02/10	19.90	32.30
2011/02/11	19.55	32.70
2011/02/12	19.00	32.90
2011/02/13	23.25	33.40
2011/02/14	18.70	32.67
2011/02/15	17.40	31.00
2011/02/16	18.05	31.07
2011/02/17	18.95	28.13
2011/02/18	19.53	28.83
2011/02/19	19.60	31.00

¹ The daily average minimum and average maximum temperatures were calculated from minimum and maximum temperatures that were taken daily at 02h00, 08h00, 14h00, and 20h00. The minimum and maximum temperatures were provided by Reliable Prognosis Ltd.

Date	Average temperature in degrees Celsius ³	
	Minimum °C	Maximum °C
<i>[This is a continuation of Table A.2 on the previous page]</i>		
2011/02/20	20.37	32.00
2011/02/21	19.93	32.17
2011/02/22	20.08	30.63
2011/02/23	20.33	30.23
2011/02/24	18.98	28.57
2011/02/25	16.73	27.70
2011/02/26	16.97	28.20
2011/02/27	17.08	29.63
2011/02/28	18.48	30.27
2011/02/01 to 2011/02/28	19.09	30.58

Table A.3 Average minimum and maximum temperatures in Mokopane in March 2011 (<http://rp5.co.za> consulted January 2012)

Date	Average temperature in degrees Celsius¹	
	Minimum °C	Maximum °C
2011/03/01	19.70	30.50
2011/03/02	17.37	30.03
2011/03/03	20.43	32.57
2011/03/04	20.13	32.00
2011/03/05	18.17	28.80
2011/03/06	16.98	29.73
2011/03/07	18.05	30.83
2011/03/08	18.83	31.85
2011/03/09	17.20	32.15
2011/03/10	19.88	32.77
2011/03/11	20.08	33.53
2011/03/12	21.25	34.37
2011/03/13	21.25	33.17
2011/03/14	21.45	30.50
2011/03/15	19.18	30.20
2011/03/16	18.55	30.40
2011/03/17	18.80	30.37
2011/03/18	19.83	32.00
2011/03/19	20.25	29.63

¹ The daily average minimum and average maximum temperatures were calculated from minimum and maximum temperatures that were taken daily at 02h00, 08h00, 14h00, and 20h00. The minimum and maximum temperatures were provided by Reliable Prognosis Ltd.

Date	Average temperature in degrees Celsius ⁴	
	Minimum °C	Maximum °C
<i>[This is a continuation of Table A.3 on the previous page]</i>		
2011/03/20	17.85	27.13
2011/03/21	17.65	27.87
2011/03/22	17.28	31.40
2011/03/23	20.55	32.93
2011/03/24	19.95	33.77
2011/03/25	21.58	33.10
2011/03/26	20.08	31.10
2011/03/27	19.95	29.43
2011/03/28	18.60	29.80
2011/03/29	18.68	31.80
2011/03/30	19.63	33.30
2011/03/31	18.20	32.07
2011/03/01 to 2011/03/31	19.32	31.26

Table A.4 Average daily temperature, humidity and water vapour pressure for Humi 2 in February 2011

Date	Average temperature in degrees Celsius	Relative humidity percentage (RH %)	Average water vapour pressure (VP) in Kpa
2011/02/17	20.65	67.20	1.63
2011/02/18	18.40	72.23	1.53
2011/02/19	18.48	75.80	1.61
2011/02/20	19.13	72.08	1.60
2011/02/21	19.03	75.60	1.66
2011/02/22	18.70	73.00	1.57
2011/02/23	18.68	72.95	1.57
2011/02/24	18.78	75.70	1.64
2011/02/25	18.35	75.08	1.58
2011/02/26	18.00	73.93	1.52
2011/02/27	17.93	70.83	1.45
2011/02/28	18.43	68.90	1.46
2011/02/17 to 2011/02/28	18.71	72.77	1.57

Table A.5 Average daily temperature, humidity and water vapour pressure for Humi 2 in March 2011

Date	Average temperature in degrees Celsius	Relative humidity percentage (RH %)	Average water vapour pressure (VP) in Kpa
2011/03/01	18.48	65.50	1.39
2011/03/02	17.88	64.23	1.31
2011/03/03	18.65	58.53	1.26
2011/03/04	19.08	66.88	1.48
2011/03/05	18.43	63.30	1.39
2011/03/06	18.30	58.80	1.23
2011/03/07	18.43	62.55	1.32
2011/03/08	18.63	62.03	1.33
2011/03/09	18.93	59.45	1.30
2011/03/10	19.30	62.93	1.41
2011/03/11	19.23	62.63	1.39
2011/03/12	19.73	61.35	1.41
2011/03/13	20.38	72.18	1.73
2011/03/14	20.20	75.73	1.79
2011/03/15	19.73	78.40	1.80
2011/03/16	19.53	72.88	1.65
2011/03/17	19.13	68.20	1.51
2011/03/18	19.55	65.25	1.48
2011/03/19	19.75	76.10	1.75
2011/03/20	19.05	78.53	1.73
2011/03/21	18.90	77.50	1.69

Date	Average temperature in degrees Celsius	Relative humidity percentage (RH %)	Average water vapour pressure (VP) in Kpa
<i>[This is a continuation of Table A.5 on the previous page]</i>			
2011/03/22	19.18	72.90	1.62
2011/03/23	19.73	74.28	1.71
2011/03/24	19.83	72.83	1.68
2011/03/25	20.30	72.58	1.73
2011/03/26	20.03	72.65	1.70
2011/03/27	22.20	68.67	1.79
2011/03/01 to 2011/03/27	19.35	68.40	1.54

Table A.6 Average daily temperature, humidity and water vapour pressure for Humi 3 in February 2011

Date	Average temperature in degrees Celsius	Relative humidity percentage (RH %)	Average water vapour pressure (VP) in Kpa
2011/02/17	22.85	60.45	1.65
2011/02/18	18.73	70.80	1.53
2011/02/19	18.75	74.55	1.61
2011/02/20	19.73	69.00	1.59
2011/02/21	19.43	73.23	1.65
2011/02/22	19.13	71.23	1.58
2011/02/23	19.13	71.18	1.58
2011/02/24	19.15	74.20	1.65
2011/02/25	18.73	73..56	1.59
2011/02/26	18.40	71.63	1.51
2011/02/27	18.33	68.95	1.45
2011/02/28	18.80	67.28	1.46
2011/02/17 to 2011/02/28	19.26	71.48	1.57

Table A.7 Average daily temperature, humidity and water vapour pressure for Humi 3 in March 2011

Date	Average temperature in degrees Celsius	Relative humidity percentage (RH %)	Average water vapour pressure (VP) in Kpa
2011/03/01	18.95	64.28	1.40
2011/03/02	18.43	62.18	1.32
2011/03/03	19.33	55.38	1.24
2011/03/04	19.53	65.58	1.51
2011/03/05	18.88	62.13	1.35
2011/03/06	18.93	56.95	1.24
2011/03/07	18.93	60.33	1.31
2011/03/08	19.10	60.90	1.34
2011/03/09	19.58	57.38	1.30
2011/03/10	19.83	61.35	1.42
2011/03/11	20.03	60.90	1.42
2011/03/12	20.45	59.30	1.42
2011/03/13	20.95	70.03	1.73
2011/03/14	20.65	74.85	1.82
2011/03/15	20.13	77.40	1.82
2011/03/16	20.10	70.93	1.66
2011/03/17	19.83	65.80	1.52
2011/03/18	20.30	63.03	1.50
2011/03/19	20.28	75.18	1.79
2011/03/20	19.53	78.18	1.78
2011/03/21	19.60	77.78	1.78

Date	Average temperature in degrees Celsius	Relative humidity percentage (RH %)	Average water vapour pressure (VP) in Kpa
<i>[This is a continuation of Table A.7 on the previous page]</i>			
2011/03/22	19.95	71.00	1.65
2011/03/23	20.55	73.23	1.77
2011/03/24	20.65	70.15	1.71
2011/03/25	21.08	70.28	1.76
2011/03/26	20.78	70.45	1.73
2011/03/27	23.20	65.70	1.79
2011/03/01 to 2011/03/27	19.98	66.69	1.56

Table A.8 Average daily temperature, humidity and water vapour pressure for Humi 4 in February 2011

Date	Average temperature in degrees Celsius	Relative humidity percentage (RH %)	Average water vapour pressure (VP) in Kpa
2011/02/17	22.70	59.50	1.63
2011/02/18	19.95	66.70	1.54
2011/02/19	20.45	67.75	1.60
2011/02/20	21.83	61.23	1.59
2011/02/21	21.18	65.75	1.64
2011/02/22	20.80	63.70	1.56
2011/02/23	21.15	62.35	1.56
2011/02/24	21.23	66.65	1.67
2011/02/25	19.93	67.83	1.58
2011/02/26	17.90	86.43	1.77
2011/02/27	18.40	72.68	1.52
2011/02/28	19.63	67.38	1.53
2011/02/17 to 2011/02/28	20.43	67.33	1.60

Table A.9 Average daily temperature, humidity and water vapour pressure for Humi 4 in March 2011

Date	Average temperature in degrees Celsius	Relative humidity percentage (RH %)	Average water vapour pressure (VP) in Kpa
2011/03/01	19.93	60.63	1.39
2011/03/02	19.44	60.05	1.34
2011/03/03	21.08	48.50	1.19
2011/03/04	21.28	58.90	1.47
2011/03/05	20.45	55.80	1.32
2011/03/06	20.33	51.43	1.20
2011/03/07	20.73	54.28	1.29
2011/03/08	21.08	53.15	1.28
2011/03/09	21.95	49.35	1.25
2011/03/10	22.38	52.63	1.39
2011/03/11	22.60	53.00	1.44
2011/03/12	24.00	48.45	1.41
2011/03/13	23.40	60.18	1.71
2011/03/14	23.28	65.68	1.85
2011/03/15	21.88	69.93	1.83
2011/03/16	22.35	61.75	1.63
2011/03/17	23.10	53.58	1.45
2011/03/18	23.98	50.13	1.46
2011/03/19	22.35	70.03	1.87
2011/03/20	20.18	92.25	2.18
2011/03/21	20.15	96.63	2.30

Date	Average temperature in degrees Celsius	Relative humidity percentage (RH %)	Average water vapour pressure (VP) in Kpa
<i>[This is a continuation of Table A.9 on the previous page]</i>			
2011/03/22	20.13	92.83	2.20
2011/03/01 to 2011/03/22	21.64	61.78	1.57

Table A.10 Average temperature in degrees Celsius (°C), relative humidity percentage (RH %) and water vapour pressure (VP) measured in kilopascals (Kpa) for Humi 2, Humi 3 and Humi 4 in February and March 2011

Date	Humi 2			Humi 3			Humi 4		
	Temperature °C	RH%	VP Kpa	Temperature °C	RH%	VP Kpa	Temperature °C	RH%	VP Kpa
02/2011	18.71	72.77	1.57	19.26	71.48	1.57	20.43	67.33	1.60
03/2011	19.35	68.40	1.54	19.98	66.69	1.56	21.64	61.78	1.57

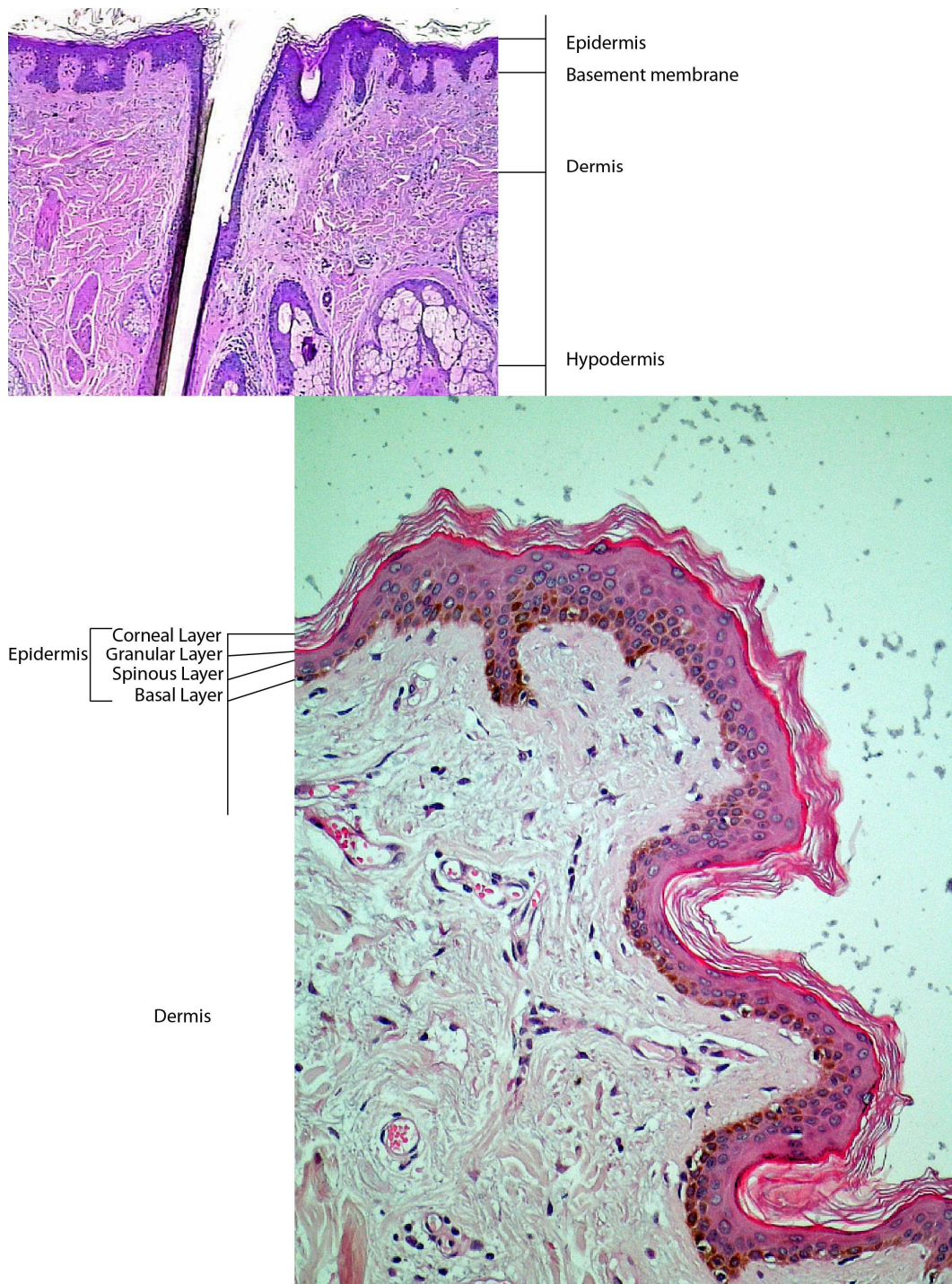


Figure A.9 The structure of normal human skin (Histological section provided by Drs Gritzman & Thatcher Laboratories)

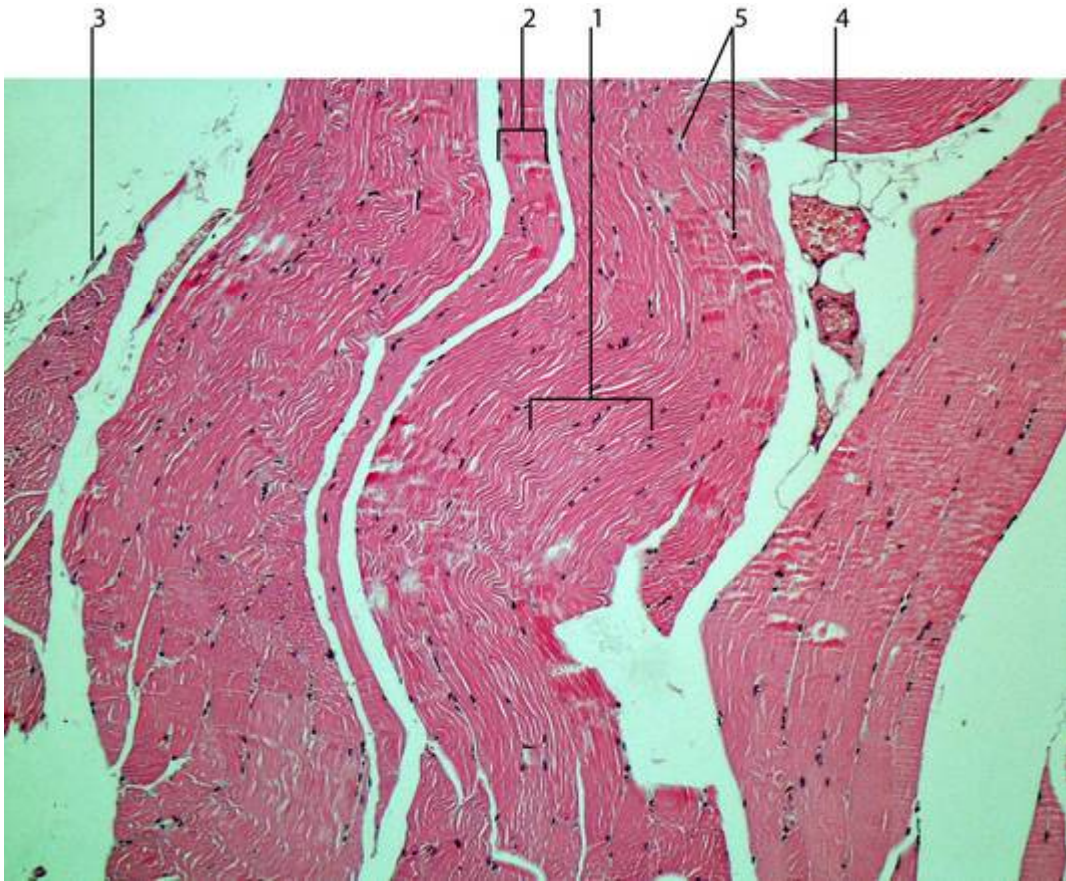


Figure A.10 Hematoxylin-eosin stain of normal human striated muscle tissue. (1) Muscle fibres; (2) fascicle or bundle of muscle fibres; (3) epimysium; (4) perimysium; and (5) nuclei (Histological section provided by Drs Gritzman & Thatcher Laboratories)

APPENDIX B



Figure B.1 The scanning electron microscope

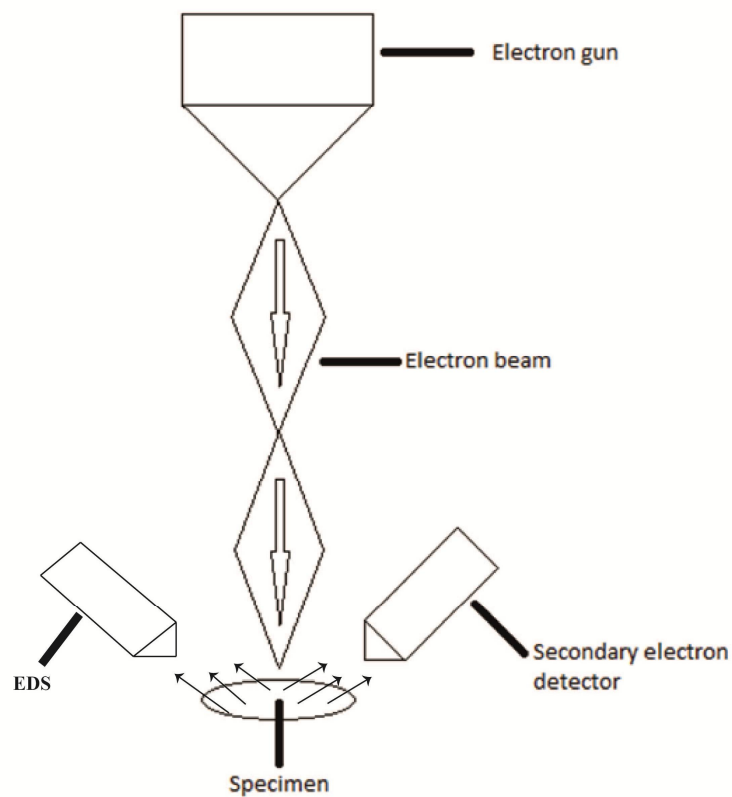


Figure B.2 Diagram illustrating how a scanning electron microscope equipped with an energy-dispersive spectrometer (EDS) works



Figure B.3 The light microscope



Figure B.4 The mandible excavated at Historic Cave (74-Dg1 M1 RBL). The desiccated tissue was sampled from the outer surface of the right side of the mandible (arrow)

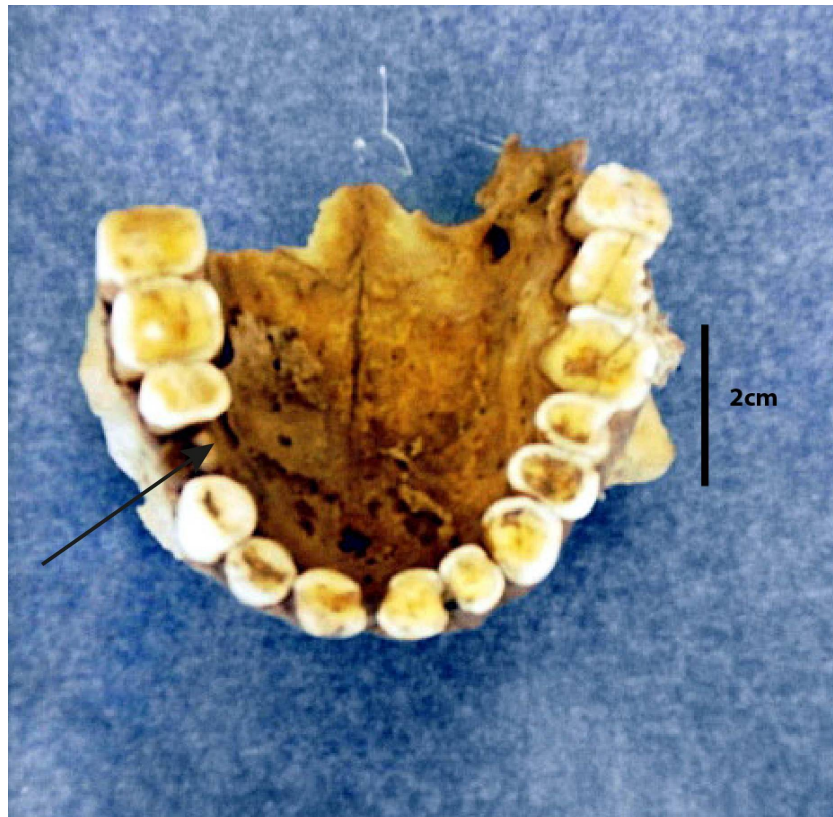


Figure B.5 The maxilla excavated at Historic Cave (69-M1 RBL). The desiccated tissue was sampled from the inferior surface of the hard palate of the superior maxilla (arrow)



Figure B.6 The rib excavated at Historic Cave (50-M1 RBL). The desiccated tissue was sampled from the inner surface of the rib (arrow)



Figure B.7 (A) The right arm of the 'Makapan Mummy' (A1081); (B) the desiccated tissue specimen was sampled from the anterior surface of the right arm (A1081-5)



Figure B.8 (A) Three desiccated tissue specimens (A1081-1 to A1081-3) were sampled from the exposed upper anterior femoral region of the 'Makapan Mummy' (A1081); (B) magnified view of the area from which the desiccated tissue specimens were sampled as shown in (A)



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9/2/257/0002

PERMIT
No. 80/10/05/009/51

Issued under Section 35(4) of the National Heritage Resources Act, Act No. 25 of 1999. Permission is hereby given:

to: Dr A Esterhuysen (ID: 6607150017081) on behalf of Shahzadee Karodia,
of: Archaeology, School of Geography, Archaeology and Environmental Studies, University of the Witwatersrand, Private Bag, WITS, 2050,
for: obtaining samples of approximately 10mm in diameter of fibrous tissue from inside the abdomen of mummified human remains excavated from Makapan and placed in the Raymond Dart collection (specimen A1081) at the Wits Medical School in 1948.

The following conditions apply:

1. Samples must be selected by the permit holder in consultation with the curator who must ensure that comparative samples are retained in the collections, and that any samples not used by the analyser are returned to the collections.
2. Sampling must be done with minimum damage to the mummified human remains and according to principles outlined in the application from the permit holder.
3. A report on the results of the analyses must be submitted to SAHRA on 1 August 2011.
4. Reprints of all published papers, or copies of theses or reports resulting from this work must be lodged with SAHRA.
5. SAHRA shall not be liable for any losses, damages or injuries to persons or properties as a result of any activities in connection with this permit.
6. SAHRA reserves the right to cancel this permit by notice to the permit holder.
7. This permit is subject to a general appeal and may be suspended should an appeal against the decisions be received by SAHRA within 14 days from the date of the permit. SAHRA may not be held responsible for any costs or losses incurred in the event of the suspension or retraction of this permit.

This permit is valid until 01 August 2011.

for CHIEF EXECUTIVE OFFICER

Date: 09 July 2010



Place: Cape Town



**SOUTH AFRICAN HERITAGE
RESOURCES AGENCY**

111 HARRINGTON STREET, CAPE TOWN, 8000
PO BOX 4637, CAPE TOWN, 8000
TEL: (021) 462 4502 FAX: (021) 462 4509

DATE: 09 July 2010
ENQUIRIES: Mrs Colette Scheermeyer
OUR REF: 9/2/257/0002

Dr Amanda Esterhuysen
School of Geography, Archaeology and Environmental Studies
University of the Witwatersrand
Private Bag
WITS
2050

Dear Dr Esterhuysen

PERMIT: No. 80/10/05/009/51

Thank you for settling your permit application fee with SAHRA. I attach as requested a permit to obtain samples of approximately 10mm in diameter of fibrous tissue from inside the abdomen of mummified human remains excavated from Makapan and placed in the Raymond Dart collection (specimen A1081) at the Wits Medical School in 1948. We have noted this permit has been issued on behalf of Shazadee Karodia, an MSc student under your supervision, who will be undertaking the sampling as part of her studies.

The SAHRA APM Unit wishes Ms Karodia every success with the project.

Yours sincerely

Mrs Nonotho Ndebochani
SAHRA: Manager of the Archaeology, Palaeontology and Meteorites Unit
For CHIEF EXECUTIVE OFFICER



Figure B.10 The stereo microscope

Table B.1 SEM procedures used in the examination of desiccated tissue from Historic Cave

Sample	Pre-sonication treatment	Sonication	Post-sonication treatment	SEM
5-2	×	×	×	Gold coated for 4 minutes before SEM
69-1	×	10 minutes in acetone	Left to dry	Gold coated for 4 minutes before SEM
69-2	×	10 minutes in acetone	Left to dry	Gold coated for 4 minutes before SEM
5-3	Fixed in absolute alcohol for 24 hours	Twice in absolute alcohol for 5 minutes	Oven dried for 2 hours	Gold coated for 4 minutes before SEM
74-2	Fixed in absolute alcohol for 24 hours	Twice in absolute alcohol for 5 minutes	Acetone for 5 minutes & left to dry for 10 minutes	Gold coated for 6 minutes before SEM
74-3	×	Twice in acetone for 5 minutes	Left to dry for 10 minutes	Gold coated for 6 minutes before SEM.

Table B.2 Histological procedures used in the examination of desiccated tissue from Historic Cave

Sample	Pre-sonication treatment	Sonication	Post-sonication treatment	Rehydration	Post-rehydration treatment	Staining
5-1	Fixed in 10% buffered formalin for 24 hours	×	×	×	×	Masson trichrome stain
50-1	Fixed in 10% buffered formalin for 7 days	×	×	×	×	Hematoxylin-eosin stain
74-1	×	Twice in absolute alcohol for 5 minutes	Oven dried for 2 hours	×	×	Masson trichrome stain
			Fixed in 10% phenol for 7 hours			Hematoxylin-eosin stain

Sample	Pre-sonication treatment	Sonication	Post-sonication treatment	Rehydration	Post-rehydration treatment	Staining
<i>[This is a continuation of Table B.2 on the previous page]</i>						
69-3	×	Twice in absolute alcohol for 5 minutes	Oven dried for 2 hours	×	×	Masson trichrome stain
			10% phenol for 7 hours			
			Fixed in 10% buffered formalin for 24 hours			Hematoxylin-eosin stain
50-2	×	Twice in absolute alcohol for 5 minutes	Oven dried for 2 hours	×	×	Masson trichrome stain
			10% phenol for 7 hours			Hematoxylin-eosin stain

Sample	Pre-sonication treatment	Sonication	Post-sonication treatment	Rehydration	Post-rehydration treatment	Staining
<i>[This is a continuation of Table B.2 on the previous page]</i>						
74-4	×	Twice in acetone for 10 minutes	×	Rehydrated in Sandison's solution for 24 hours	Fixed in 10% buffered formalin for 24 hours	Masson trichrome stain
						Hematoxylin-eosin stain
74-5	×	Twice in acetone for 10 minutes	×	Rehydrated in glycerine for 7 days	Fixed in 10% buffered formalin for 24 hours	Masson trichrome stain
						Hematoxylin-eosin stain
69-4	×	Twice in acetone for 10 minutes	×	Rehydrated in Sandison's solution for 24 hours	Fixed in 10% buffered formalin for 24 hours	Masson trichrome stain
						Hematoxylin-eosin stain

Sample	Pre-sonication treatment	Sonication	Post-sonication treatment	Rehydration	Post-rehydration treatment	Staining
<i>[This is a continuation of Table B.2 on the previous page]</i>						
69-5	×	Twice in acetone for 10 minutes	×	Rehydrated in Ruffer's solution for 24 hours	Fixed in 10% buffered formalin for 24 hours	Masson trichrome stain
						Hematoxylin-eosin stain
50-4	×	Twice in absolute alcohol for 5 minutes	×	Rehydrated in Ruffer's solution for 24 hours	Fixed in 10% buffered formalin for 24 hours	Aldehyde hematoxylin-eosin stain
						Verhoeff's stain
50-5	×	Twice in absolute alcohol for 5 minutes	×	Rehydrated in Sandison solution for 24 hours	Fixed in 10% buffered formalin for 24 hours	Aldehyde hematoxylin-eosin stain
						Verhoeff's stain

Sample	Pre-sonication treatment	Sonication	Post-sonication treatment	Rehydration	Post-rehydration treatment	Staining
<i>[This is a continuation of Table B.2 on the previous page]</i>						
50-7	×	Twice in absolute alcohol for 5 minutes	×	Rehydrated in 10% buffered formalin for 24 hours	×	Aldehyde hematoxylin-eosin stain
						Verhoeff's stain
A1081-1	×	Twice in absolute alcohol for 5 minutes	×	Rehydrated in Ruffer's solution for 24 hours	Fixed in 10% buffered formalin for 24 hours	Masson trichrome stain
						Hematoxylin-eosin stain
A1081-2	×	Twice in absolute alcohol for 5 minutes	×	Rehydrated in glycerine for 7 days	Fixed in 10% buffered formalin for 24 hours	Masson trichrome stain
						Hematoxylin-eosin stain

Sample	Pre-sonication treatment	Sonication	Post-sonication treatment	Rehydration	Post-rehydration treatment	Staining
<i>[This is a continuation of Table B.2 on the previous page]</i>						
A1081-3	×	Twice in absolute alcohol for 5 minutes	×	Rehydrated in Sandison's solution for 24 hours	Fixed in 10% buffered formalin for 24 hours	Masson trichrome stain
						Hematoxylin-eosin stain

APPENDIX C

Table C.1 Table illustrating the cleaning procedure, the SEM results, and the EDS results for the desiccated tissue from Historic Cave

Sample	Pre-sonication treatment	Sonication	Post-sonication treatment	SEM results	EDS results
5-2	×	×	×	Dust	High counts of C, O, Na, Mg, K, Ca, Si
				Epidermal keratinocytes in patches	
5-3	Fixed in absolute alcohol for 24 hours	Twice in absolute alcohol for 5 minutes	Oven dried for 2 hours	Epidermal keratinocytes in patches	Low counts of C, O, Na, Mg, K, Ca, Si
				Dust	
69-1	×	10 minutes in acetone	Left to dry	Dust	High counts of C, O, Na, Mg, Si, K, Ca
69-2	×	10 minutes in acetone	Left to dry	Dust	High counts of C, O, Na, Mg, Si, K, Ca
74-2	Fixed in absolute alcohol for 24 hours	Twice in absolute alcohol for 5 minutes	Acetone for 5 minutes & left to dry for 10 minutes	Epidermal keratinocytes in patches	Mg, P, K, Ca
				Hair shaft holes	
				Hair	
74-3	×	Twice in acetone for 5 minutes	Left to dry	Dust	K & Ca present. Decreased counts for C, Mg, & Si
				Hair shaft holes	
				Hairs	

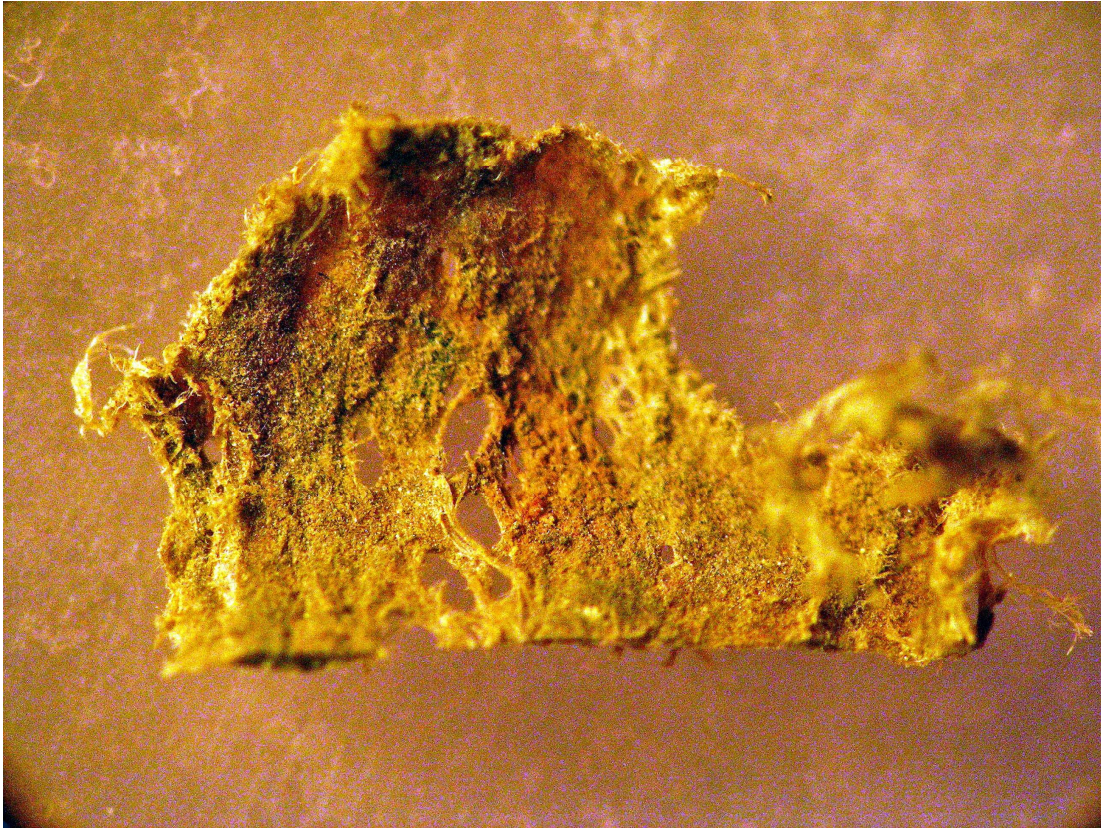


Figure C.1 Stereo microscope observation of the dorsal surface of the desiccated skin (sample 5-2). The sample was not cleaned. Dust is observed on the surface of the sample

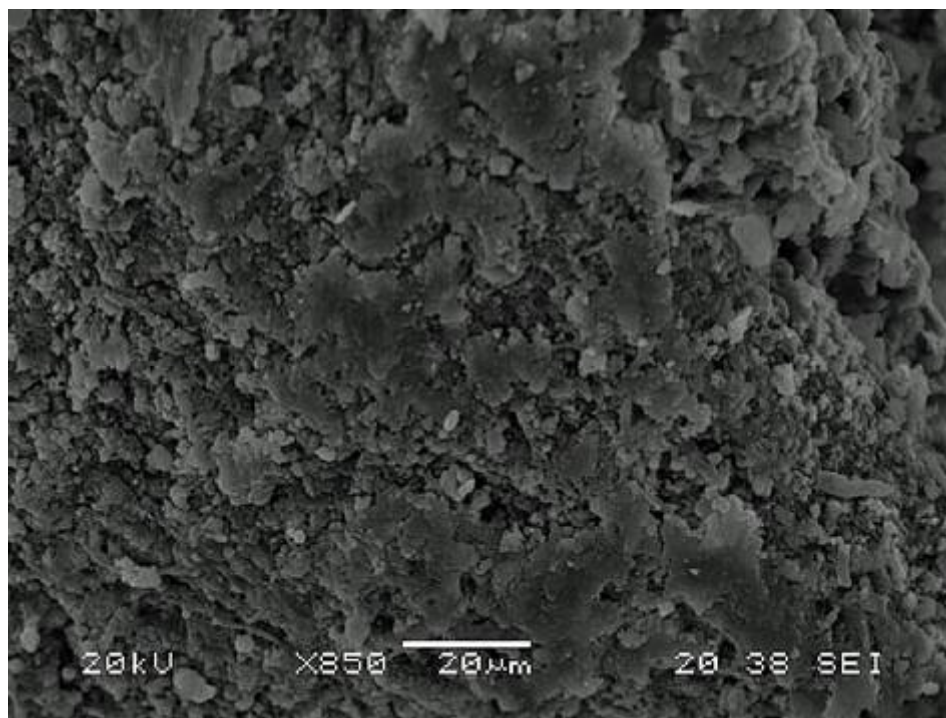


Figure C.2 SEM observation showing dust and debris coating the surface of the skin (sample 5-2). This sample was not cleaned

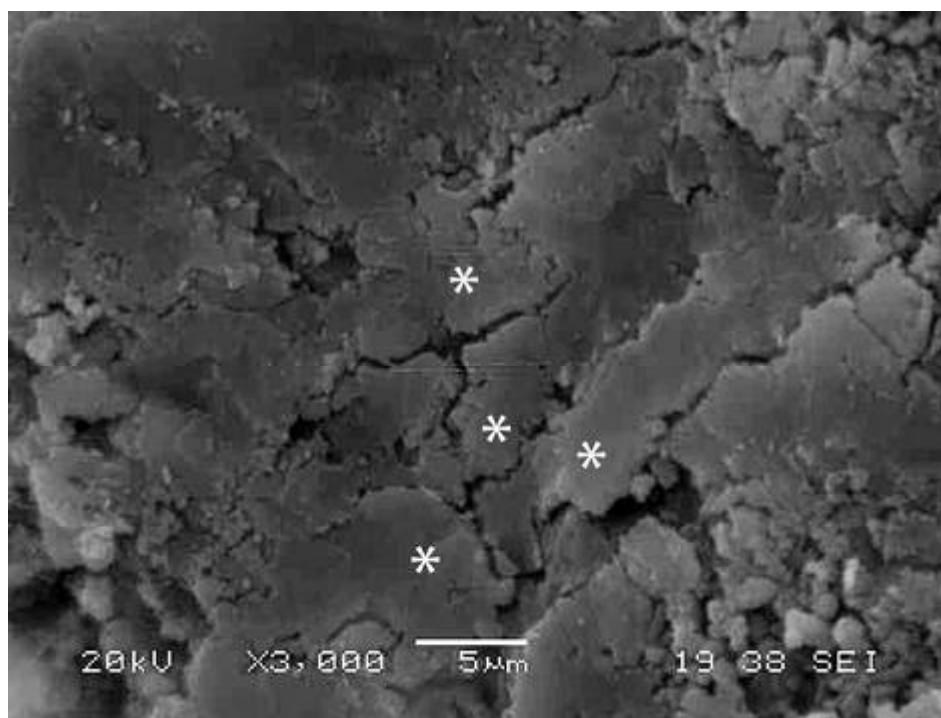


Figure C.3 SEM observation of flattened epidermal keratinocytes (asterix) on the surface of the skin (sample 5-2). This sample was not cleaned

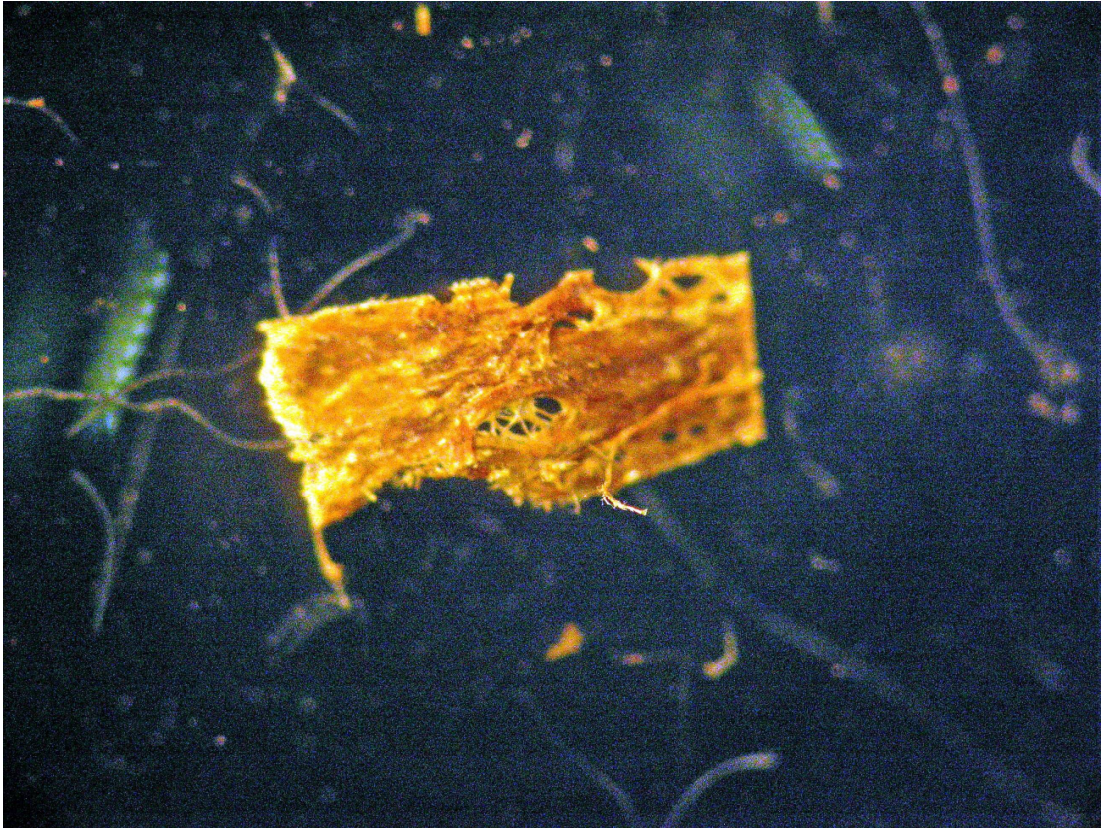


Figure C.4 Stereo microscope observation of the ventral surface of the desiccated skin (sample 5-3). The sampled was cleaned in absolute alcohol. A layer of dust and debris was observed on the surface of the tissue

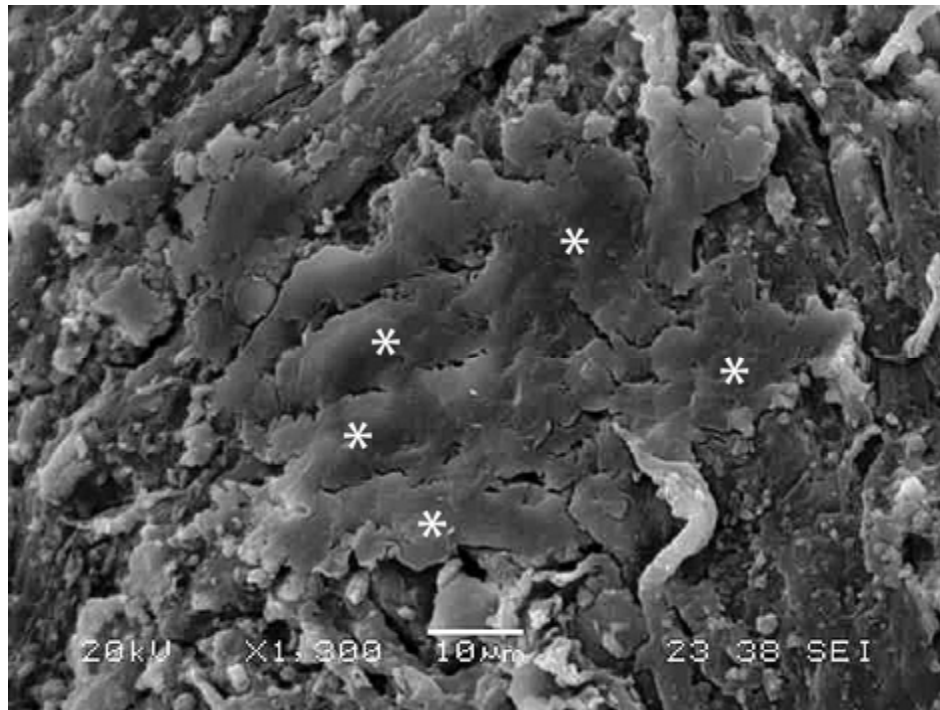


Figure C.5 SEM observation of flattened epidermal keratinocytes (asterix) on the surface of the skin (sample 5-3)

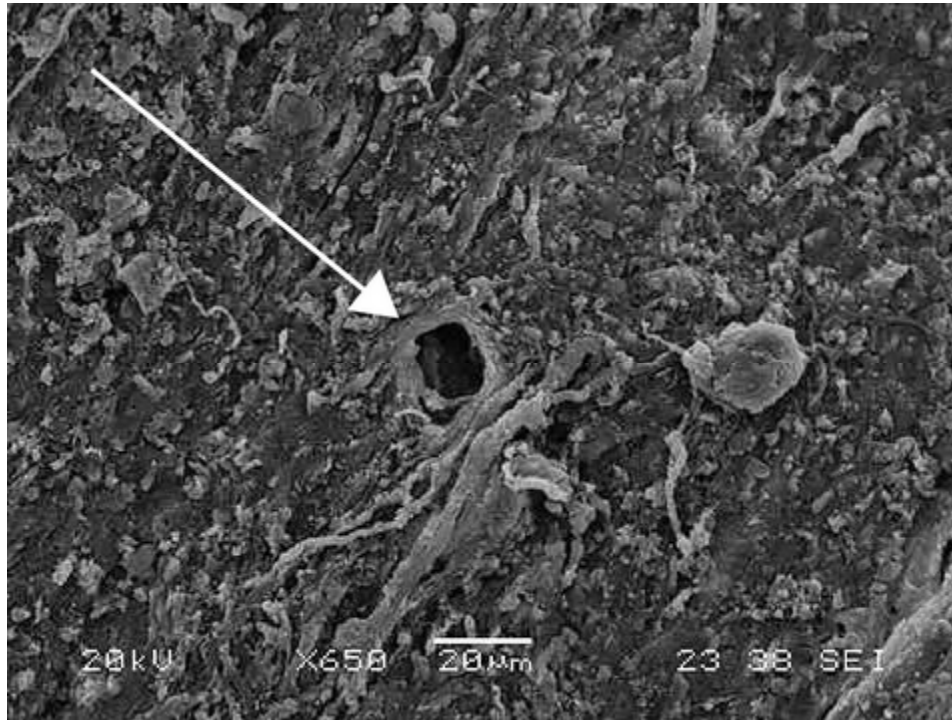


Figure C.6 SEM observation of a hair shaft hole (arrow) on the surface of the skin (sample 5-3)



Figure C.7 A foreign particle (asterix) on the surface of the skin (sample 5-3). The particle showed a high calcium count when analysed with EDS

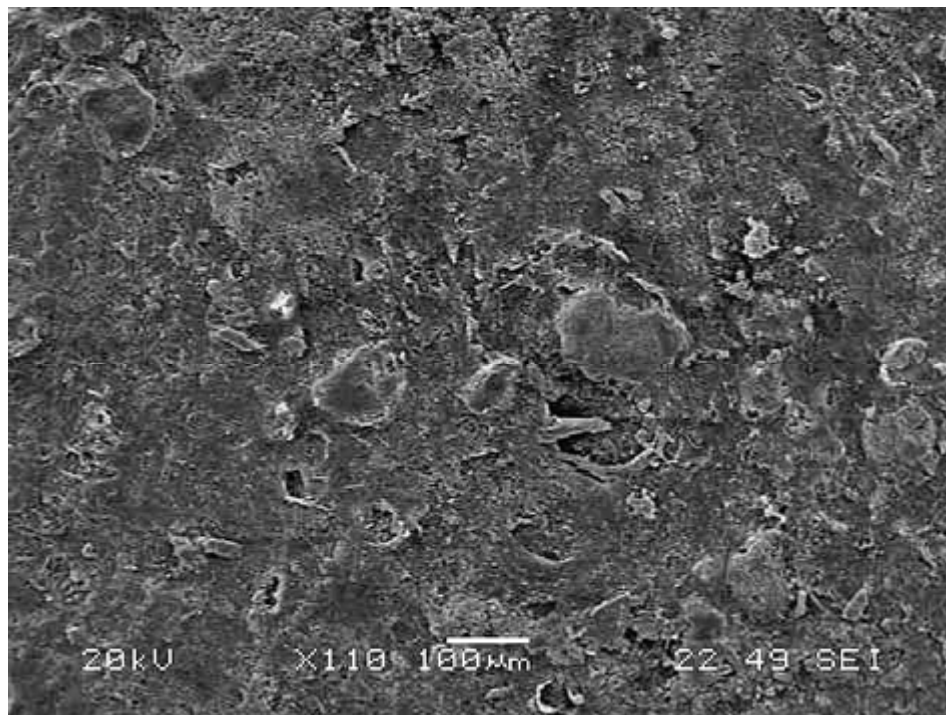


Figure C.8 SEM observation of the surface of the skin (sample 74-2). The sample was cleaned absolute alcohol. Most of the dust and debris was removed

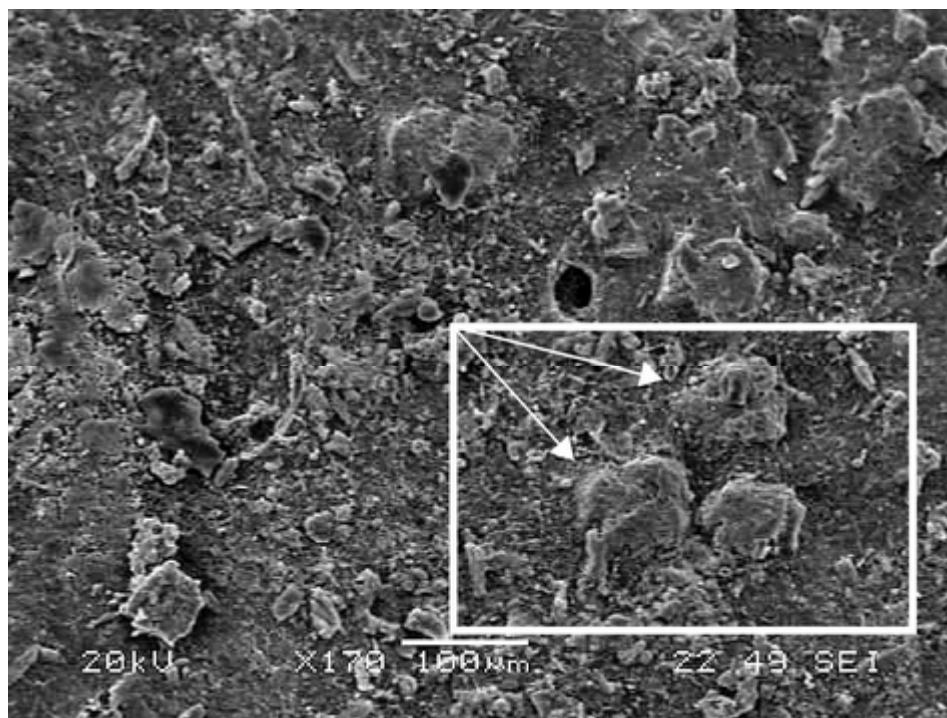


Figure C.9 SEM observation of flattened epidermal keratinocytes (arrows) on the skin (sample 74-2)

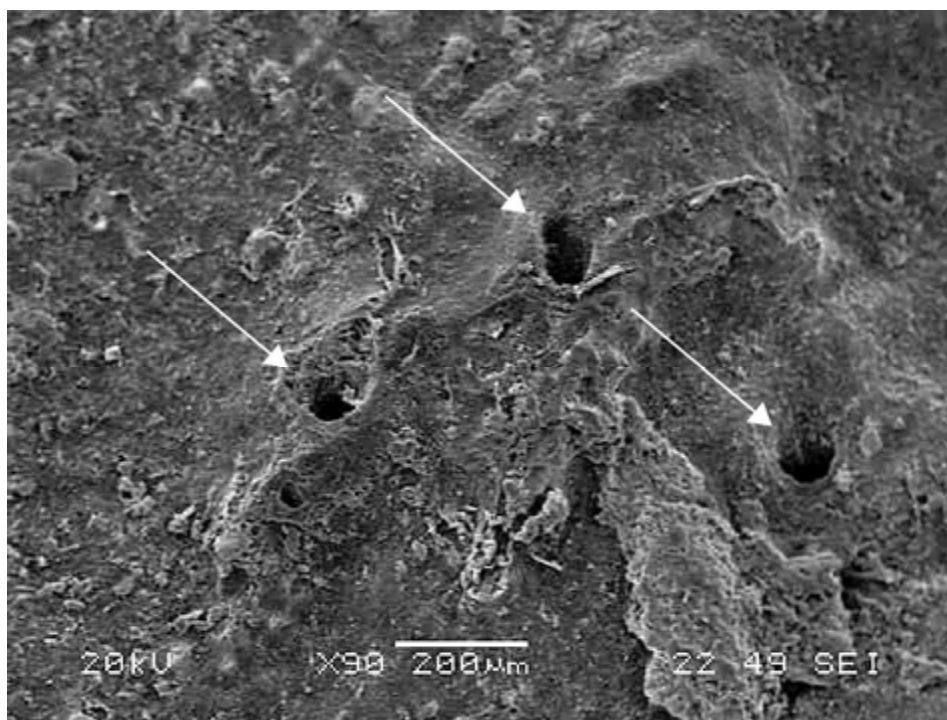


Figure C.10 SEM observation of hair shaft holes (arrows) on the skin (Sample 74-2)

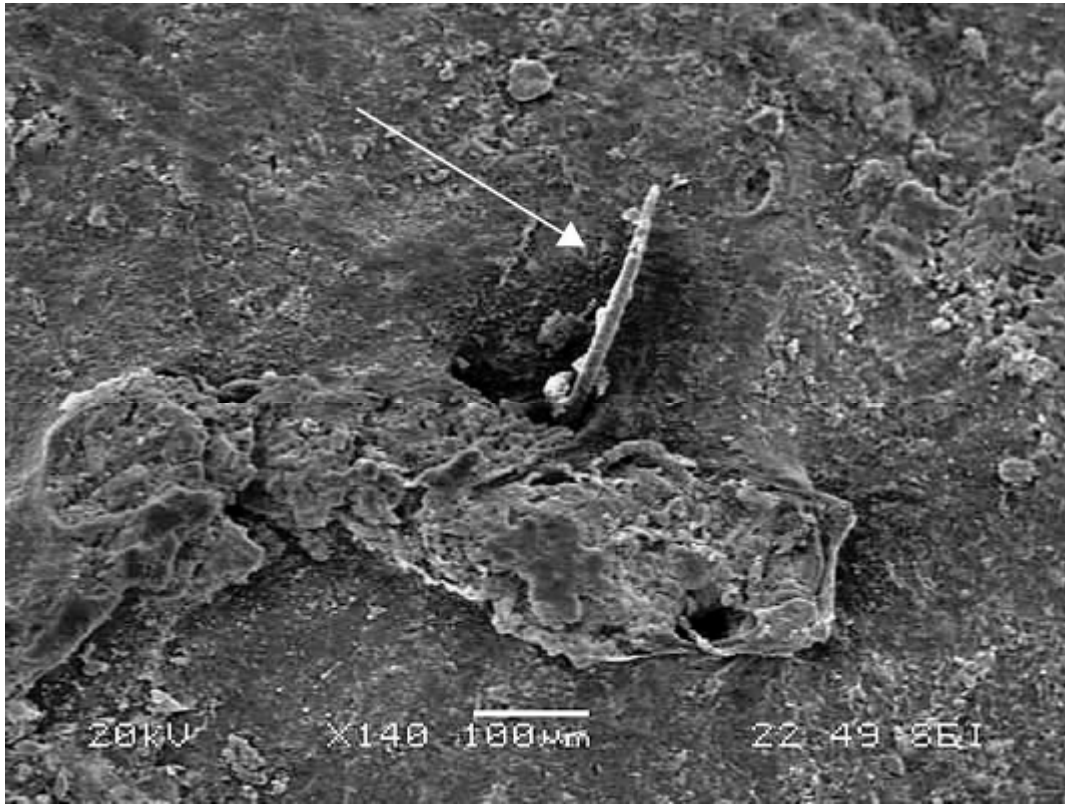


Figure C.11 SEM observation of a minute structure (arrow) resembling a hair on the skin (sample 74-2)

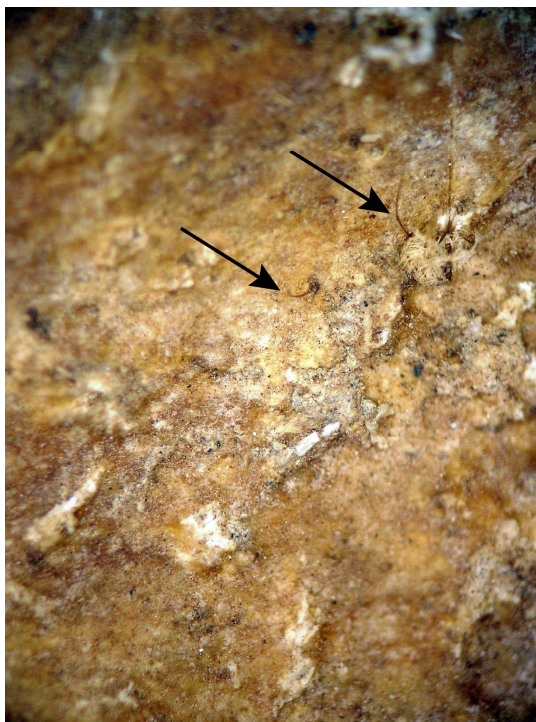
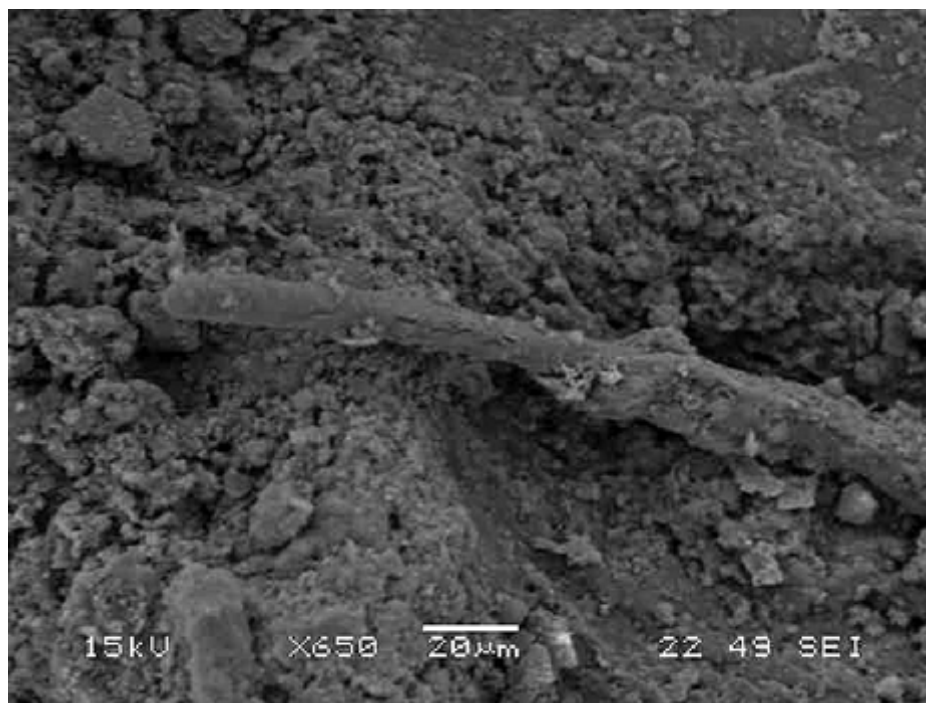


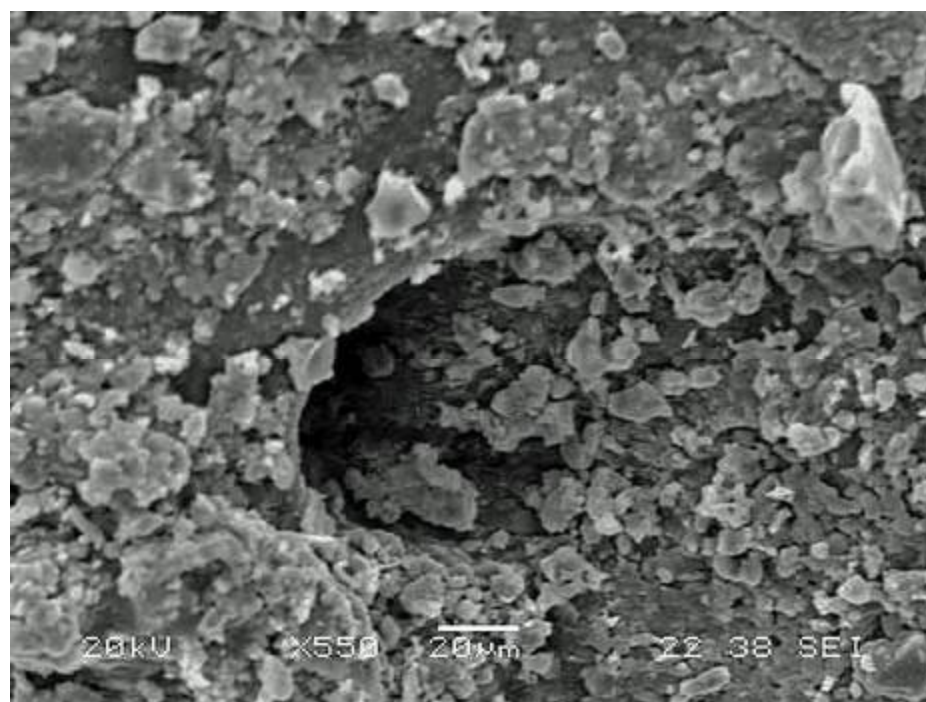
Figure C.12 Stereo microscope observation showing minute structures (arrows) resembling hairs on the surface of the skin (sample 75-3) prior to the sonication treatment



Figure C.13 Magnified image of the minute structures (arrows) shown in Figure C.12



**Figure C.14 SEM observation of dust on the surface of the skin (sample 74-3).
The sample was cleaned in acetone**



**Figure C.15 SEM observation of dust on the surface of the skin (sample 74-3).
The sample was cleaned in acetone**

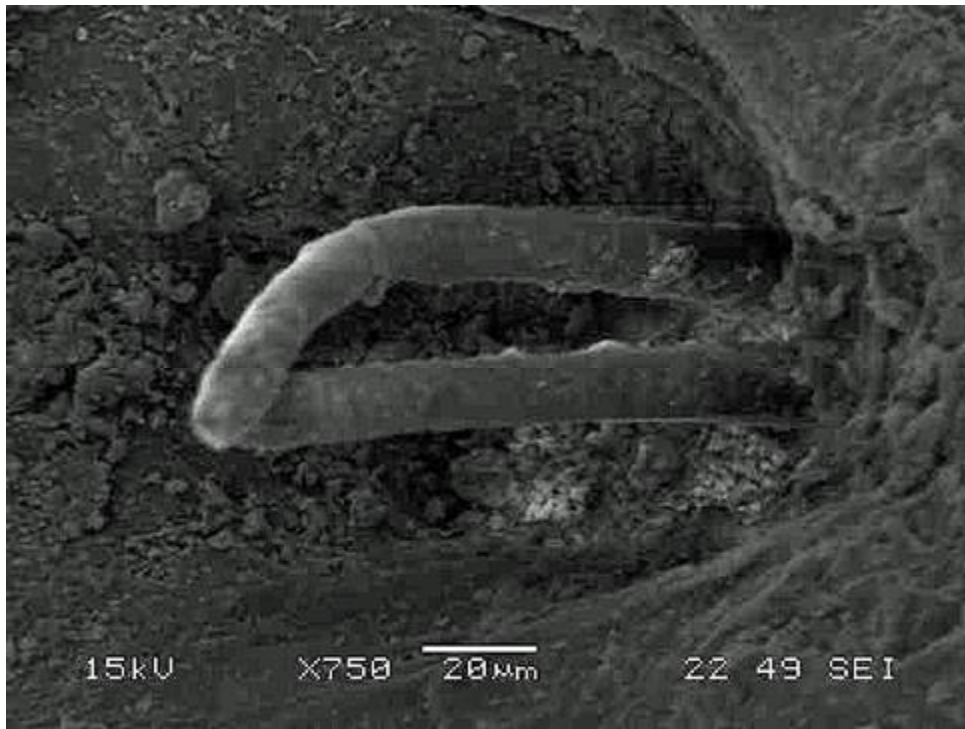


Figure C.16 Hair on the surface of the skin (sample 74-3)



Figure C.17 Hair on the surface of the skin (sample 74-3)



Figure C.18 (A) Hair on the surface of the skin (sample 74-3); (B) Magnified image of the hair in (A)

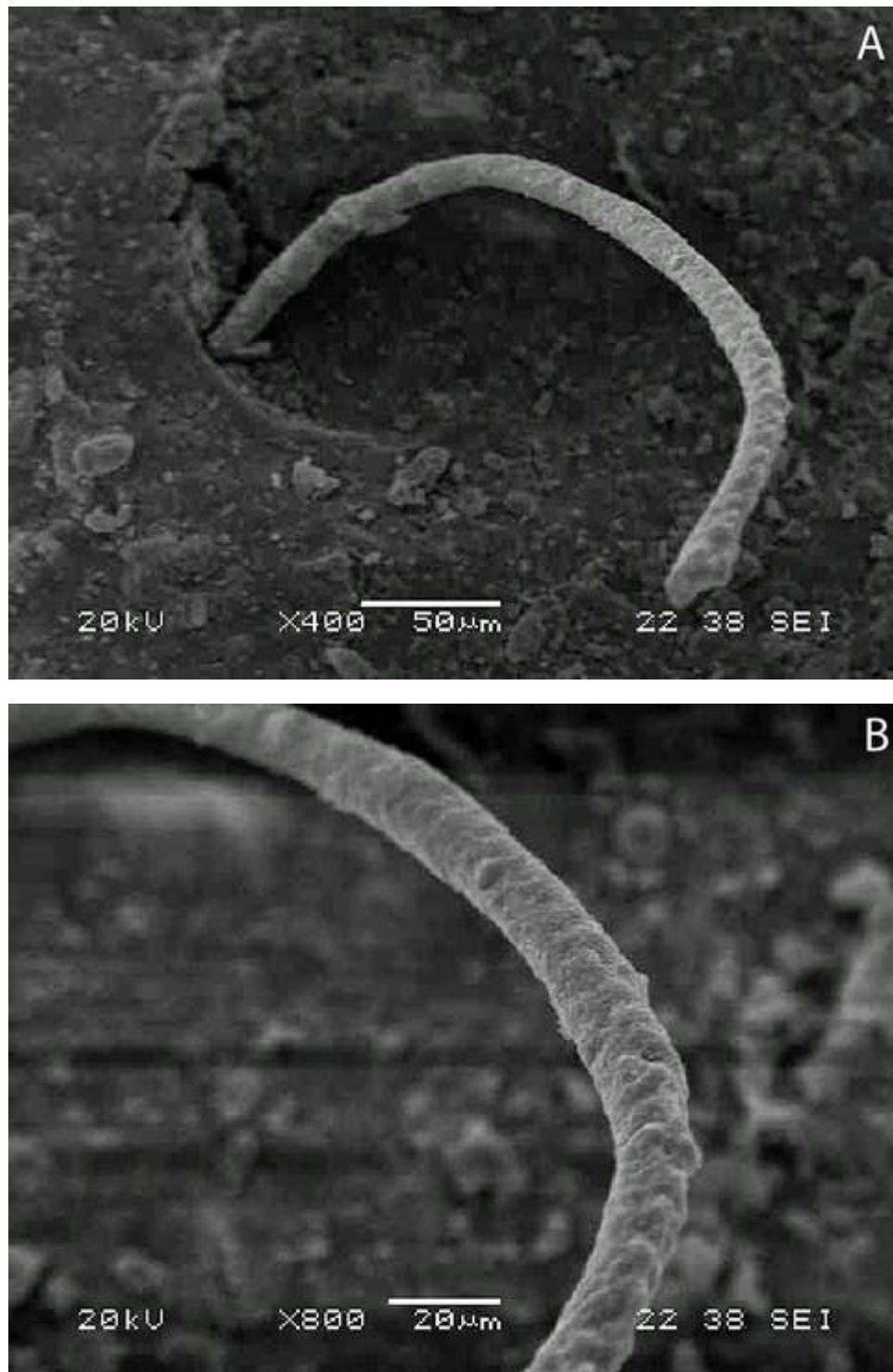


Figure C.19 (A) Hair on the surface of the skin (sample 74-3); (B) Magnified image showing keratinised scales on the hair shown in (A)

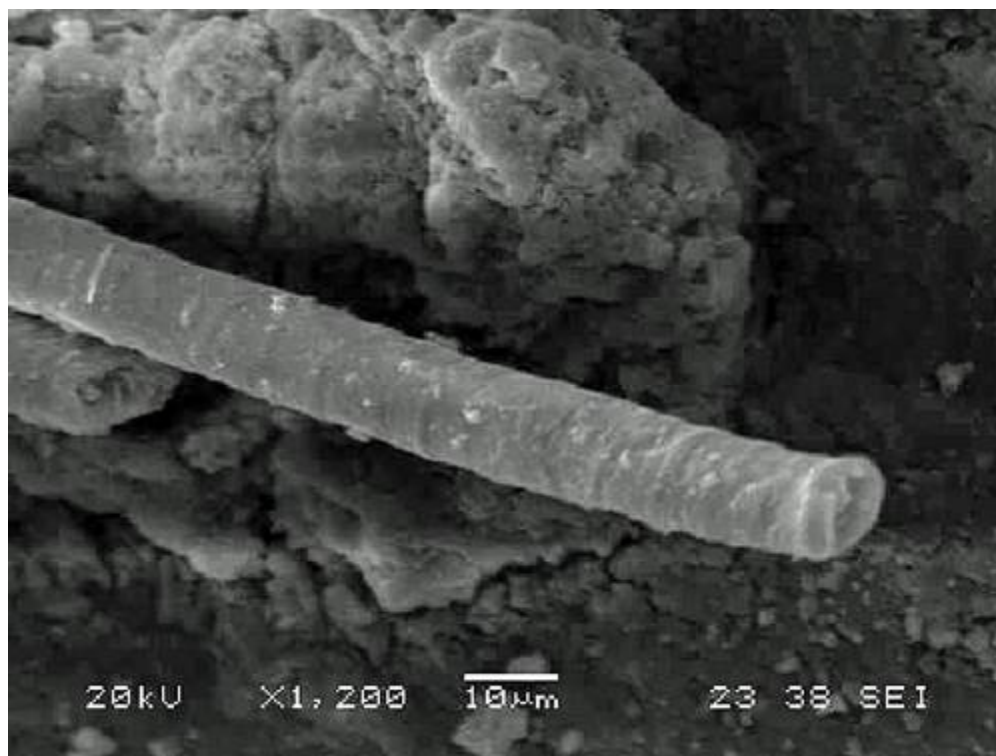


Figure C.20 Hair on the surface of the skin (sample 74-3)

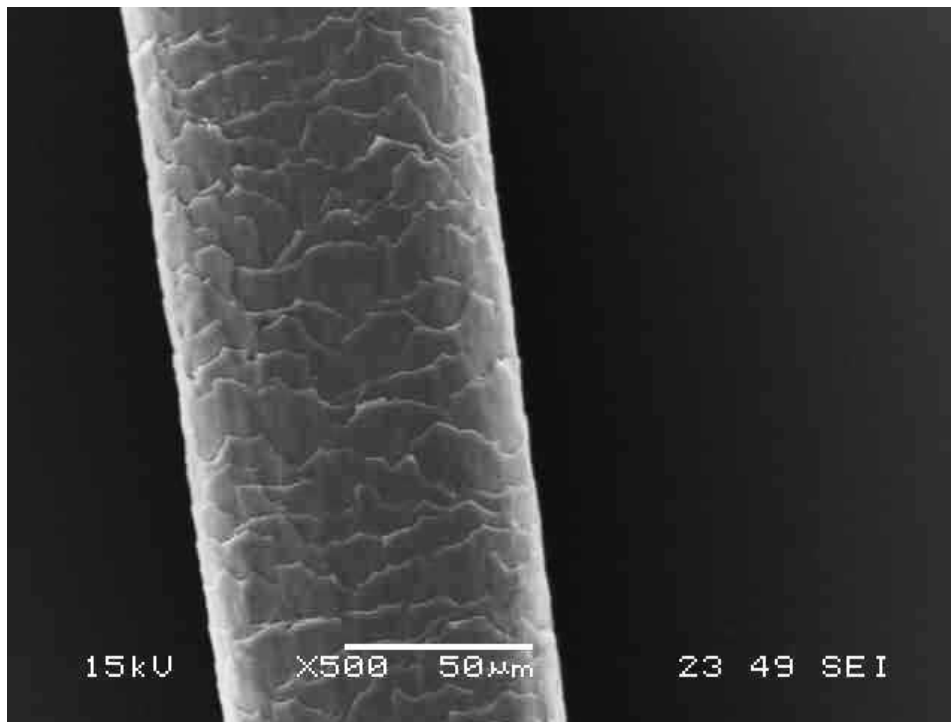


Figure C.21 Keratin scales on the cuticle of a normal human hair from the scalp

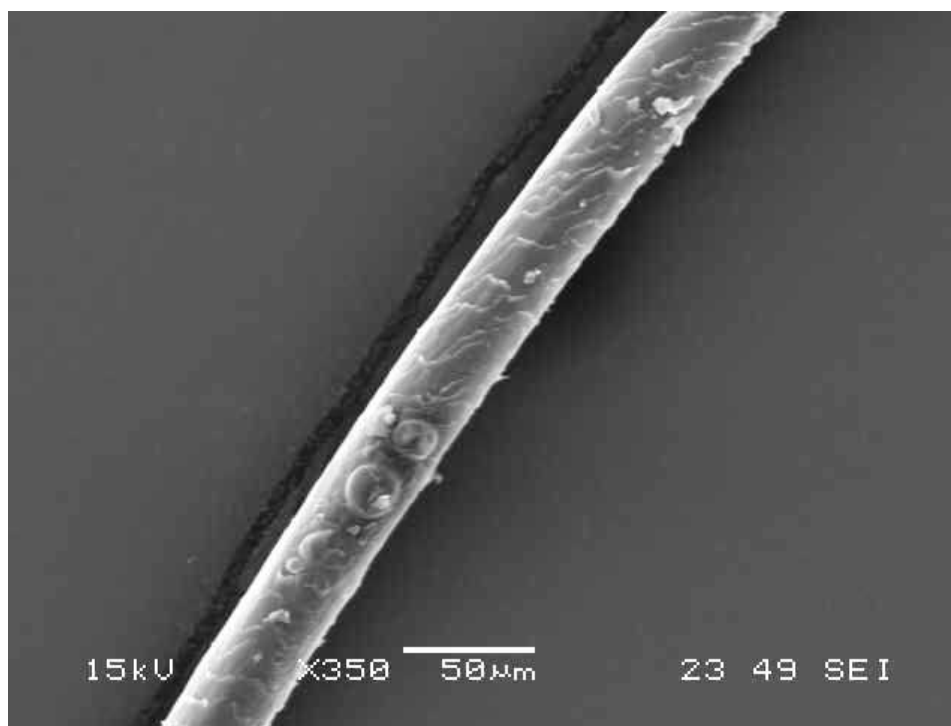


Figure C.22 SEM observation of the hair from the arm

Table C.2 Table illustrating the histological procedures and the results for the desiccated tissue from Historic Cave

Sample	Sonication	Rehydration	Stain	Results
50-1	Fixed in 10% buffered formalin for 7 days	×	Hematoxylin-eosin	Section stained pink
				Dust
				Fascicles were observed but individual muscle fibres & nuclei could not be identified
				Blood vessels could be identified
50-2	Twice in absolute alcohol		Masson trichrome	Sections stained red & green
	Oven dried for 2 hours			Fascicles were observed but individual muscle fibres & nuclei could not be identified
	Fixed in 10% phenol for 7 hours			The epimysium & perimysium were identified

Sample	Sonication	Rehydration	Stain	Results	
<i>[This is a continuation of Table C.2 on the previous page]</i>					
50-4	Twice in absolute alcohol	Ruffer’s solution for 24 hours	Aldehyde hematoxylin-eosin	Section stained deep pink	
				Fascicles were observed but individual muscle fibres & nuclei could not be identified	
				The connective tissue could not be identified	
	Left to dry		Verhoeff’s stain	Poorly preserved tissue	
50-5	Twice in absolute alcohol for 5 minutes	Sandison’s solution for 24 hours	Aldehyde hematoxylin-eosin	Section stained deep pink	
				Fascicles were observed but nuclei could not be identified	
				The connective tissue could not be identified	
	Left to dry		Verhoeff’s stain	Sections stained yellow	
				Fascicles were observed but individual muscle fibres & nuclei could not be identified	
				The connective tissue was identified	

Sample	Sonication	Rehydration	Stain	Results
<i>[This is a continuation of Table C.2 on the previous page]</i>				
50-7	Twice in absolute alcohol for 5 minutes	10% buffered formalin for 24 hours	Aldehyde hematoxylin-eosin	Poorly preserved tissue
	Left to dry		Verhoeff's stain	Poorly preserved tissue
69-3	Twice in absolute alcohol for 5 minutes	×	Masson trichrome	Section stained red & green
				Muscle tissue stained red
	Oven dried for 2 hours			Collagen fibres were poorly preserved
	10% phenol for 7 hours		Hematoxylin-eosin	Section stained pink
	Fixed in 10% buffered formalin for 14 hours			Fascicles were observed but individual muscle fibres & nuclei could not be identified
				The perimysium was identified

Sample	Sonication	Rehydration	Stain	Results
<i>[This is a continuation of Table C.2 on the previous page]</i>				
69-4	Twice in acetone for 10 minutes	Sandison’s solution for 24 hours	Masson trichrome	Section stained red & green
				Connective tissue with muscle & collagen fibres
				The epithelium detached from the underlying connective tissue
	Left to dry		Hematoxylin-eosin	Section stained pink
				Connective tissue with muscle & collagen fibres
				Epithelium could be identified

Sample	Sonication	Rehydration	Stain	Results
[This is a continuation of Table C.2 on the previous page]				
69-5	Twice in acetone for 10 minutes	Ruffer’s solution for 24 hours	Masson trichrome	Sections stained red & green
				Dust
				Connective tissue with muscle & collagen fibres
				Epithelial tissue could not be identified
	Left to dry		Hematoxylin-eosin	Sections stained pink
				Poorly preserved tissue

Sample	Sonication	Rehydration	Stain	Results
[This is a continuation of Table C.2 on the previous page]				
74-1	Twice in absolute alcohol for 5 minutes	×	Masson trichrome	Sections stained red & green
				Fascicles were observed but individual muscle fibres & nuclei could not be identified
				The perimysium could be identified
	Oven dried for 2 hours		Hematoxylin-eosin	Sections stained pink
	Fixed in 10% phenol for 7 hours			Fascicles were observed but individual muscle fibres & nuclei could not be identified
				The perimysium could be identified

Sample	Sonication	Rehydration	Stain	Results
<i>[This is a continuation of Table C.2 on the previous page]</i>				
74-4	Twice in acetone for 10 minutes	Sandison's solution for 24 hours	Masson trichrome	Sections stained red & green
				Dust
				Fascicles were observed but individual muscle fibres & nuclei could not be identified
				The epimysium & perimysium were identified
	Left to dry		Hematoxylin-eosin	Sections stained pink
				Dust
				Fascicles were observed but individual muscle fibres & nuclei could not be identified
				The perimysium could be identified

Sample	Sonication	Rehydration	Stain	Results
<i>[This is a continuation of Table C.2 on the previous page]</i>				
74-5	Twice in acetone for 10 minutes	Glycerine for 7 days	Masson trichrome	Sections stained red & green
				Dust
				The epidermis and dermis could be identified
	Left to dry		Hematoxylin-eosin	Dust
				Poorly preserved tissue
A1081-1	Twice in absolute alcohol for 5 minutes	Ruffer's solution for 24 hours	Masson trichrome	Dust
				Poorly preserved tissue
	Left to dry		Hematoxylin-eosin	Dust
				Poorly preserved tissue

Sample	Sonication	Rehydration	Stain	Results
<i>[This is a continuation of Table C.2 on the previous page]</i>				
A1081-2	Twice in absolute alcohol for 5 minutes	Glycerine for 7 days	Masson trichrome	Dust
				Poorly preserved tissue
	Left to dry		Hematoxylin-eosin	Dust
				Poorly preserved tissue
A1081-3	Twice in absolute alcohol for 5 minutes	Sandison’s solution for 24 hours	Masson trichrome	Sections stained red & green
				Fascicles were observed but individual muscle fibres & nuclei could not be identified
				The perimysium could be identified
	Left to dry		Hematoxylin-eosin	Sections stained pink
The tissue was identified as striated muscle tissue that is poorly preserved				

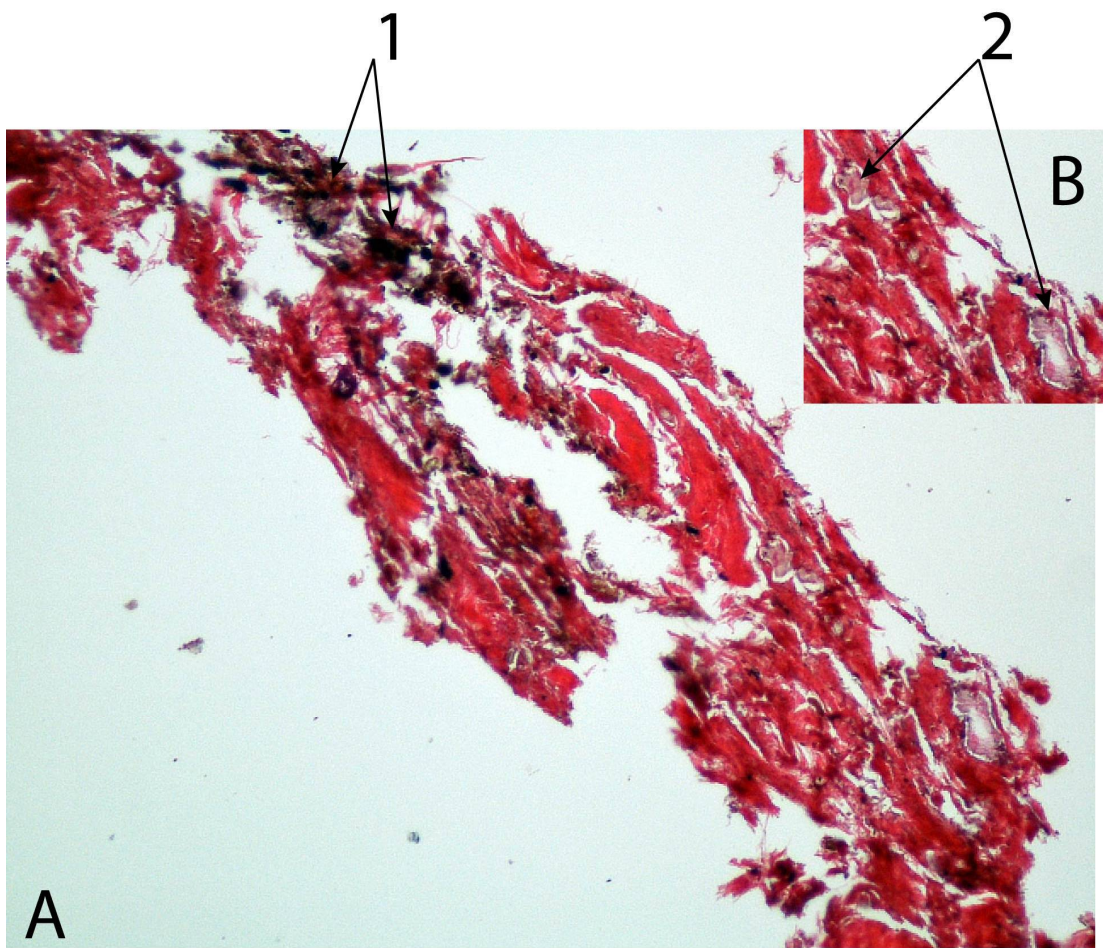


Figure C.23 (A) Hematoxylin-eosin stain of the desiccated muscle tissue from the rib (sample 50-1) with dust is embedded in the tissue (1). (B) Muscle fibres with blood vessels (2). $\times 20$ objective lens

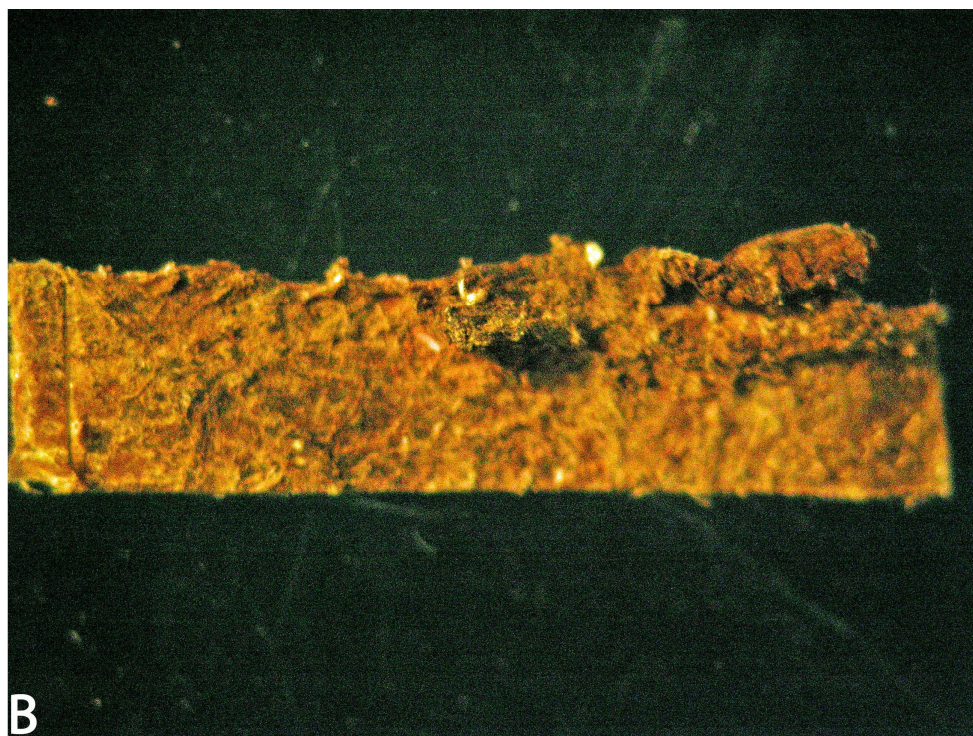
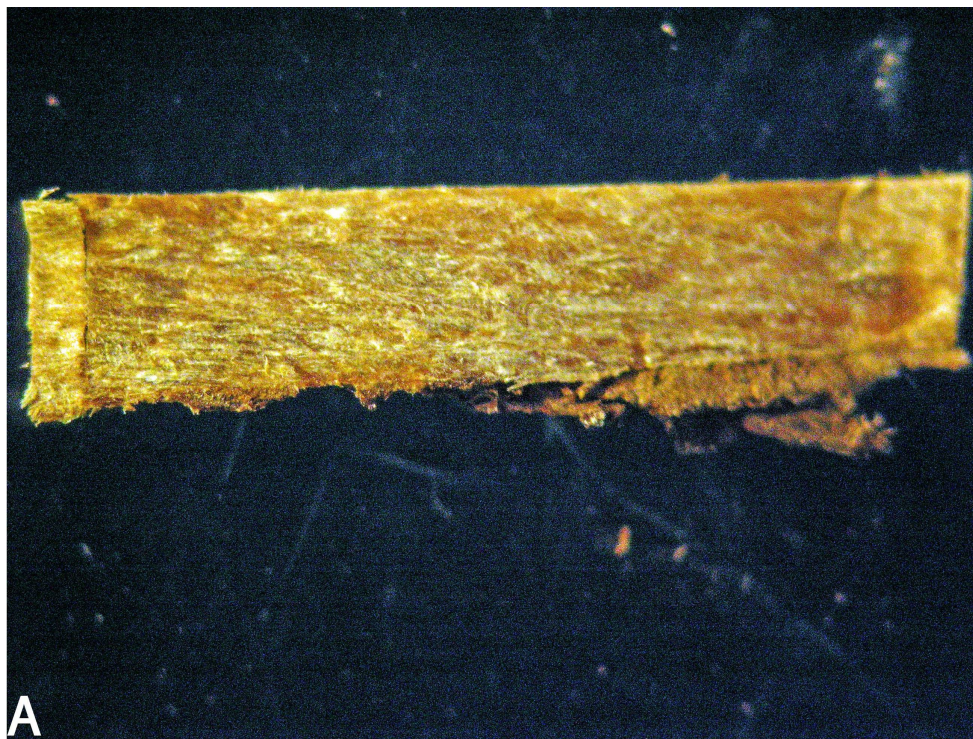


Figure C.24 Stereo microscope observation of the dorsal (A) and the ventral (B) views of the desiccated tissue from the rib (sample 50-2). A layer of dust and debris was observed on the surface of the tissue

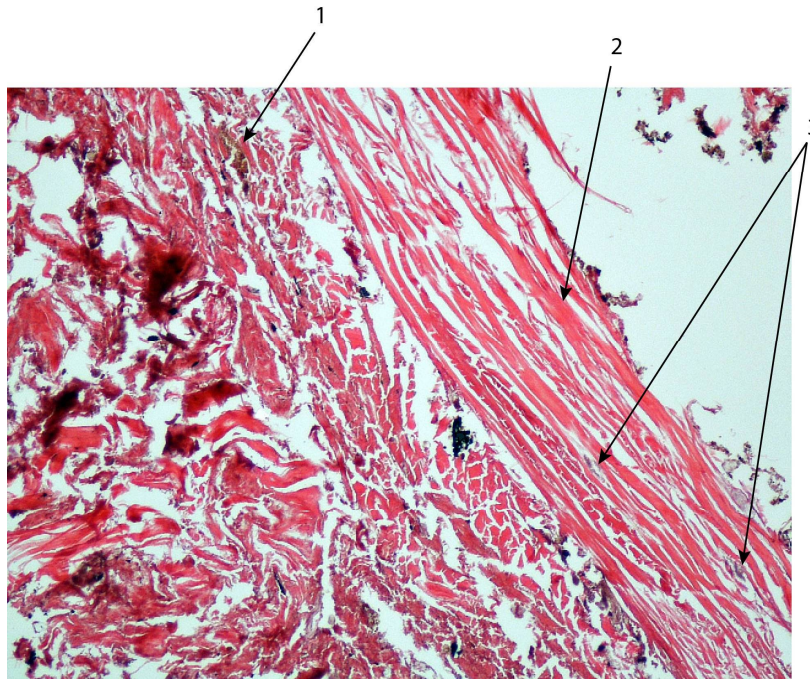


Figure C.25 Hematoxylin-eosin stain of the desiccated tissue from the rib (sample 50-2). Dust is embedded in the muscle tissue (1) which is composed of fascicles (2) and the perimysium (3). $\times 10$ objective lens

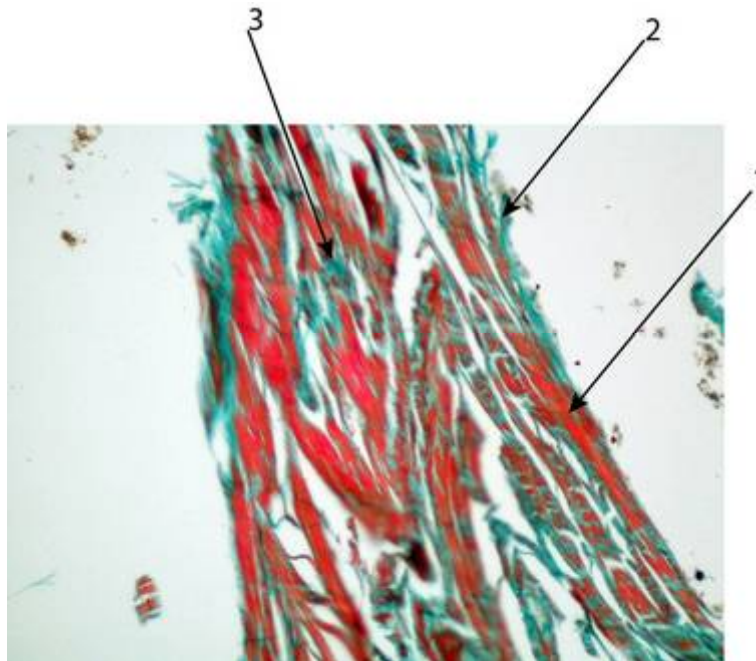


Figure C.26 Masson trichrome stain of the desiccated tissue from the rib (sample 50-2). The muscle tissue is composed of fascicles (1), the epimysium (2) and the perimysium (3). $\times 20$ objective lens



Figure C.27 Stereo microscope observation of the surface of the desiccated tissue from the rib (sample 50-4). The stereo microscope observation showed that much of the dust was removed

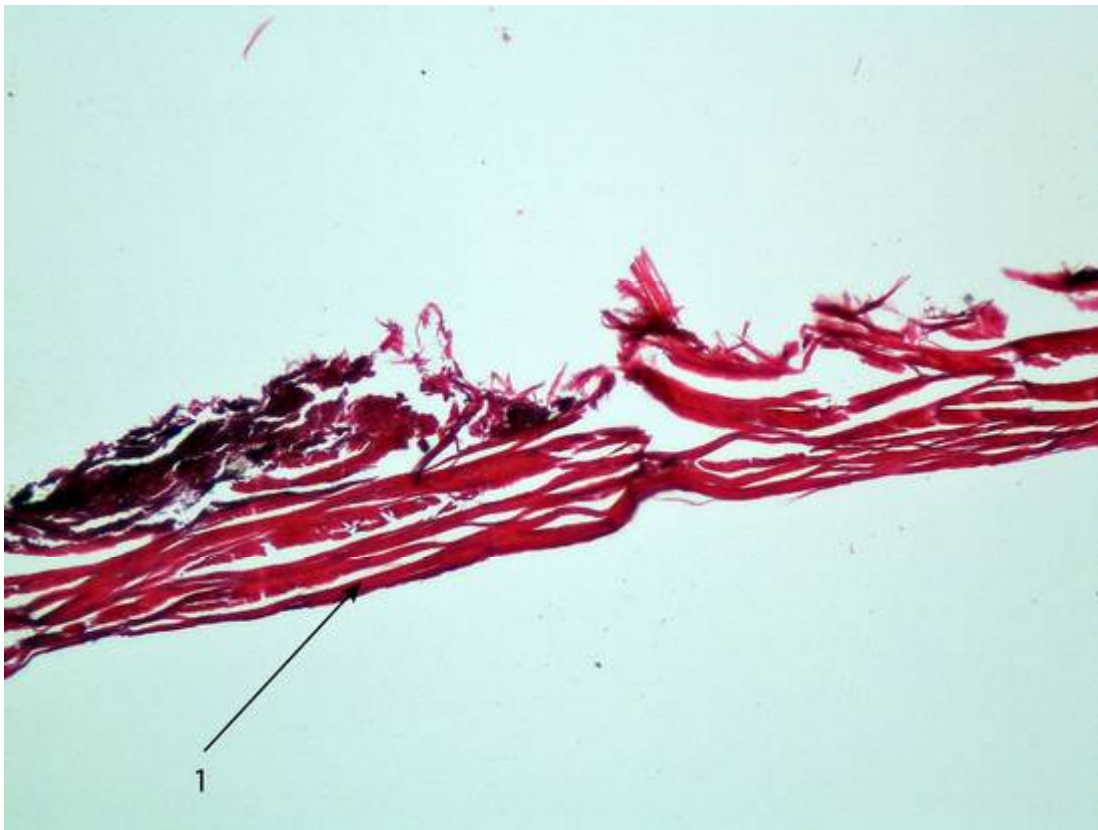


Figure C.28 Aldehyde hematoxylin-eosin stain of the desiccated tissue from the rib (sample 50-4). The muscle tissue is arranged as a series of fascicles (1). ×20 objective lens

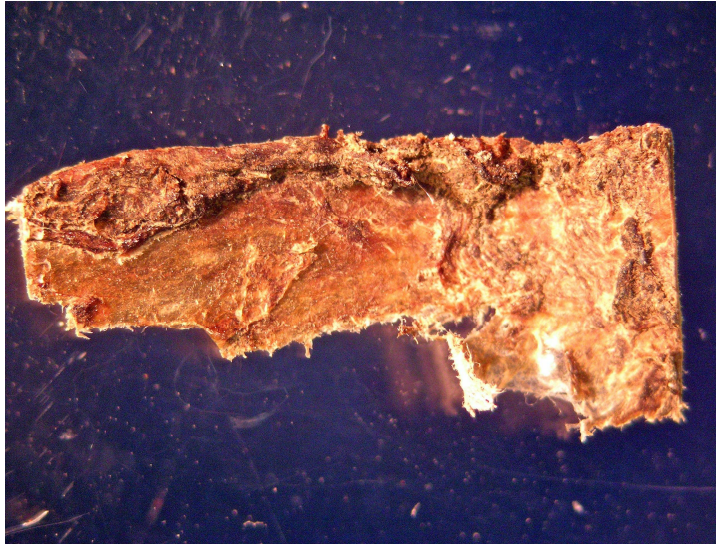


Figure C.29 Stereo microscope observation of the surface of the desiccate tissue from the rib (sample 50-5). Much of the dust was removed after cleaning

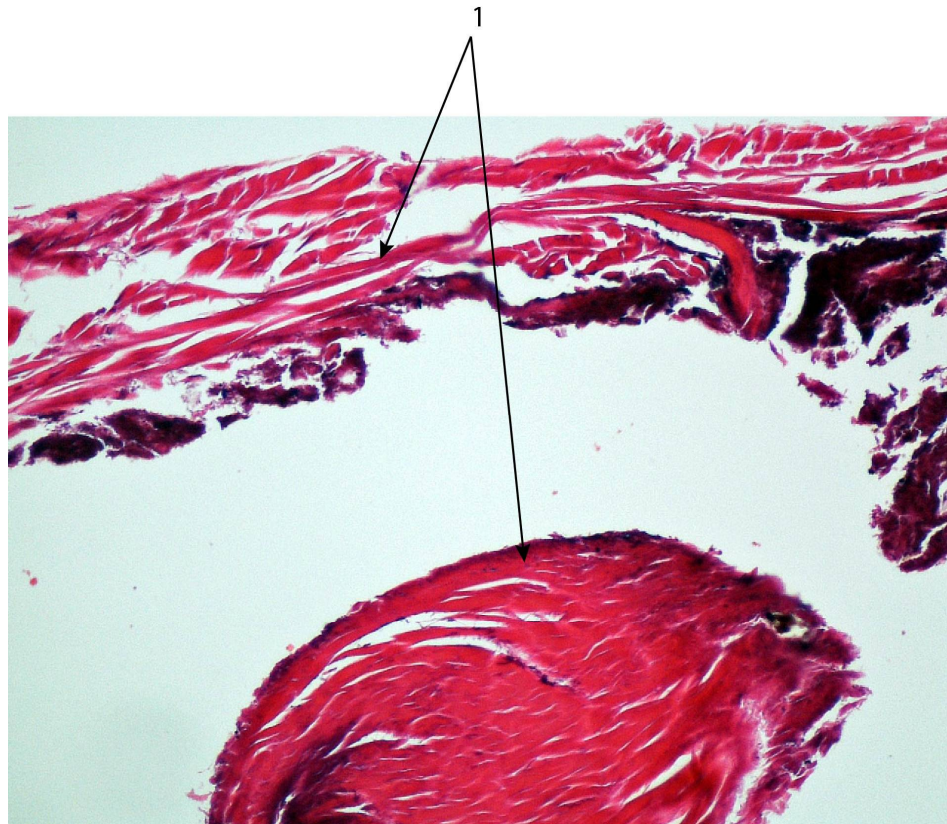


Figure C.30 Aldehyde hematoxylin-eosin stain of the desiccated tissue from the rib (sample 50-5). Fascicles are identified (1). ×20 objective lens

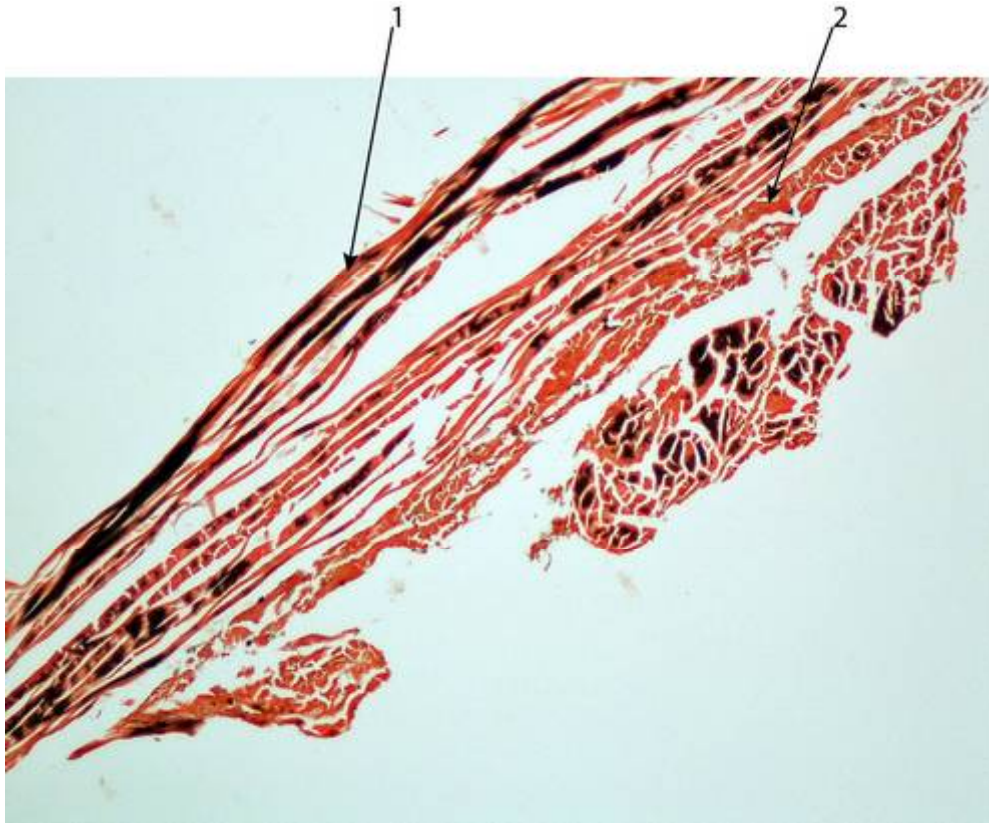


Figure C.31 Verhoeff's stain of desiccated muscle tissue from the rib (sample 50-5). The muscle tissue is arranged as a series of fascicles (1); connective tissue is stained yellow (2). $\times 10$ objective lens



Figure C.32 Stereo microscope observation of the desiccated tissue from the palate (sample 69-3). Dust is observed on the surface of the sample

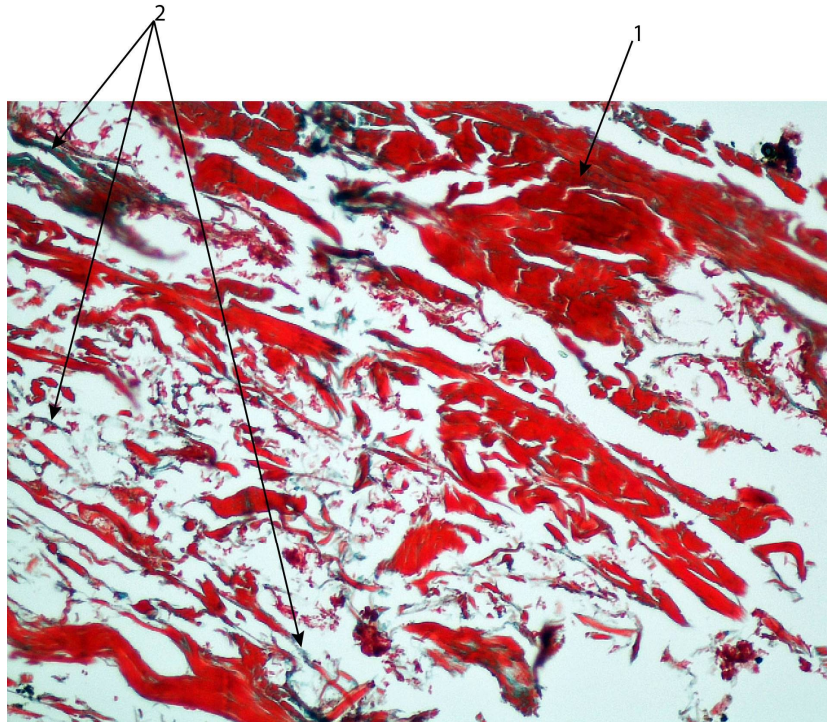


Figure C.33 Masson trichrome stain of the desiccated tissue from the palate (sample 69-3). Muscle fibres (1) and collagen fibres (2) are identified. $\times 20$ objective lens

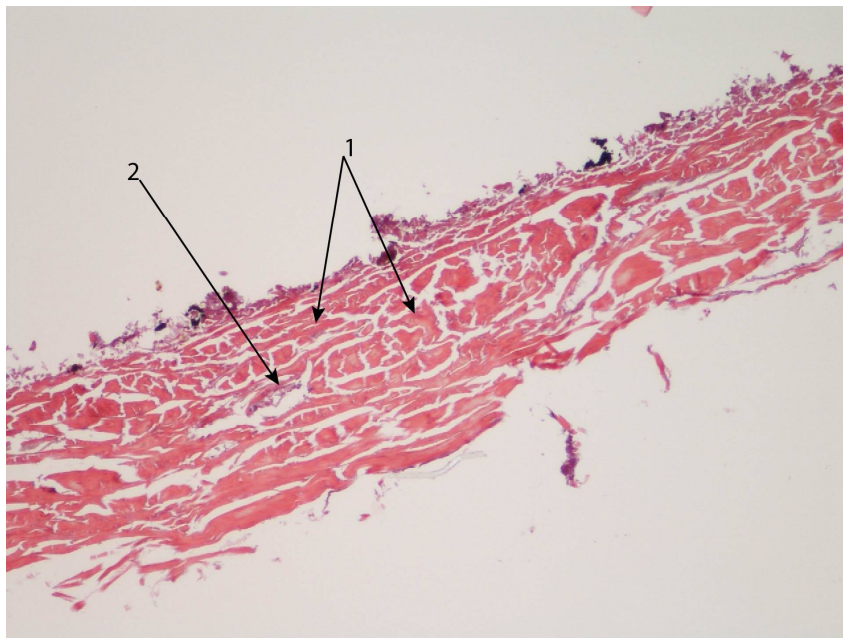


Figure C.34 Hematoxylin-eosin stain of the desiccated muscle tissue from the palate (69-3). Fascicles (1) and the perimysium (2) are identified. $\times 10$ objective lens

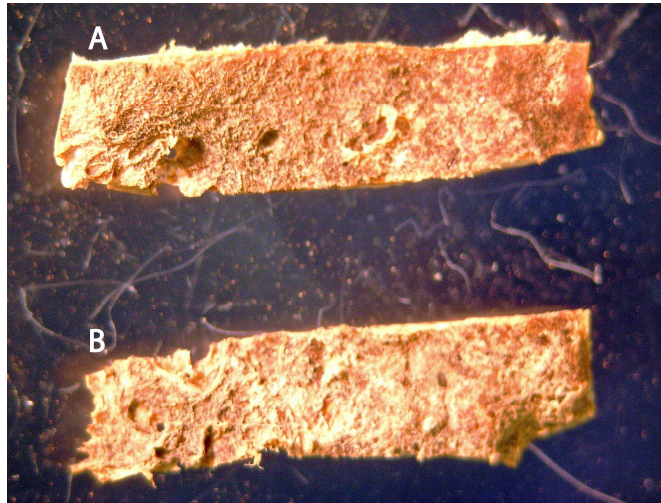


Figure C.35 Stereo microscope observation of the desiccated tissue from the palate. (A) Sample 69-4 and (B) sample 69-5. Dust was observed on the surfaces of the samples

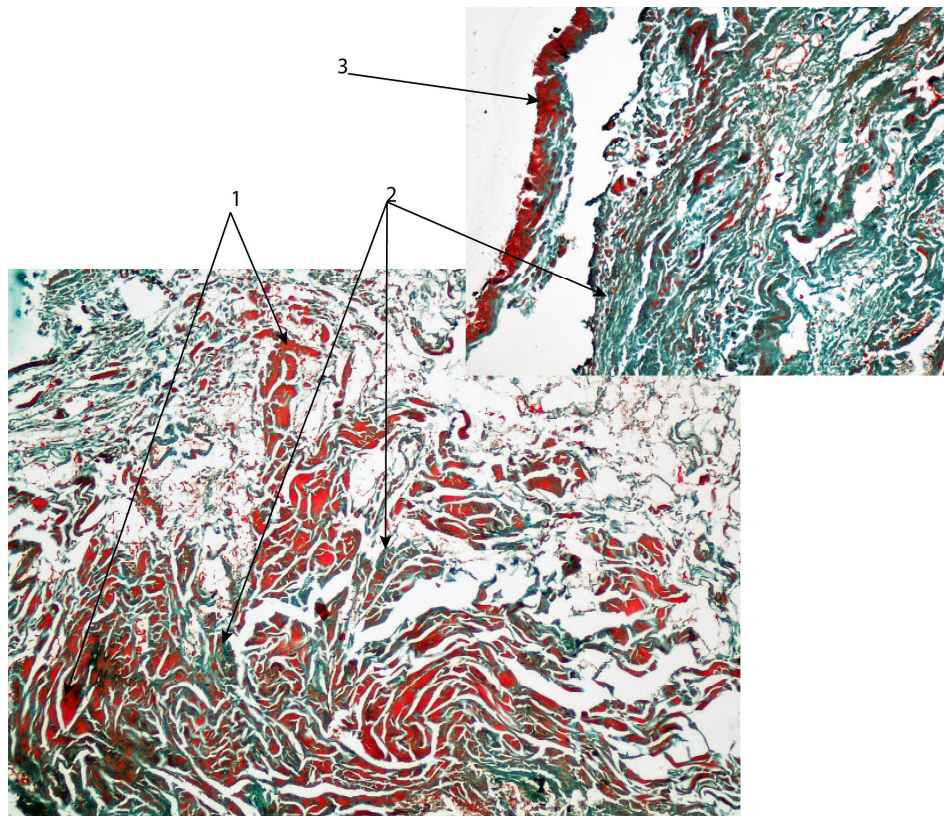


Figure C.36 Masson trichrome stain of the desiccated tissue from the palate (sample 69-4). Muscle tissue is stained red (1); connective tissue is stained green (2); the epithelium is stained red (3). ×10 objective lens

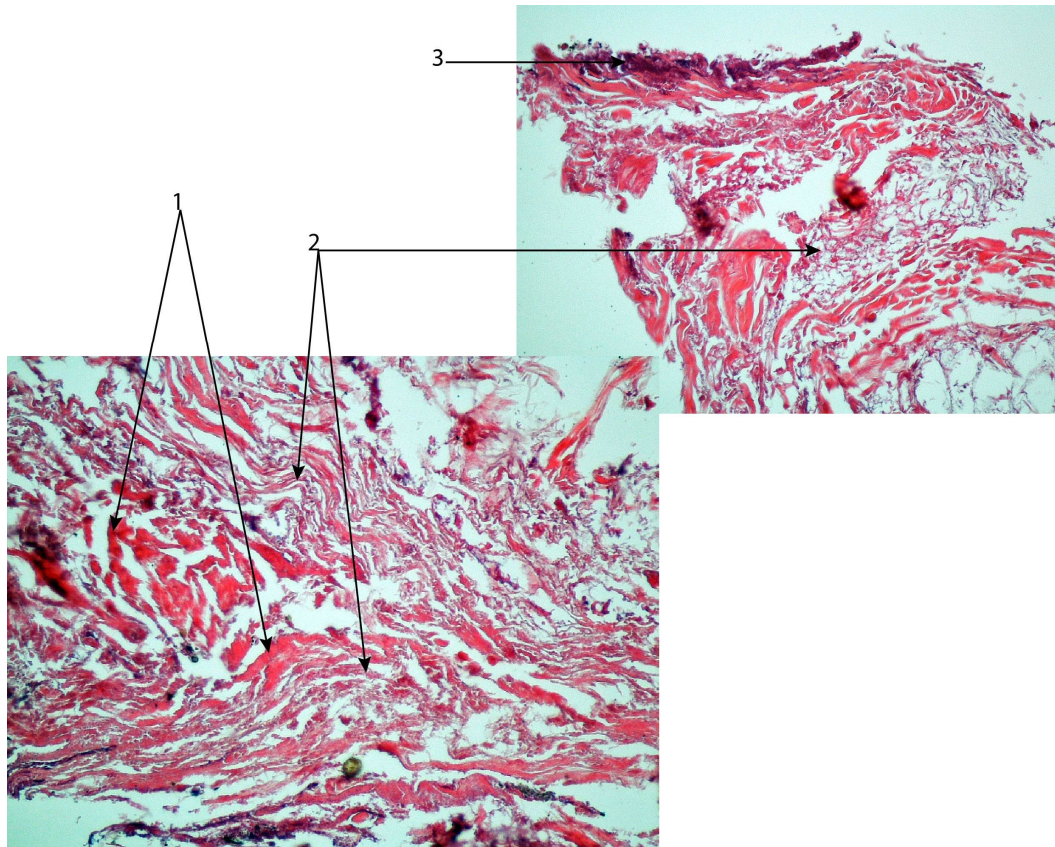


Figure C.37 Hematoxylin-eosin stain of the desiccated tissue from the palate (sample 69-4). Connective tissue composed of muscle fibres (1) and collagen fibres (2). The connective tissue lies beneath the epithelium (3). ×10 objective lens

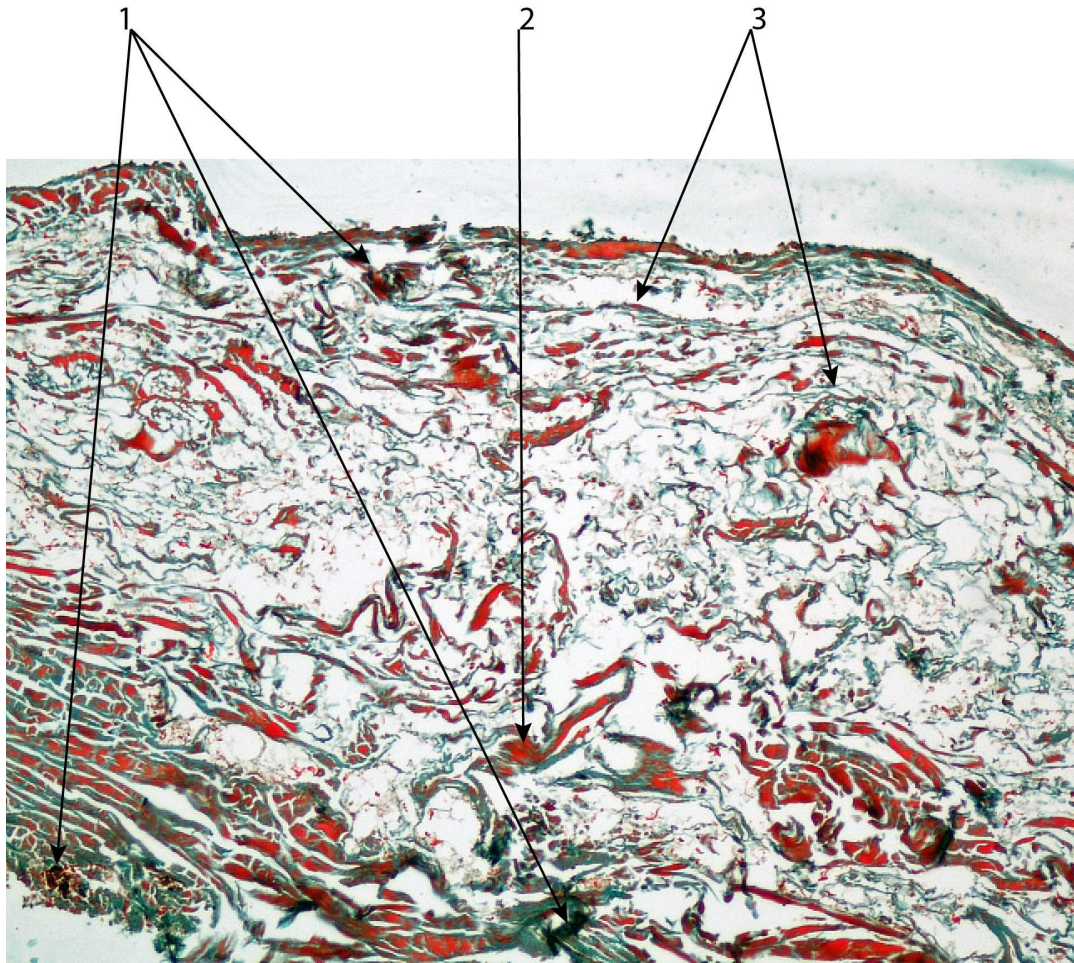


Figure C.38 Masson trichrome stain of the desiccated tissue from the palate (sample 69-5). Dust is embedded in the tissue (1); identified as connective tissue with muscle fibres (2) and collagen fibres (3). $\times 10$ objective lens



Figure C.39 Stereo microscope observation of the surface of the desiccated tissue from the mandible (74-Dg1 M1 RBL). SEM confirmed that the tissue is skin

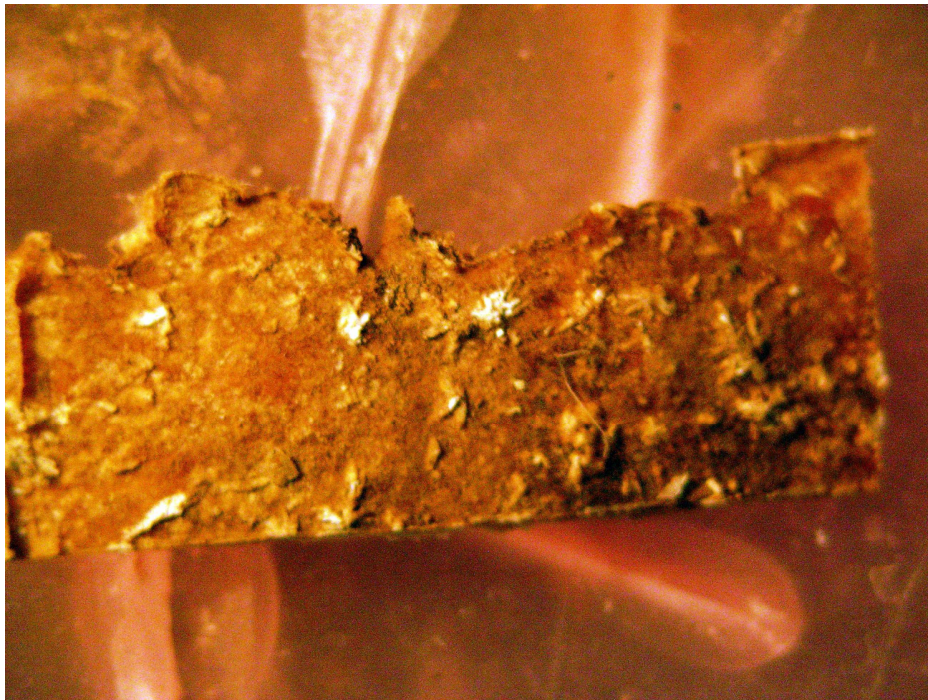


Figure C.40 Stereo microscope observation of the desiccated skin from the mandible (sample 74-1). The stereo microscope observation showed the sample was covered by a layer of dust

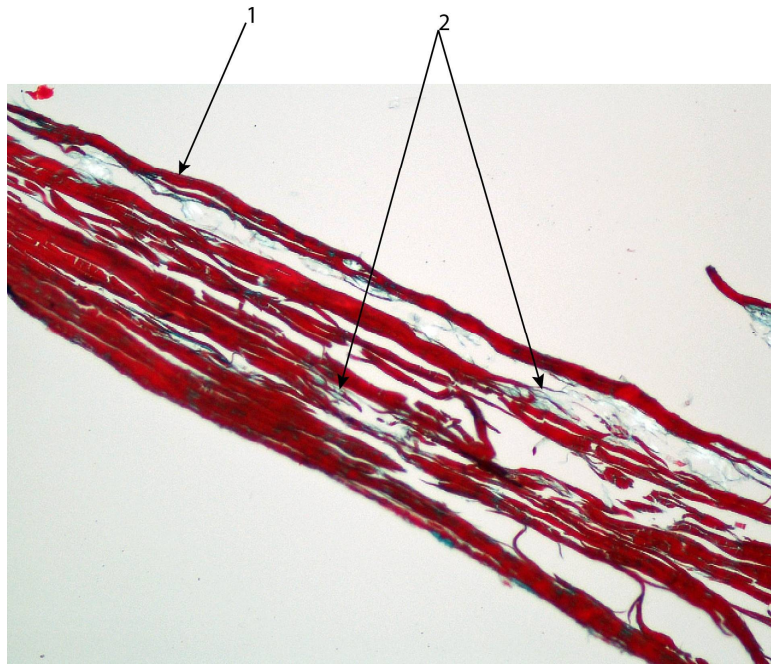


Figure C.41 Masson trichrome stain of desiccated skin from the mandible (sample 74-1). The tissue is striated muscle tissue with fascicles (1) and connective tissue known as the perimysium (2). ×20 objective lens

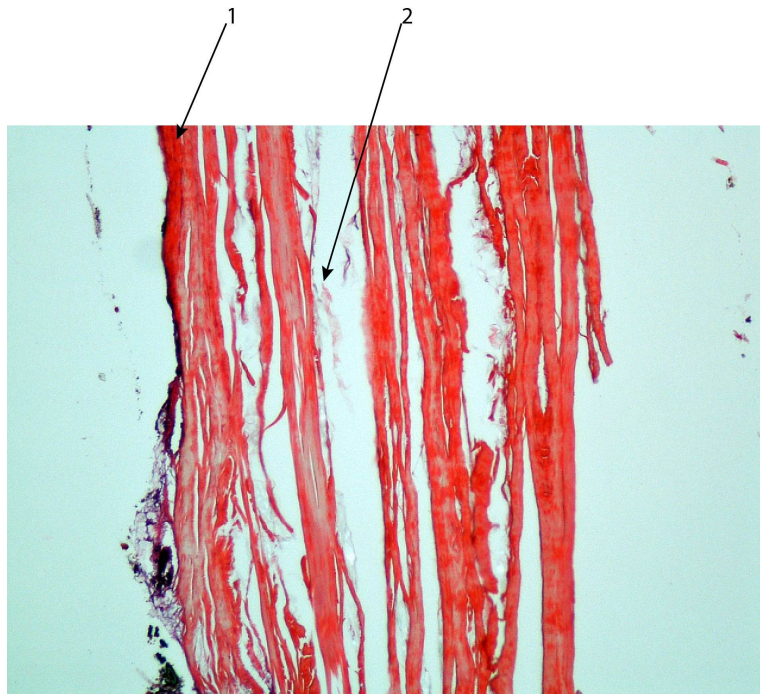


Figure C.42 Hematoxylin-eosin stain of the desiccated skin from the mandible (sample 74-1). The tissue is striated muscle tissue with fascicles (1) and connective tissue known as the perimysium (2). ×20 objective lens



Figure C.43 Stereo microscope observation of the desiccated skin from the mandible (sample 74-4). Dust was observed on the surface of the sample



Figure C.44 Masson trichrome stain of the desiccated skin from the mandible (sample 74-4). Dust is embedded in the tissue (1). The tissue is striated muscle tissue with fascicles (2) and the epimysium (3) and perimysium (4). ×20 objective lens

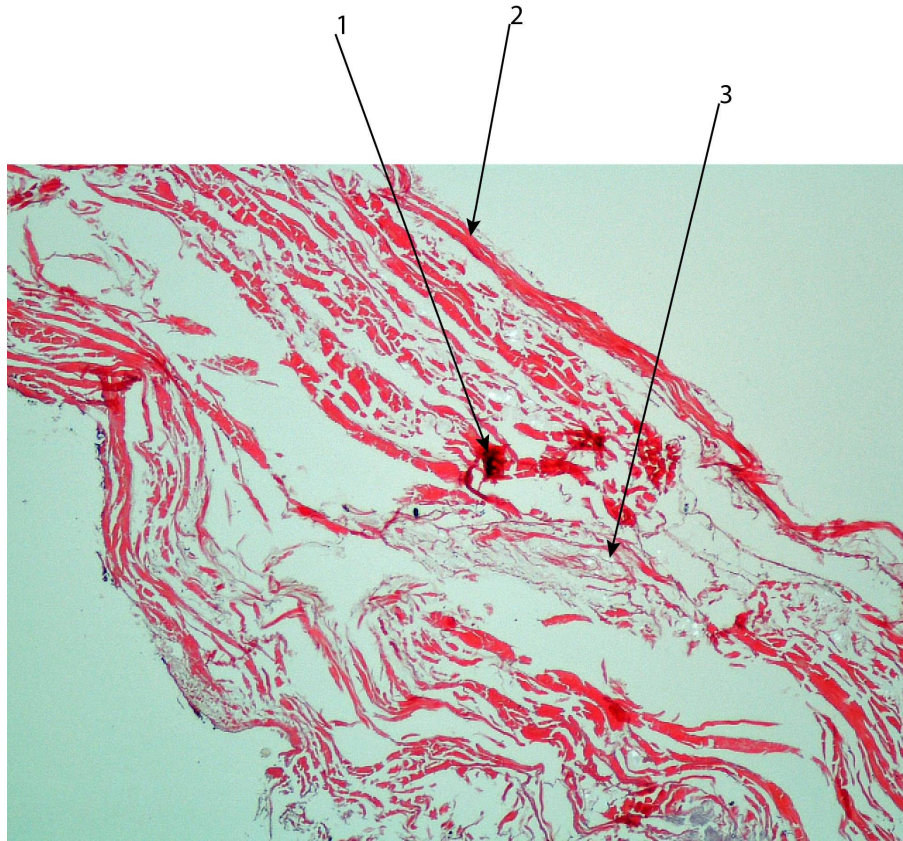


Figure C.45 Hematoxylin-eosin stain of desiccated skin from the mandible (sample 74-4). Dust is embedded in the tissue (1). The striated muscle tissue has fascicles (2) and connective tissue known as the perimysium (3). $\times 10$ objective lens



Figure C.46 Stereo microscope observation of the desiccated skin from the mandible (sample 74-5). The stereo microscope observation showed that a layer of dust covered the surface of the sample

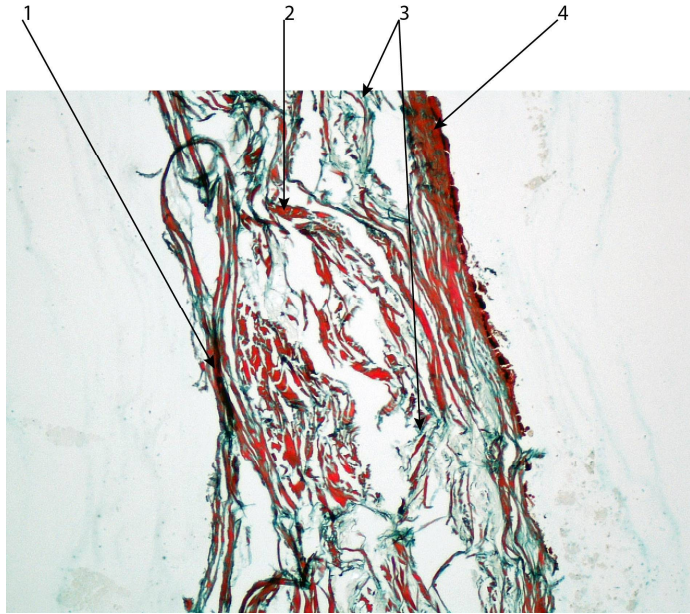


Figure C.47 Masson trichrome stain of desiccated skin from the mandible (sample 74-5). Dust is embedded in the tissue (1). The dermis comprises muscle fibres (2) and collagen fibres (3); the skin tissue is well-preserved with an epidermis (4). $\times 10$ objective lens

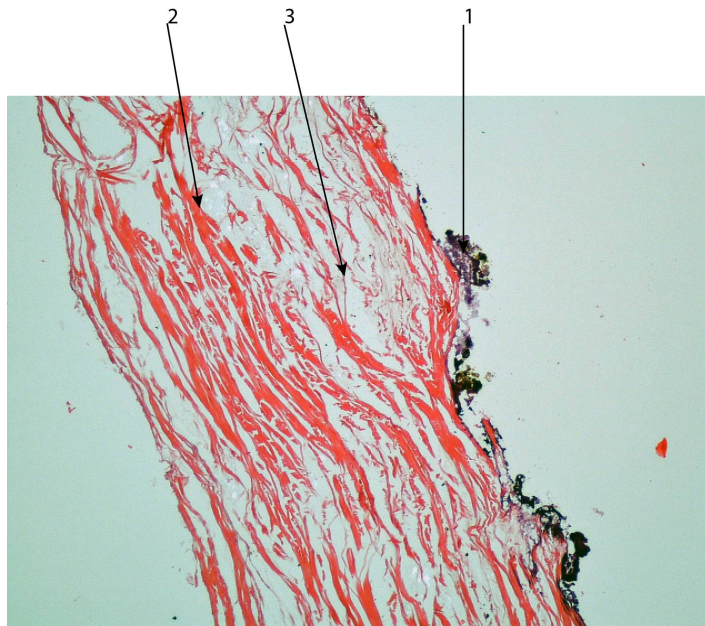


Figure C.48 Hematoxylin-eosin stain of desiccated skin from the mandible (sample 74-5). The muscle fibres and collagen fibres of the remaining dermis were stained pink. $\times 10$ objective lens



Figure C.49 Stereo microscope observation of the surface of the desiccated tissue from the mummy (A1081-1). The surface of the specimen is covered by a layer of dust and debris



Figure C.50 Stereo microscope observation of the surface of the desiccated tissue from the mummy (A1081-2). The surface of the specimen is covered by a layer of dust and debris

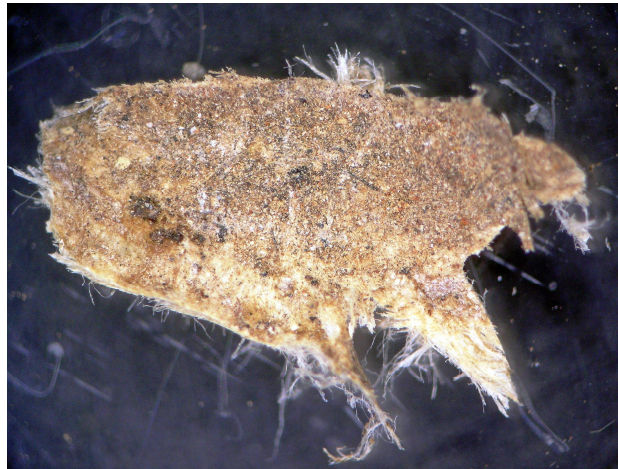


Figure C.51 Stereo microscope observation of the surface of the desiccated tissue from the mummy (A1081-3). The surface of the specimen is covered by a layer of dust and debris

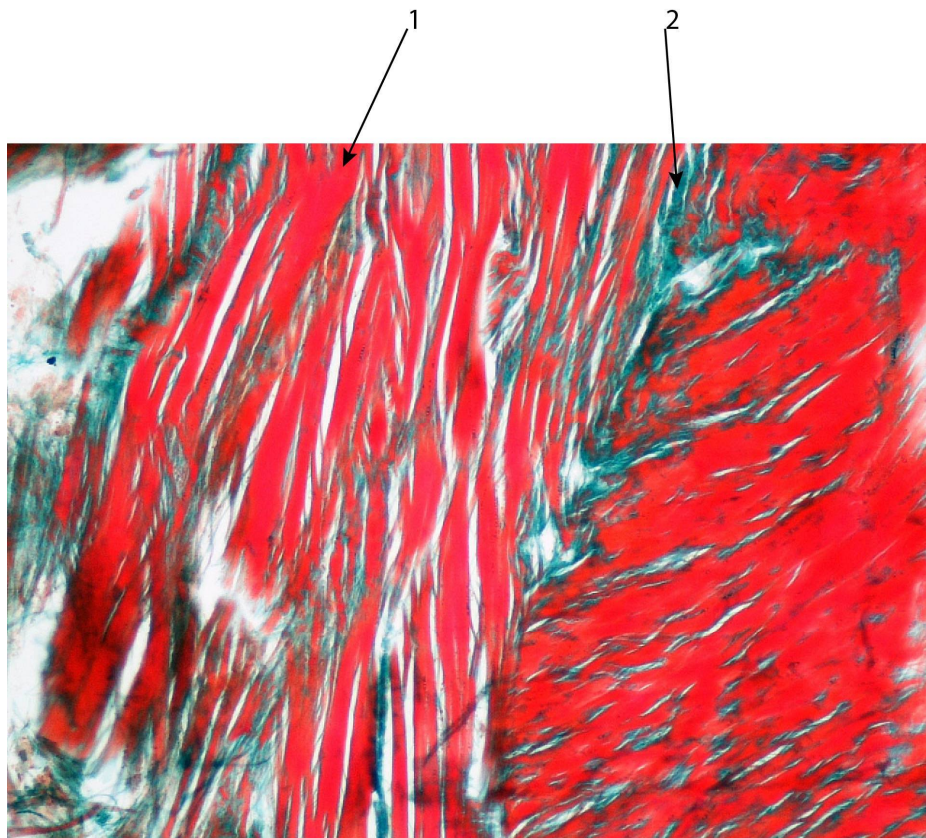


Figure C.52 Masson trichrome stain of the desiccated muscle tissue from the mummy (A1081-3). The striated muscle tissue has fascicles (1); there is connective tissue known as the perimysium in between the fascicles (2). ×40 objective lens

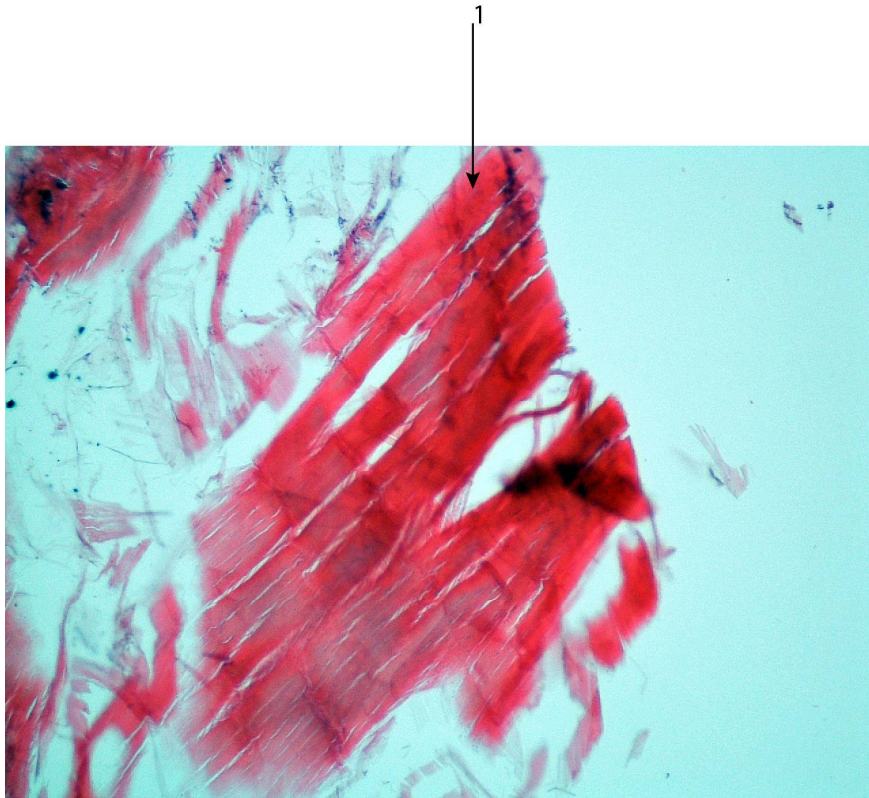


Figure C.53 Hematoxylin-eosin stain of the desiccated muscle tissue from the mummy (A1081-3). The striated muscle tissue can be observed (1). $\times 40$ objective lens



Figure C.54 Stereo microscope observation of the desiccated tissue from the palate (69 – M1 RBL) showing dust and debris on the surface of the tissue

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