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Palaeocytology in Skeletal Remains: Microscopic Examination of Putrefaction Fluid Deposits and Dental Calculus of Skeletal Remains from French Archaeological Sites

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ABSTRACT The relatively elusive nature of preserved human cells in fossilised tissues was recognised prior to the description of fossilised sickle cells by Maat (1991) and Maat & Baig (1991). Preserved neural cells were also noted in mummified brain tissues by Hauswirth *et al.* (1991) and Doran *et al.* (1986). Exceptional circumstances of preservation were used to explain these rare observations (Briggs & Kear, 1993). In this study, dental calculus and endocranial putrefaction fluid were rehydrated for 12 hours in 0.4 M acetic acid solution at room temperature, smear stained with May-Grunwald-Giemsa and examined using light microscopy. Remnants of leucocytes, epithelial cells and other cells are presented and discussed. Copyright © 2007 John Wiley & Sons, Ltd.

Key words: dental calculus; putrefaction fluid deposits; preserved human cells; rehydration; France; 4th–12th centuries AD

Introduction

One of the major limits to the study and interpretation of past humans is the post-mortem autolysis of cells through decay processes. To date, only a few publications have reported the preservation of blood cells (Maat, 1991; Maat & Baig, 1991) or the restitution of the morphological appearance of neural cells (Doran *et al.*, 1986; Hauswirth *et al.*, 1991). Briggs & Kear (1993) initiated an experiment with living shrimps and hypothesised the underlying reason for the preservation of cellular details in fossilised remains. A similar method was used for remineralisation of dental calculus matrixes, indicating that above a certain phosphorus and calcium level, remineralisation occurs under optimal conditions (Little *et al.*, 1966).

In this paper, we present results obtained with a new and simple method of rehydration applied to

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deposits of putrefaction fluid (PFD) inside the skull and to dental calculus. The method was originally developed by one of the authors (Charlier, 2005a) on identical material. Both PFD and dental calculus (DC) are often preserved in skeletal remains and may be assumed to contain numerous preserved organic inclusions. Few studies have focused on the rehydration of dental calculus for microscopic examination and dietary reconstruction (Dobney & Brothwell, 1986; Dobney, 1987, 1994). Results showed muscle fibres, plant cells and remains of bacteria but, interestingly, they failed to demonstrate any preserved human cells. Furthermore, PDF inside or outside skeletal cavities such as the skull appeared to be another potential source of cell preservation. These deposits were previously attributed to the desiccation of putrefaction fluids inside 'air-tight' burials (Payen *et al.*, 1988; Molleson & Cox, 1993; Crubézy & Dieulafait, 1996).

Figure 1. Duisans 1036: putrefaction liquid deposition is seen as a distinct line crossing the interior of the cranial vault. This figure is available in colour online at www.interscience.wiley.com/journal/oa.

Material

Eight individuals were selected from the Arras-Duisans site (Duisans 1022, 1024, 1029, 1036, 1037, 1040, 1047, 1050) which all had deposits of putrefaction fluid inside the skull cavity. The Arras-Duisans site (Jacques & Gaillard, 2006) is a family cemetery dating from the end of the 4th century AD. Skeletal preservation is good. Most of the bodies have been buried, but not exclusively, in coffins. Fragmentary skulls of those coffin burials disclose indications of the original levels of putrefaction fluid in the form of brown linear deposits of varying thickness (Figure 1). The surface of the deposits varied between granular to smooth. The deposits could not be due to an accumulation of groundwater since the local groundwater level was situated between 5 m and 10 m below the position of the skulls.

Dental calculus samples were obtained from the so-called Douai-St Amé 257 child skeleton dating back to the late medieval period excavated by E. Louis 2006, the skeleton of Jean I d'Avesnes exhumed from the Dominican church of Valenciennes by V. Maliet in 1991 and dating from the 12th century AD, and the skeleton of Gertrude, Abbess of the Hamage monastery dating from the 7th century AD, excavated by E. Louis in 2002.

Method

All endocranial and calculus deposits were removed with the aid of sterile disposable scalpels, and a mask and sterile gloves were worn. Samples weighing 0.05–0.1 mg were removed onto a sterile blanket. The samples were ground in a mortar and dissolved in 2 ml of 0.4 M acetic acid solution in a test tube. Subsequently the solution was shaken vigorously and the tube was then left for 12 hours at room temperature. Centrifugation or filtration was not used. The most superficial layer of sediment was gently removed using a pipette and deposited on a large glass slide and allowed to dry. Cells were stained using the May-Grundwald-Giemsa stain since this is widely used for the examination of blood cells. Finally, the prepared samples were mounted on a glass slide, covered with a coverslide, and observed using a Nikon Labophot microscope at 200× magnification.

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Results

PFD samples

Samples were taken from the eight individuals from the Arras-Duisans site. Three of them (Duisans 1024, 1029, 1036) revealed complete, incomplete and deformed remains of leucocytes (Figures 3, 6, 10) as well as epithelial cells (Figure 8). All cells displayed well-known morphological features (Figures 9, 14). Most of the leucocytes showed enlarged or fragmented nuclei which were adhering to the cell membrane. They appeared similar to leucocytes observed in vaginal smears. Clusters of yeasts in some fields mimicked the shape and staining properties of erythrocytes (Figures 2, 7, 8), but since both human and yeast cells are identical in size and appearance, it seemed logical to use a higher power microscope and chemical analyses to differentiate between them.

In the Duisans 1024 (Figure 4), Duisans 1029 and Duisans 1036 samples, remains of pollen and spores were also identified. In two of the samples (Duisans 1024 and 1036) numerous species of Uromyces (rust) fungal spores were found. Among the pollens, graminaceae (poaceae) and plantago major (plantaginaceae) were recognised.

In two other samples (Duisans 1022 and 1050), morphological features of blood cells were not recognisable, but remains of fragmented cells and isolated cell nuclei were present. In all samples, the different types of cells appeared to cluster in different parts of the slide, thereby facilitating their observation.

DC samples

The dental calculus sample from the Saint Amé 257 medieval child showed leucocytes (Figure 11) and yeasts that mimicked the appearance of erythrocytes (Figure 5) in section, although smaller in width (6 μm). They also had a similar shape to those observed in the endocranial samples. Shrinkage of some yeast to the shape of a shell was a feature already encountered in the PFD samples. A large number of crystals were visible throughout the slide (Figures 11, 12).

The sample of dental calculus from Jean I d'Avesnes exhibited few leucocytes and numerous folded epithelial cells (Figures 13, 15). Moreover, muscle fibres (Figure 15) and one mite (Figure 17) were observed.

The dental calculus samples from Gertrude of Homage exhibited needle-shaped crystals (Figure 18), numerous clusters of bacteria and large folded cells with a large nucleus containing basophilic plasma (Figure 16).

Discussion

Why does cell preservation occur in dental calculus in the oral cavity as well as inside the cranial cavity during the long process of decomposition? Although the specific reasons have yet to be analysed and experimentally reproduced, it should be emphasised that not all areas of the body suffer similar physical and/or chemical conditions during putrefaction. This can be seen in the forensic examination of decomposed bodies, where the surface putrefaction deposit fluid contains fat and lipid acids (Cabirol *et al.*, 1998). The putrefactive fluid may have a protective effect on cells. Briggs & Kear (1993) conducted experiments that explored the decay of tissues in living shrimps to explain the remarkable fossilisation of cellular details in soft tissues. By assuming that the cell fossilisation process required elevated concentrations of phosphates in sediment pore-waters, they studied the formation of calcium phosphate from the shrimps themselves and noted that mineralisation occurred within 2 to 4 weeks. Progressive calcification and fixation of the cell surface may be one of the mechanisms at work in the preservation of cells in ancient samples. Although the loss of cellular details and different staining properties indicates incomplete preservation of these cells, it is assumed that the cellular phospholipid membrane architecture is sufficiently preserved to allow rehydration. The mild acid acetic solution used here, originally meant to be a light decalcifier, may have been of crucial importance in restoring an ionic balance through the cell membrane and thus promoting the restoration of the morphology of the cells.

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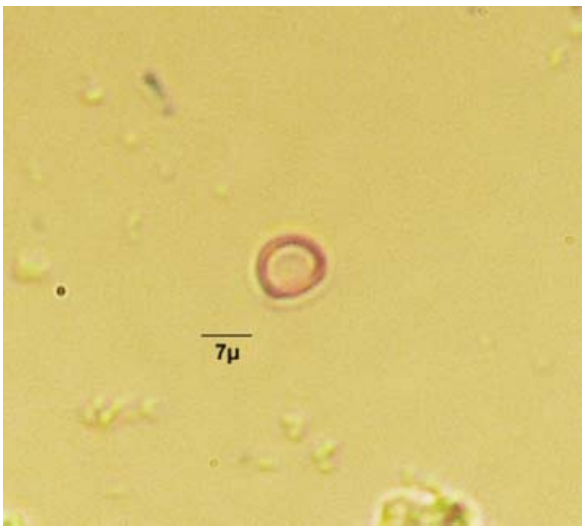

Figure 2. Duisans 1024: erythrocyte or yeast mimicking an erythrocyte (May-Grundwald-Giemsa staining).

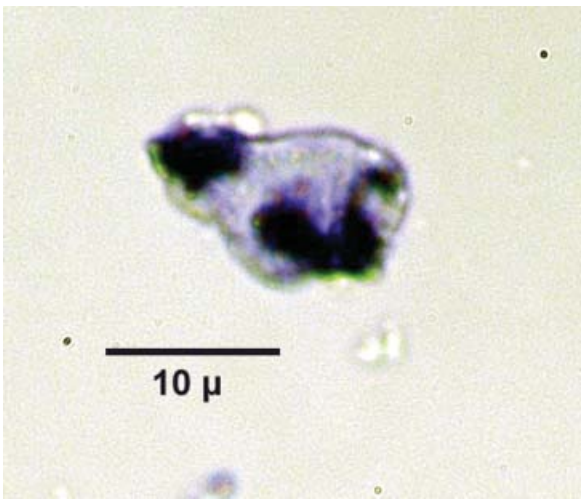

Figure 3. Duisans 1029: leucocyte with a lobulated nucleus (May-Grundwald-Giemsa staining).

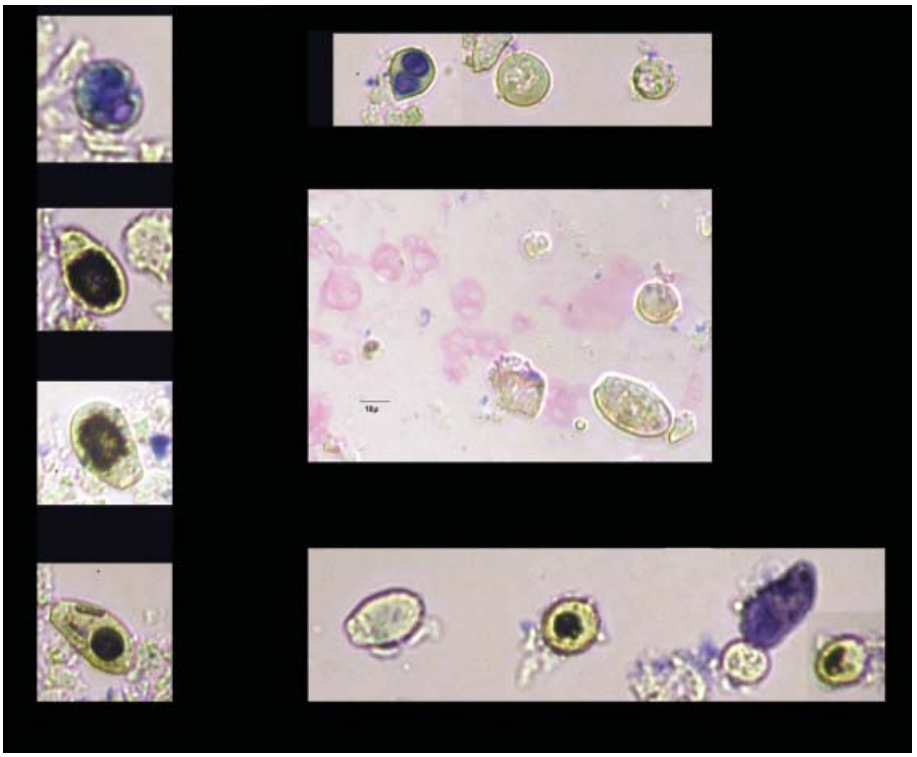

Figure 4. Duisans 1024 PFD: example of spores (mainly fungus and rusts), pollen and diatoms from putrefaction fluid deposits (×200, May-Grundwald-Giemsa staining).

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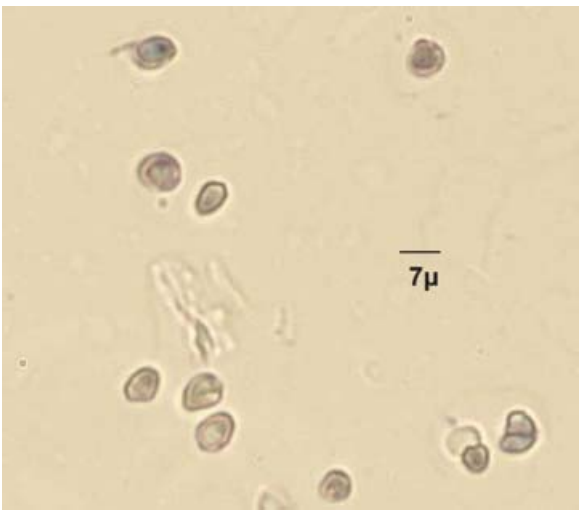

Figure 5. Saint Amé 257 DC: colony of yeasts in dental calculus (May-Grundwald-Giemsa staining). This figure is available in colour online at www. interscience.wiley.com/journal/oa.

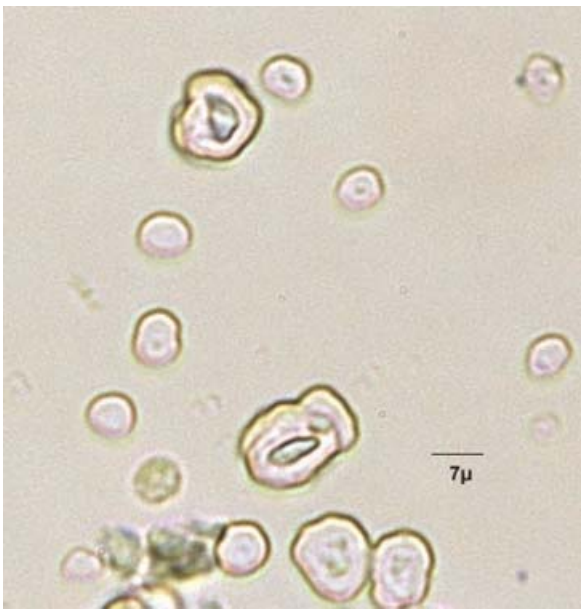

Figure 7. Duisans 1029 PFD: colony of yeast, mimicking erythrocytes (May-Grundwald-Giemsa staining). This figure is available in colour online at www.interscience.wiley.com/journal/oa.

Preserved white blood cells may originate not only from the individual, but also from dietary intake, particularly through the ingestion of red meat and sauces containing meat. Cells preserved in dental calculus that have a small nucleus and basophilic plasma (Figures 13,15 and 16) can easily be attributed to remains of epithelial tissues in the mouth. The presence of colonies of yeasts is probably due to post-mortem contamination or possibly a result of the method of rehydration (organic decoction during 12 hours at room temperature). Thus they cannot be identified as true ancient yeast inclusions. The presence of parasites may conversely indicate particular types of food being eaten (Charlier, 2006). Muscle fibres (Figure 15) and the mite (Figure 17) could indicate a rich diet combining meats and cheese.

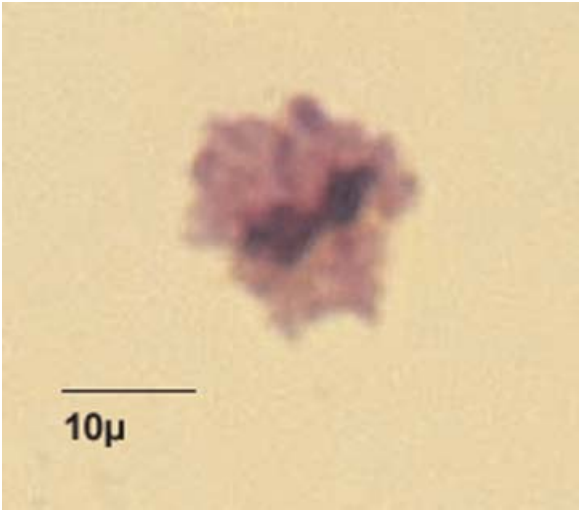

Figure 6. Duisans 1029 PFD: shrunken eosinophil (May-Grundwald-Giemsa staining). This figure is available in colour online at www. interscience.wiley.com/journal/oa.

A similar method was used by Dobney & Brothwell (1986) and Dobney (1987, 1994) in analysing dental calculus for identifying food debris from 94 individuals from the late medieval cemetery at Jewbury (near York, UK) and others from earlier periods. The authors did not find any remains of cells. Similar studies of calculus have been made without rehydration of samples by single TEM (transmission electron microscopy; Charlier, 2006), and of blood residues dated to the 19th century on Dogon statues from the

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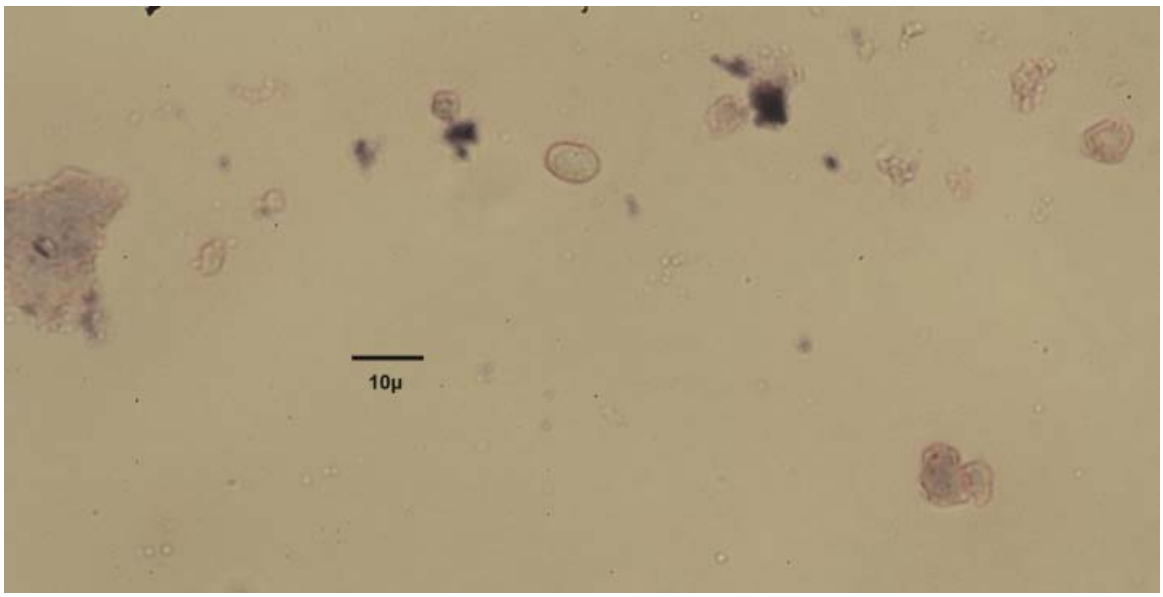

Figure 8. Duisans 1036 PFD: epithelial cell at the left, with yeasts and cellular debris (May-Grundwald-Giemsa staining). This figure is available in colour online at www.interscience.wiley.com/journal/oa.

Louvres Museum (Mazel *et al.*, 2006). Even if long-term degradation of samples by desiccation led to post-mortem change, morphological analysis was still possible. That was also the case for the samples from the Ikaros Hellenistic individuals studied by Maat (1991, 1993) and

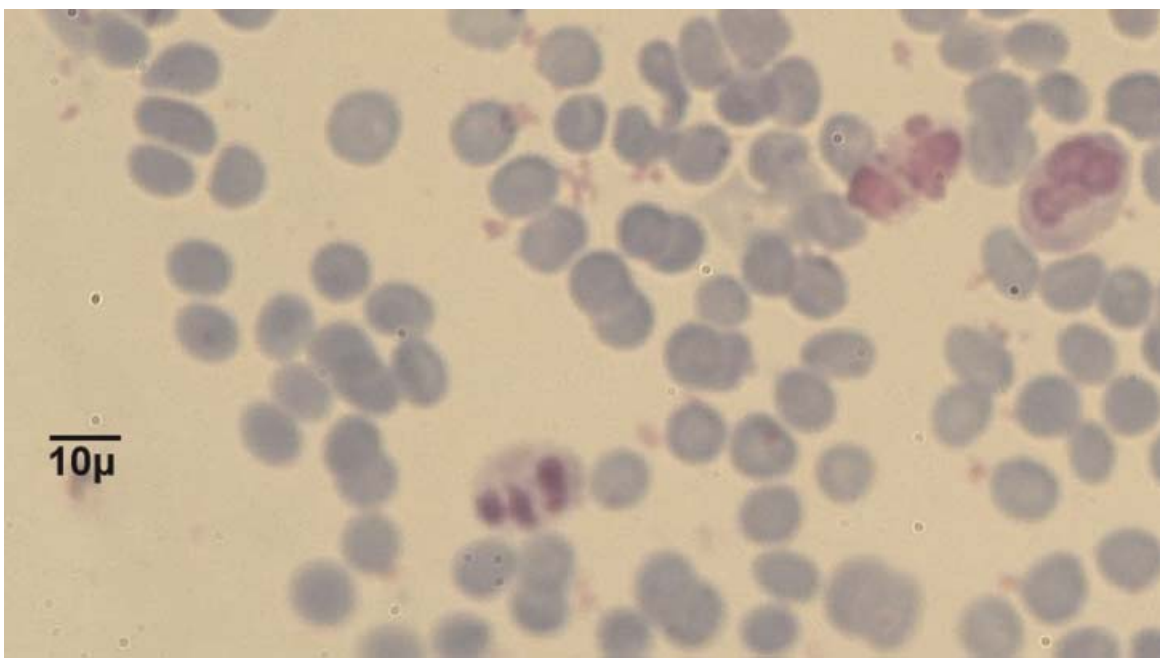

Figure 9. One of the author's blood smears for comparison with the above and following pictures (May-Grundwald-Giemsa staining). This figure is available in colour online at www.interscience.wiley.com/journal/oa.

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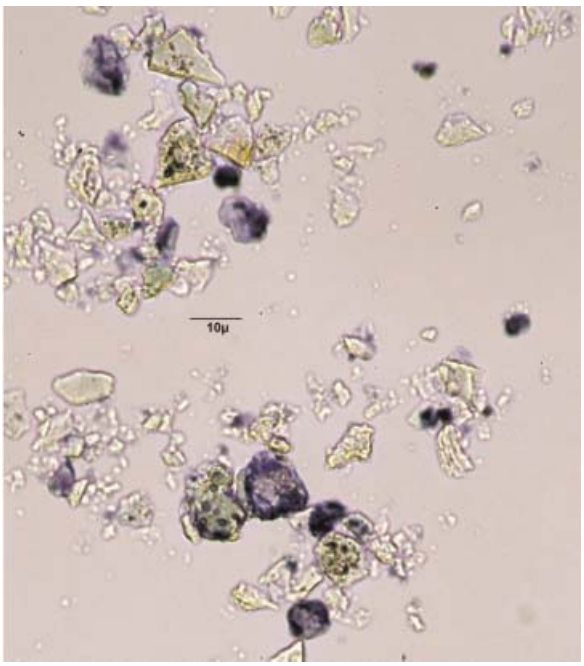

Figure 10. Duisans 1029 PFD: aspects of leucocytes and other cellular debris (May-Grundwald-Giemsa staining). This figure is available in colour online at www.interscience.wiley.com/journal/oa.

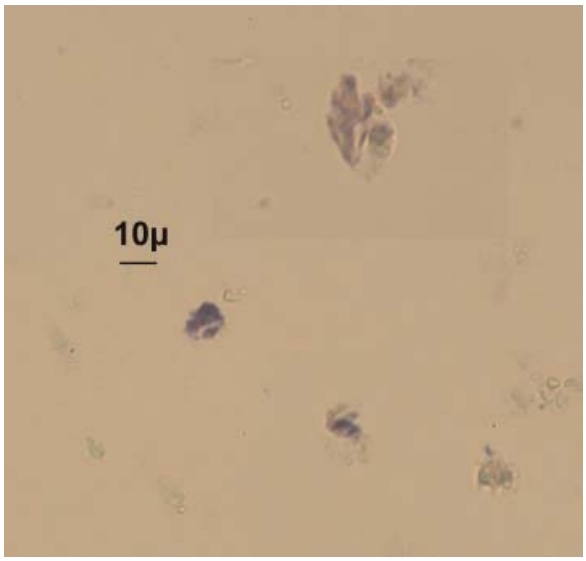

Figure 11. Saint Amé 257 DC: leucocyte, debris and crystals in dental calculus (May-Grundwald-Giemsa staining). This figure is available in colour online at www.interscience.wiley.com/journal/oa.

Figure 12. Saint Amé 257 DC: crystals in dental calculus under polarised light (×200). This figure is available in colour online at www.interscience.wiley.com/journal/oa.

Maat & Baig (1991). Needle-like crystals radiating from the remineralisation nodules in the experiment of Little *et al*. (1966) on dental calculus matrixes are comparable to the crystals exhibited during this study. In our study, these are probably crystals of calcium phosphate, often associated with cellular debris and clusters of bacteria. They could be evidence of remineralisation.

The presence of pollen and fungi in the putrefaction fluid deposits shows that the liquid has been in contact with its burial surroundings and that the process of cell preservation did not only occur inside the cranial cavity. Moreover, identified spores and pollen suggest that the season of burial was between July and October. Numerous spores were enlarged at the top and flattened at the base. They are teleutospores of rusts, specifically those from the genus *Uromyces*. These are parasites of many plants and are probably associated with grasses, flowers (mullein, valeriana) or fruits placed inside the coffin at burial at the end of the summer. Further experiments and comparative analysis on geographically and temporally different samples may differentiate between long- and short-term contamination from the burial environment. Furthermore, these preserved remains may originate from the embalming techniques used, as proposed in the study of the putrefaction deposit and dental calculus of the burial of Agnes Sorel (Charlier, 2005b,c).

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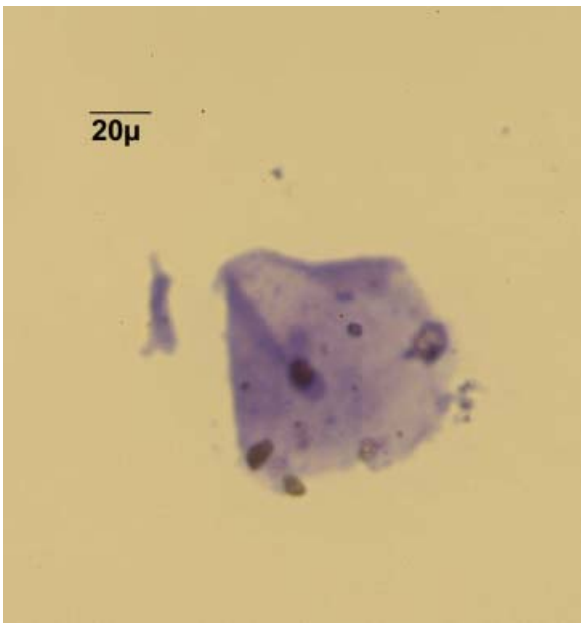

Figure 13. Jean I d'Avesne DC: epithelial cell in dental calculus (May-Grundwald-Giemsa staining).

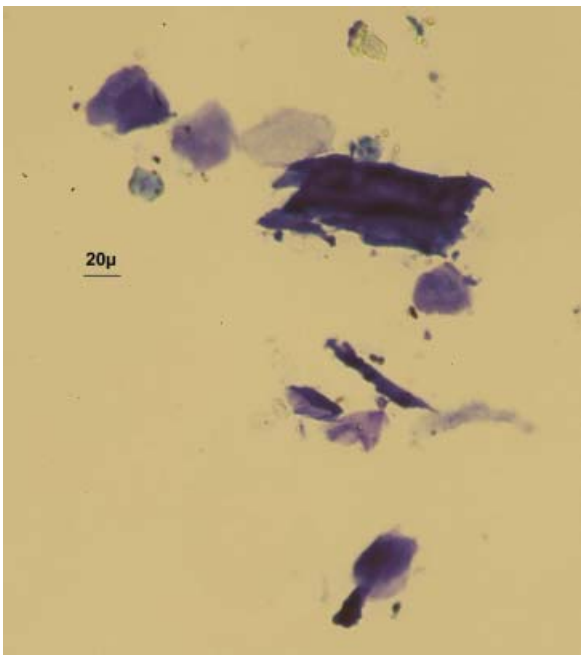

Figure 15. Jean I d'Avesne DC: folded epithelial cells with a muscle fibre in dental calculus (May-Grundwald-Giemsa staining).

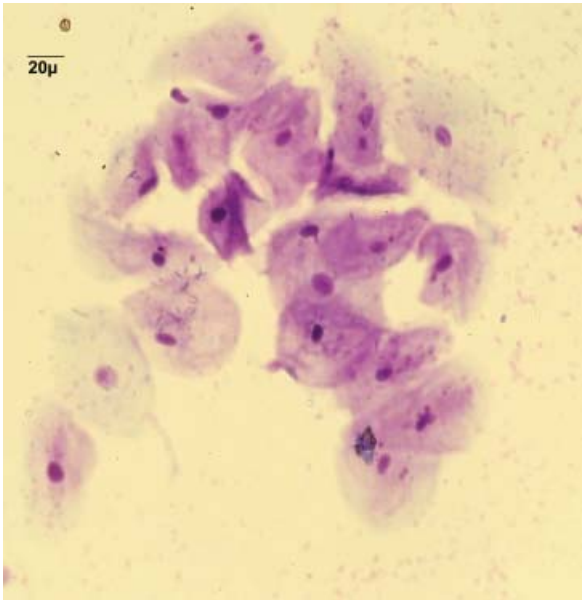

Figure 14. Buccal smear of one of the authors for comparison with the above and following pictures (May-Grundwald-Giemsa staining).

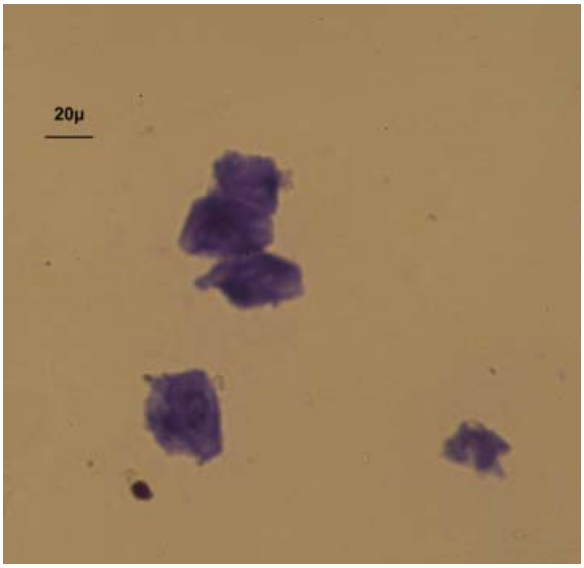

Figure 16. Gertrude of Hamage DC: folded epithelial cells (May-Grundwald-Giemsa staining).

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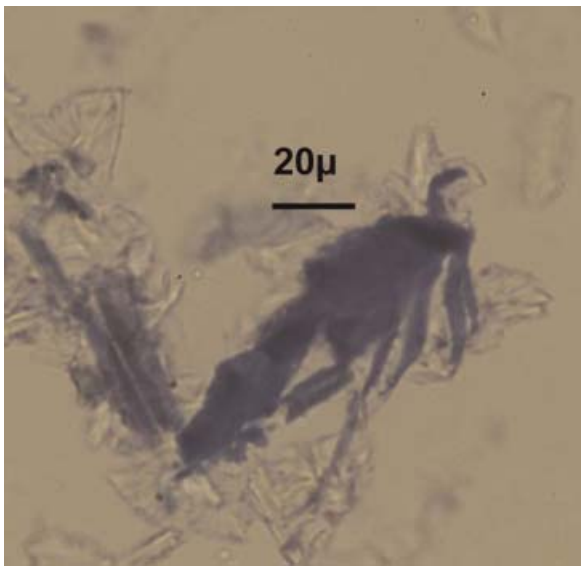

Figure 17. Jean I d'Avesne DC: remains of a mite in dental calculus (May-Grundwald-Giemsa staining). This figure is available in colour online at www.interscience.wiley.com/journal/oa.

Conclusion

It is suggested that this method of analysis may be further used to diagnose diseases of the blood, such as leukaemia, in both skeletal and mummified remains. But other parasitic and infectious diseases may also be identified using the method. Another application could be for studying the processes of cellular decomposition before autolysis and, through environmental SEM (scanning electron microscopy), identifying membranes and organelles. The state of plasma and chromatin in such preserved cells might also facilitate the work of proteomic and aDNA identification. The study of the preservation of cells in dental calculus is another important field for further studies. As dental calculus develops over a long time period, this might allow the recovery of cellular and proteomic remains from the individual long before death. Modern samples of dental calculus could also be analysed and used to explain the unexpected preservation of cellular microstructures enclosed within dental calculus.

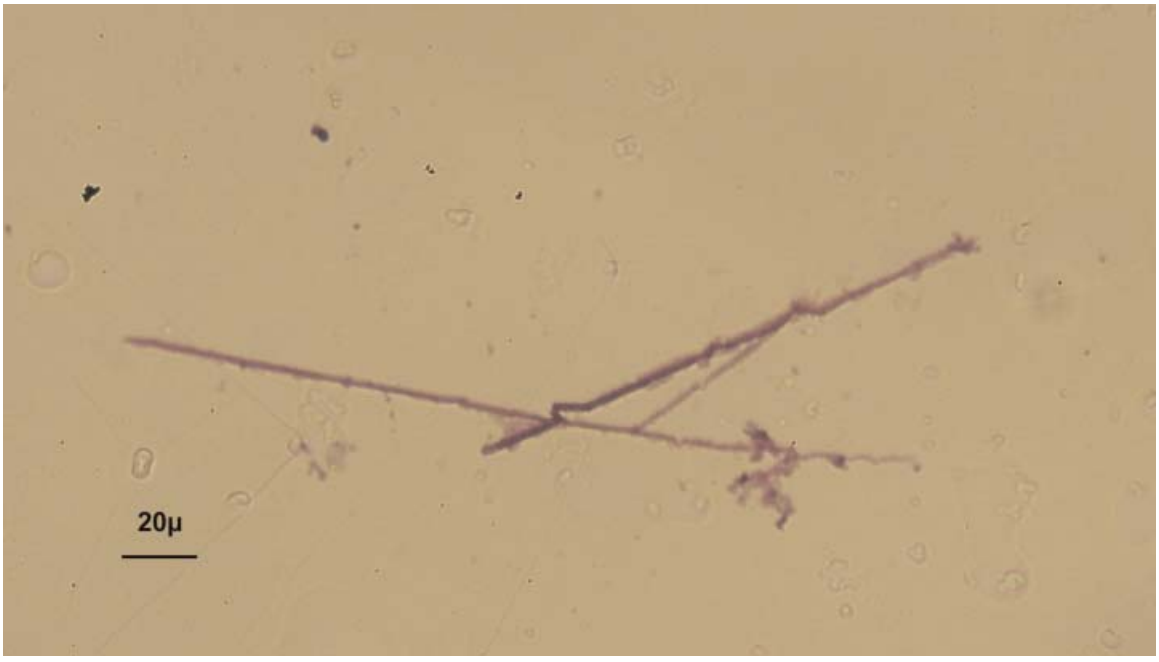

Figure 18. Gertrude of Hamage DC: needle-shaped crystals with aggregates of bacteria and yeast on the left (May-Grundwald-Giemsa staining). This figure is available in colour online at www.interscience.wiley.com/journal/oa.

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