Dry bone histology : technicalities, diagnostic value and new applications

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Chapter 1

GENERAL INTRODUCTION

Physical anthropology, forensic anthropology and palaeopathology

Physical anthropology (also known as biological anthropology) is the academic discipline that studies man as a biological species. As such it studies human origin, evolution and variability, the latter both in relation to time and space (geography). For comparative reasons, physical anthropologists sometimes extend their research to other mammals, primarily primates.

Physical anthropologists work in all scientific fields in which human anatomy, variability and adaptation are central themes. Well-known examples are palaeoanthropology, which studies human evolution, and comparative anatomy, which compares human anatomy with that of other mammals. Also human growth and development, as well as geographical phenotypical variation and sexual dimorphism (i.e. the anatomical differences between males and females) are part of the physical anthropological domain. The research in this thesis focuses on two subspecialties of physical anthropology: forensic anthropology and palaeopathology. Due to the wide scope of the discipline, the contemporary physical anthropologist can be found as a colleague among archaeologists, biologists and medical doctors.

Forensic anthropologists apply physical anthropological knowledge in a forensic setting (e.g. Reichs, 1998). An example hereof is the identification of human remains that are unsuitable for 'regular' identification methods such as facial recognition, odontology, finger printing or straightforward DNA-profiling. In such cases, forensic anthropologists use knowledge on human anatomical variation with regard to sex, age at death, stature and ancestry to define a biological profile. This may lead to a positive identification. Because of their specialized knowledge, forensic anthropologists are often asked to participate in 'disaster victim identification' (DVI) units, in which they help to identify for instance airplane crash victims. Furthermore, they are often requested to assist forensic pathologists and scene of crime officers by analyzing decomposed or cremated human remains related to an unnatural death. In those cases, thorough knowledge on anatomical and pathological (including traumatological) patterns are essential.

Palaeopathology is the study of diseases in ancient remains. Palaeopathologists combine knowledge on human variability, effects of ageing and pathological processes to diagnose diseases in skeletonized or mummified remains. Subsequently, demographic knowledge on the individuals is used to formulate epidemiological

hypotheses. By diagnosing and studying diseases in the past, palaeopathologist not only provide a valuable glimpse into the living conditions of past populations, but they also improve our understanding of disease mechanisms (e.g. Ortner, 2003). This in turn might aid in managing disease in the present and future (e.g. Donoghue *et al*. 2004, Halperink, 2004). Although palaeopathology may also encompass diseases in animals, this thesis focuses on human palaeopathology.

Forensic anthropologists and palaeopathologists differ with regard to the context in which they operate (i.e. forensic versus historical), but they share a common ground. Both work with human remains, often in an advanced state of decomposition. The remains may be completely skeletonized, incomplete or commingled. Sometimes they are combusted or even deteriorated to a degree almost beyond recognition. Secondly, both share a focus on anatomical (age at death, sex, stature, ancestry, anomalies) and pathological (disease, trauma, cause of death) features. Lastly, both are frequently confronted with a general lack of objective contextual information.

Since forensic anthropologist and palaeopathologists encounter the same challenges, approaches and applied methods are often very similar. The principles used for age at death and sex assessment are for instance the same. This also holds for methods on the identification of diseases or on the analysis of trauma. Advancements in forensic anthropological methods have therefore often a direct bearing on palaeopathological practice and vice versa. This can be illustrated by the use of the same scientific journals and the attendance at the same scientific conferences. Nevertheless, only a few workers practice both disciplines in combination. The situation in the Netherlands is no exception thereof.

Due to analytical limitations associated with forensic anthropological and palaeopathological research, workers combine multiple methodological approaches to increase the reliability of their diagnoses. The research in this thesis centers around one of those diagnostic modalities: the use of the light microscope. To understand its potential we will review the histology of bone tissue in short.

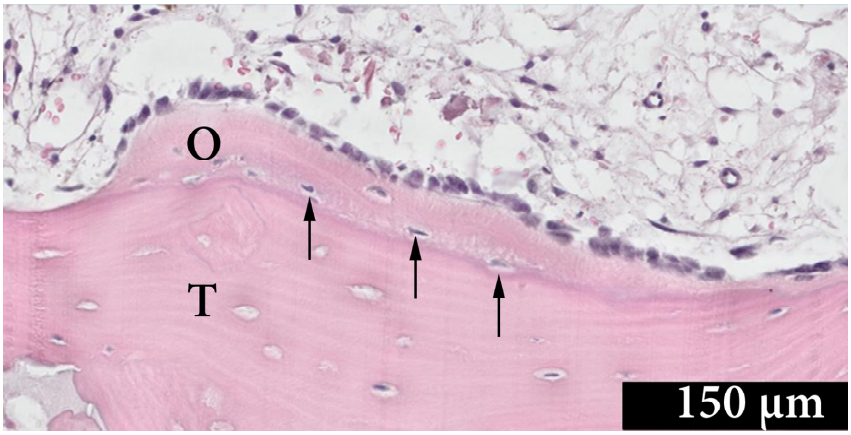
Bone tissue histology

Bone tissue is not a static entity but a metabolically active tissue, constantly communicating with its surroundings and adapting to the physical demands. Julius Wolff already identified this relation in the 19th century when studying trabecular bone morphology. He came to the conclusion that bone tissue has a 'well motivated architecture, which grants every one of its trabeculae a mechanical meaning […] as a building block […] in the grand structure that is the bone' (Wolff, 1870). This insight

was eloquently conmemorated and summarized by Paul Ernst-Heidelberg in 1931 when he stated that 'Struktur [verhält zich] zu Funktion wie Form zu Kraft. […] Es besteht zwischen Struktur und Funktion ein Abhängigkeitsverhältnis zweier veränderlicher Größen, demzulfolge eine Veränderung der einen Größe eine Veränderung der andern bedingt, wodurch über ihr Kausalitätsverhältnis nog nichts ausgesagt ist' (Ernst-Heidelberg, 1931). Harold Frost further refined and elaborated on this idea in the 'Utah paradigm of bone physiology' (Frost, 1960; 2000). In summary the basic idea was unchanged: bone morphology is regulated by, and thus a direct effect of biomechanical demands. The underlying regulatory mechanisms are complex and not fully understood, and are beyond the scope of this thesis. Still, bone tissue growth and adaptation is primarily a cellular and microarchitectural one. A major role is reserved for the three types of bone tissue cells. These cells and their main functions will be introduced shortly. For a more detailed description the reader is referred to a general histology book (e.g. Ross *et al.*, 1995).

Bone tissue is formed by osteoblasts, mononuclear cells from the fibroblast cell lineage (Fig. 1). Osteoblasts produce an extracellular bone matrix progenitor called osteoid, which is subsequently mineralized by the same osteoblasts. After

Figure 1.

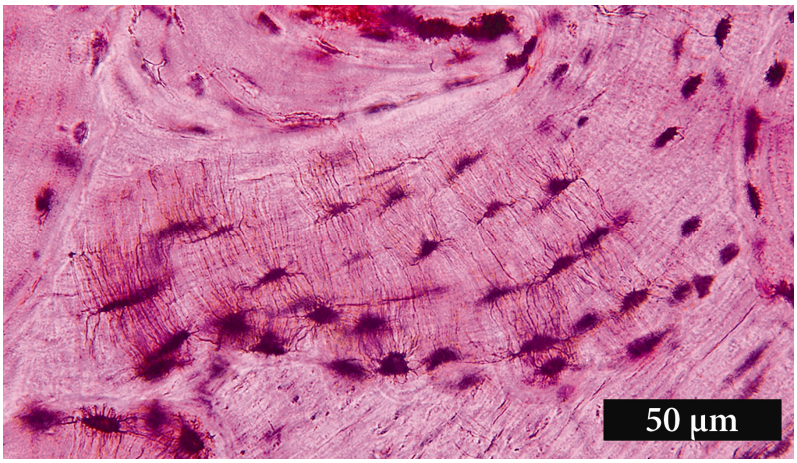


Decalcified section of fresh human bone trabecula, stained with haematoxylin and eosin. Bar indicates size. The trabecula (T) is covered with osteoid (O). The osteoid was produced by the lining string of osteoblasts. Apparently, a number of osteoblasts have become incorporated by their own product (arrows). After mineralization of the surrouding osteoid, these osteoblasts differentiate into osteocytes. They reside in savings called osteocyte lacunae.

mineralization the bone tissue matrix is 'ready', for instance for mechanical loading and metabolic demands. Due to their matrix production, osteoblasts become incorporated within their own product. Once incorporated, osteoblasts differentiate into osteocytes. Osteocytes reside in (matrix) lacunae and communicate with each other by their surrounding cellular processes that project through the matrix in so-called 'canaliculi' (i.e. little canals, see Fig. 2). One of the main functions of the osteocytes is the monitoring of their surrounding bone tissue matrix. As such, they play a major role in monitoring bone turnover (see below), whereas recent results suggest they also play a role in maintaining endocrine homeostasis (Noble, 2008). Bone tissue is resorbed by osteoclasts. These are polynuclear cells from the mononuclear phagocytic lineage. Once activated, these multinuclear cells adhere to the bone matrix surface and excrete erosive hydrogen ions. As a result, the adjacent bone tissue dissolves, creating a resorption bay (Fig. 3). Such a resorption bay is called a 'Howship's lacuna', named after the 19th century British anatomist John Howship.

In mature trabecular bone, resorption and apposition take place at the trabecular surface, while in mature cortical bone they mainly take place by means of 'basic multicellular units' (BMUs). A BMU is essentially a drilling and filling unit that

Figure 2.

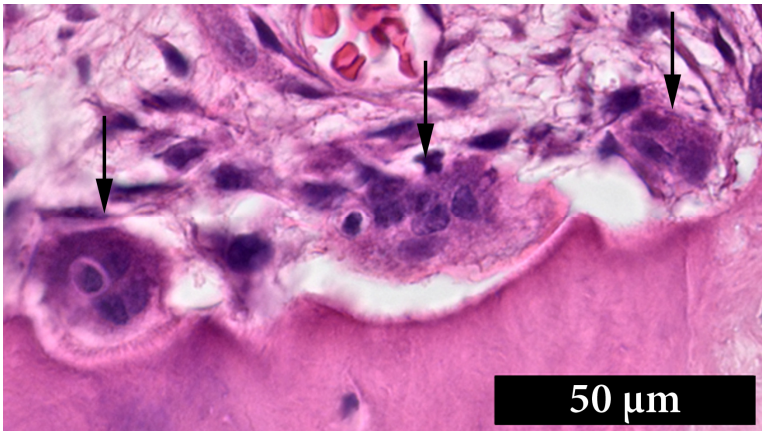


Undecalcified ground section of human dry bone, stained with haematoxylin and eosin. Due to the accumulation of grinding debris in the remaining space after cell decomposition, osteocyte lacunae and their interconnecting canaliculi become clearly visible. During life, the canaliculi contain cellular projections by which the osteocytes monitor their surroundings.

transverses through cortical bone, remodeling it on the way. At the front end of the unit, osteoclasts reabsorb bone tissue. The interior of the thus extending tunnel is subsequently lined by osteoblasts, which deposit and mineralize osteoid in a lamellar way at the rear end. The result is a 'stave' of bone tissue, composed of concentrically deposited lamellar bone fibres, called a Haversian system (or osteon). The Haversian system is essentially a thick walled cylinder of 'new' bone with a narrow central canal, the Haversian canal. The latter contains blood vessels and a little connective tissue. In a transverse microscopy section, the perimeter of a Haversian system shows a cement line, which demarcates the end of the initial osteoclastic resorption. In a normal situation, bone tissue is continuously replaced by newly formed bone. This balanced resorption and apposition process annually replaces approximately 10% of the total bone mass, thus gradually increasing the number of Haversian systems during life.

The degree of osteoclastic and osteoblastic activity is tightly regulated by many factors, such as by hormonal changes (e.g. by the parathyroid hormone), electrolyte changes (e.g. of calcium and phosphate) and mechanical changes (see e.g. Vigorita, 2007). Osteoblastic and osteoclastic activity can also be affected by pathological conditions, such as by trauma, vitamin deficiencies or hormonal imbalances.

Figure 3.



Polynuclear osteoclasts, adherent to a bone trabecula (arrows). By secreting erosive substances, the osteoclasts resorb the underlying bone tissue. This creates scalloped reabsorption pits, so-called 'Howship's lacunae'. Decalcified section of fresh human bone, stained with haematoxylin and eosin.

Irrespective of the causal factor, bone tissue reaction is limited to an osteoblastic, osteoclastic and osteocytic response. With these mechanisms in mind, the potential of the light microscope for the investigation of bone tissue can be understood.

Histology of dry bone tissue in forensic anthropology and palaeopathology

The potential of microscopic investigation of dry bone tissue did not remain unnoticed by forensic anthropologists and palaeopathologists. After a somewhat hesitant start, the use of histology gradually increased over the past decades and is nowadays applied for a variety of purposes. An example is the study of microarchitectural differences to differentiate between human and animal bone (e.g. Hillier and Bell, 2007; Cuijpers, 2009). Another application is the use of the life-long remodeling process and its resulting increase in Haversian systems within cortical bone to estimate the age at death of individuals (e.g. Kerley, 1965; Stout and Simmons, 1979; Maat *et al.*, 2006). The histological research on the gradual decomposition of human bone by soil, ground water, insects and microbes became applicable to assess the postmortem time interval of interred and non-interred corpses (e.g. Hedges *et al.*, 1995; Jans *et al.*, 2004).

Such examples illustrate that histology can be a useful and valuable tool in the study of human remains. But, like all methods, microscopy has its diagnostic limitations and technical challenges. Many workers, unfamiliar with the microscope as a tool, assume that histological research is expensive, difficult and probably of little additional value to gross anatomical and pathological study. Yet, this thesis will focus on these principal aspects to demonstrate the various methodological approaches, as well as the potential and restrictions of dry bone histology.

Part 1: Technicalities associated with dry bone histology

If a pathology laboratory produces histological sections of 'fresh' bone tissue, the material is decalcified and in most cases paraffin-embedded, after which it is sectioned with a microtome. Such an approach is unsuitable for dry bone material, as decalcification will result in the total dissolving of the specimen. The production of sections of undecalcified dry bone material is problematic, since its hardness and brittleness will cause it to shatter upon direct sectioning. Several costly and time consuming methods have been proposed to overcome these problems, but they were scarcely accepted (e.g. Xipell *et al.*, 1974, Wallin *et al.*, 1985). The introduction of a

'cheap and quick' method by Maat *et al.* in 2001 overcame a great deal of the objections and made histological research of dry bone more accessible to all (Maat *et al.*, 2001).

Chapter 2 of this thesis will focus on an useful addition to this latter method, viz. the use of histochemical staining. Traditionally, forensic anthropologists and palaeopathologists produce and perform their investigations on unstained bone sections. This in contrast to pathologists, who always apply stainings to improve the visibility of microarchitecture and cells. We will discuss the potential of histochemical staining of dry bone, and propose a relatively easy method to stain undecalcified dry bone sections.

Chapter 3 tackles a 'shortcoming' of Maat's method, as it was thought to be less suitable for the production of sections of fragile/trabecular bone material (Beauchesne and Saunders, 2006). Several earlier approaches to handle extremely fragile bone tissue were proposed, but their production times and costs appeared to run out of proportion (e.g. Schultz, 1998). Besides, these methods did not allow for histochemical staining. In this chapter we provide a relatively quick and easy method to produce embedded sections of dry bone, with the optional possibility to apply histochemical stains.

Part 2: The diagnostic value of dry bone histology

The lack of soft tissue in dry bone remains poses a huge problem for diagnosing disorders, since all characteristic soft tissue architecture and cytonuclear characteristics are missing. This problem is further increased by the usual lack of contextual data on the case, such as medical history details, resulting in an ongoing discussion on the diagnostic usefulness of dry bone histology (Waldron, 2009; Weston, 2009; Schutskovski and Fernandez-Gil, 2010; Van der Merwe *et al.*, 2010).

In chapter 4 we briefly review the varying opinions on the value of dry bone histology diagnoses. Then, the existing literature on the histopathological diagnoses of various groups of disorders in dry bone material is reviewed and reflected on in the light of up-to-date knowledge on the pathogenesis of these disorders. By doing so, we aim to define which diseases do or do not have a pathognomonic dry bone histomorphology.

In chapter 5 we summarize these findings and propose, specifically for archaeologists, robust methods for section production and histological age assessment, in order to make dry bone histology more accessible to those less familiar with microscopy.

A recent case of how dry bone histology can aid in palaeopathological and archaeological analysis is illustrated in chapter 6. It describes the investigation of alleged scurvy in crew members of the lost Franklin Expedition of 1845.

Part 3: The use of histology for the detection of features of mechanical injury in dry bone

Traumatic lesions are amongst the most common findings in human archaeological and forensic remains. Consequently, a large body of literature exists on their interpretation. In a substantial number of papers a 'best guess' is made on the time relation between the moment of a traumatic event and eventual death, as it may shed light on to what extend the traumatic event may have affected the individual's life. Usually, lesions are only described as being antemortem, postmortem and -in indifferent cases- perimortem (Lovell, 1997). In antemortem lesions, a further analysis of the bone changes could lead to an estimation of the time laps between the traumatic event and eventual death. This 'posttraumatic time interval' is generally only roughly characterized by mentioning whether the lesion is healing (usually interpreted as a short posttraumatic time interval) or healed (usually interpreted as a long posttraumatic time interval) (e.g. Brickley, 2006). A more detailed 'dating' of a lesion would be desirable, as it would aid in the interpretation of facets such as medical status, medical care and the timing/sequence of multiple traumas. In forensic practice, the interpretation of alleged cases of torture and child abuse would benefit from such a method.

Chapter 7 examines the feasibility and objectivity of a more detailed assessment of the posttraumatic time interval in dry bone tissue. The study extrapolates on recent forensic pathological practice, in which healing features are used as an intrinsic indicator of posttraumatic time interval (Maat, 2008; Maat and Huls, 2010). The study assesses which microscopic and radiologic features are still reliably detectable in dry bone material.

The potential of the approach is illustrated in chapter 8 en 9. Chapter 8 describes the analysis of mechanical traumas in a 19th century mining population from Kimberly, South Africa. Chapter 9 focuses on a selection of mechanical traumas found in soldiers of the army of Napoleon from his 1812 field campaign in Russia.

References

Beauchesne, P., Saunders, S., 2006. A test of the revised Frost's 'rapid manual method' for the preparation of bone thin sections. International Journal of Osteoarchaeology 16, 82–87.

Brickley M., 2006. Rib fractures in the archaeological record: a useful source of sociocultural information? International Journal of Osteoarchaeology 16, 61-75.

Cuijpers, S., 2009. Distinguishing between the bone fragments of medium-sized mammals and children. A histological identification method for archaeology. Anthropologischer Anzeiger 67, 181-203.

Donoghue, H. D., Spigelman, M., Greenblatt, C. L., Lev-Maor, G., Kahila Bar-Gal, G., Matheson, C., Vernon, K., Nerlich, A.G., Zink, A.R., 2004. Tuberculosis: from prehistory to Robert Koch, as revealed by ancient DNA. The Lancet Infectious Diseases 4, 584-592.

Ernst-Heidelberg, P., 1931. Struktur und funktion. Verhandlungen der Deutschen Orthopedischen Gesellschaft. 15-31.

Frost, H.M., 1965. The Utah paradigm of skeletal physiology. Vol. 1 and 2. International Society of Musculoskeletal and Neuronal Interactions.

Frost, H.M., 2000. The Utah paradigm of skeletal physiology: an overview of its insights for bone, cartilage and collagenous tissue organs. Journal of Bone and Mineral Metabolism 18(6), 305-16.

Halperink, E.C., 2004. Paleo-oncology: the role of ancient remains in the study of cancer. Perspectives in Biology and Medicine 47, 1-14.

Hedges, R.E.M., Millard, A.R., Pike, A.W.G., 1995. Measurements and relationships of diagenetic alteration of bone from three archaeological sites. Journal of Archaeological Science 22, 201-209.

Hillier, M. L. and Bell, l. S., 2007. Differentiating human bone from animal bone: a review of histological methods. Journal of Forensic Science 52, 249–263.

Jans, M.M.E., Nielsen-Marsh, C.M., Smith, C.I., Collins, M.J., Kars, H., 2004. Characterisation of microbial attack on archaeological bone. Journal of Archaeological Science 31, 87-95.

Kerley, E.R., 1965. The microscopic determination of age in human bone. American Journal of Physical Anthropology 23 (2), 149-163.

Lovell, N.C., 1997. Trauma analysis in paleopathology. Yearbook of physical anthropology, 104, 139-170.

Maat, G.J.R., Van den Bos, R.P., Aarents, M.J., 2001. Manual preparation of ground sections for the microscopy of natural bone tissue: update and modification of Frost's 'rapid manual method'. International Journal of Osteoarchaeology 11, 366-374.

Maat, G.J.R., Maes, A., Aarents, M.J., Nagelkerke, N.J.D., 2006. Histological age prediction from the femur in a contemporary Dutch sample. Journal of Forensic Science 51(2), 230-237.

Maat, G.J.R., 2008. Case study 5.3: Dating of fractures in human dry bone tissue - the Berisha case. Skeletal trauma, Kimmerle E.H., Baraybar JP (Eds.). Taylor & Francis Group: Boca Raton, FL, 245–254.

Maat G.J.R., Huls N., 2010. Histological fracture dating of fresh and dried bone. In: Forensic aspects of pediatric fractures. Bilo, R.A.C., Robben, S.G.F. and Van Rijn, R. (Eds.). Springer-Verlag: Berlin – Heidelberg. 194-201.

Noble, S.N., 2008. The Osteocyte Lineage. Archives of Biochemistry and Biophysics 473, 106-111.

Ortner, D.J., 2003. Identification of pathological conditions in human skeletal remains. Academic Press: San Diego.

Reichs, K. 1998. Forensic osteology, advances in the identification of human remains. 2nd edition. Charles C. Thomas publisher, Springfield, IL, USA.

Ross, M.H., Kay, G.I., Pawlina, W., 1995. Histology, a text and atlas, 4th edition. Lippincot, Williams and Williams, Baltimore. ISBN 0-683-30242-6.

Schultz, M., 1988. Methoden der Licht—und Elektronenmikroskopie. In: Handbuch der vergleichenden Biologie des Menschen. Knussmann, R., (Ed.). Gustav Fischer Verlag: Stuttgart, 698–730.

Schutkowski, H., Fernandez-Gil, M., 2010. Histological manifestation of mycobacterial bone lesions. Paper presented at 18th European meeting of the paleopathology association, Vienna, Austria.

Stout, S.D., and Simmons, D.J., 1979. Use of histology in ancient bone research. Yearbook of Physical Anthropology 22, 228-249.

Van der Merwe, A.E., Maat, G.J.R., Steyn, M., 2010. Ossified haematomas and infectious bone changes on the anterior tibia: histomorphological features as an aid for accurate diagnosis. International Journal of Osteoarchaeology 20, 227-239.

Vigorita, V.J., 2007. Orthopaedic Pathology. 2nd edition. Philadelphia: Lippincott Williams & Wilkins.

Wallin, J.A., Tkocz, I., Levinsen J., 1985. A simplified procedure for preparation of undecalcified human bone sections. Biotechnic & Histochemistry 60, 331–336.

Waldron, T., 2009. Palaeopathology. Cambridge University press, Cambridge.

Weston, D.A., 2009. Brief communication: paleohistopathological analysis of pathology museum specimens: can periosteal reaction microstructure explain lesion etiology? American Journal of Physical Anthropology 140, 186-193.

Wolff, J., 1987. Ueber die innere architectur der knochen und ihre bedeutung für die frage vom knochenwachstum. (translated into English by Heller *et al*.: on the inner architecture of bones and its importance for bone growth. Clinical Orthopedics and Related Research 468(4), 1056–1065.

Xipell, J., Makin, H., McKinnon, P., 1974. A method for the preparation of undecalcified bone sections for light microscopy and microradiography. Stain Technology 49, 69–76