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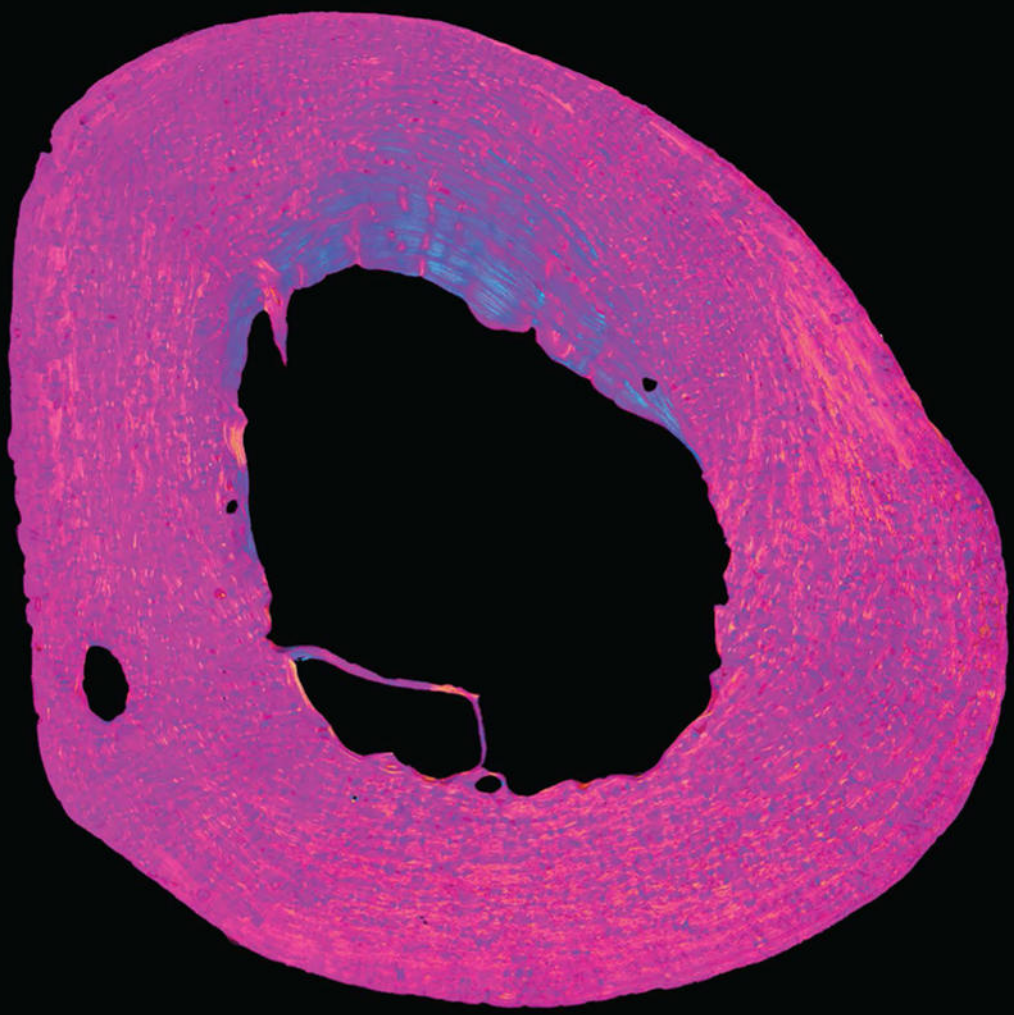
SECOND EDITION

Bone Histology

A Biological Anthropological Perspective

Edited by Sam D. Stout and Christian Crowder



Bone Histology

Bone Histology: A Biological Anthropological Perspective, Second Edition builds on the success of the first edition, recognizing the significant advances that have occurred in bone biology, histology, and histological techniques and methods in subsequent years.

Bones and teeth are of considerable importance for anthropological and related research, due to their nature as hard tissues. The physical remains of humans available to biological anthropologists, bioarchaeologists, paleopathologists, and paleontologists are, with exception to forensic anthropology, limited to skeletal material; fortunately, the same characteristics of hard tissues that lead to their persistence after death make them a storehouse of information about biological processes experienced during the life of the individual. This book covers important aspects of bone biology which underlie the microstructure of hard tissues that are crucial for histological analysis. This includes an overview of two major metabolic processes, bone remodeling and modeling, and their importance for understanding and interpreting bone histomorphology. Subsequent chapters apply histological methods to the biological profile, such as estimation of age and evaluation of pathological conditions that affect the skeleton, or to determine whether remains are human or nonhuman. Finally, there is a discussion of current research trends in bone histology, with a focus on technological advances in imaging and methods. Reviews of four well-documented skeletal collections – developed specifically for bone histological and imaging research – are discussed, as well as the importance of such collections for future research.

Bone Histology, Second Edition has assembled a collection of contributing authors, with extensive experience and expertise in various aspects of hard tissue biology, to provide readers with an overview of the current state of research and potential applications of histological analysis in biological anthropology, forensic anthropology, and skeletal biology. It serves as a valuable resource for students, researchers, and practitioners in these and related disciplines.

Bone Histology

A Biological Anthropological Perspective

Second Edition

Edited by
Sam D. Stout
Christian Crowder

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Dr. Sam D. Stout:

I dedicate this book to two mentors whose sage advice has guided me throughout my academic career. John Eaton profoundly influenced me early in my career, revealing the excitement of doing research, and "thinking outside the box." Steven Teitelbaum introduced me to the field of bone biology and histomorphological analysis, and inculcated within me an appreciation for the importance of rigor in research. My appreciation goes, too, to Clark Larsen who brought me into the Department of Anthropology at The Ohio State University, supported my research, and continues to be a valuable colleague and friend. Finally, I am thankful to the many outstanding graduate students with whom I have had the privilege to work with over the years, and who have gone on to become colleagues and collaborators. Indeed, several are authors of chapters in this book.

Dr. Christian Crowder:

I would also like to dedicate this book to two mentors who helped me find balance between my career paths as a forensic practitioner and a researcher / academic. Dr. Dana Austin who took me under her wing as a graduate student and eventually as an intern at the Tarrant County Medical Examiner's Office. She showed me the exciting world of applied anthropology as a forensic anthropologist. Dr. Susan Pfeiffer accepted me as her PhD student at the University of Toronto and influenced my passion for bone histology and skeletal biology. She created the most positive atmosphere within our cohort, and I fondly think back at our discussions during "Pfeiffer club" and the wisdom she passed on to us. Finally, I would like to thank Dr. Sam Stout. Although I was never his student, he always had the time to discuss bone histology with me which led to us becoming colleagues and friends. I am also thankful for the graduate students and researchers who have worked with me on bone histology projects over the years, especially Janna Andronowski, Victoria Dominguez, Sophia Mavroudas, Deborrah Pinto, and Zoe Morris.

Contents

Preface

Because of their nature as hard tissues, bones and teeth are of considerable importance for anthropological and related research. Except for forensic anthropology and the relatively rare cases of mummification, the physical remains of humans available to bioarchaeologists, paleopathologists, and paleontologists are limited to skeletal material. Fortunately, the same characteristics of hard tissues that lead to their persistence after death make them a storehouse of information about biological processes experienced during the life of the individual. Unlike for the soft tissues of the body, the activity of cells involved in growth and development, tissue maintenance, and adaptation of hard tissues are encoded in their microstructure. Recognition of our ability to extract biologically important information from hard tissues emerged through the work of researchers such as Amprino, Currey, Enlow, Frost, Johnson, and Marotti, and for teeth the work of Dean, Reid, Boyde, Beynon, Bromage, and Shellis. We now understand that while bone serves an important metabolic function relating to mineral homeostasis, its primary function is biomechanical. The first edition of *Bone Histology: An Anthropological Perspective* addressed the need for a comprehensive volume covering theoretical and applied aspects in histological analysis of skeletal tissue. The stimulus for this updated and expanded second edition arose from the success of the first edition, and recognition that significant advances have occurred in bone biology, histology, and histological methodology since the first edition was published. While the focus of the book is the application of histological analysis in bioarchaeology, forensic anthropology, paleontology, and paleopathology, relevant aspects of basic bone biology that are essential for interpretation of histomorphometric data are also included.

Organization and Content

The first three chapters of the book cover important aspects of bone biology that underlie the microstructure of hard tissues and are crucial for histological analysis. They provide an overview of two major metabolic processes, bone remodeling and modeling, and their importance for understanding and interpreting bone histomorphology. Furthermore they discuss pediatric and adult bone histomorphology and its response to various intrinsic and extrinsic factors.

Chapter 1, *Bone Remodeling and Its Histomorphological Products*, provides a broad overview of the theoretical foundations, cellular activities, microstructural products, and histomorphometric interpretations of bone remodeling, including principles and models of bone functional adaptation, mechanotransduction of mechanical stimuli into bone remodeling, and a brief overview of bone cell activity during each phase of bone remodeling. It also presents considerations for interpreting remodeling activity from histomorphometry,

including methodological guidelines, identifying osteon types, sources of intra- and inter-skeletal variation, techniques for static and dynamic histomorphometry, and method reliability.

Related, but distinct in important ways from bone remodeling, is bone modeling. In Chapter 2, *Making the Mold: A Microstructural Perspective on Bone Modeling during Growth and Mechanical Adaptation*, Cory and Isabel Maggiano define bone modeling, and discuss how it acts in concert with remodeling during growth and mechanical adaptation. This well-illustrated and innovative chapter concludes with a discussion of current methods employed to investigate how bones change in morphology and envisions possible future directions in research to be explored.

In Chapter 3, *Pediatric Bone Histomorphology and Environmental Stresses*, Goldman and Schug put forward a novel and timely methodology by which bone microstructure can be used to assess the effects of biocultural and environmental stresses on pediatric health in past populations and provide insights into the effects of modern climate change and other environmental issues.

The next two chapters cover important aspects of bone biomechanics and how bone adapts under mechanical loading. These chapters delve into structure and function, with insight into research models and applications of load history interpretation in forensic anthropology and bioarchaeology.

In Chapter 4, *Skeletal Fracture: Biomechanics and Forensic Perspectives*, Harden, Hunter, and Agnew discuss how the application of histological methods and analyses in skeletal trauma research can enhance our ability to relate bony responses to traumatic loading and fracture outcomes, and effectively substantiate the advantages of histological analysis as component of human osteological examinations, specifically in forensic anthropology and bioarchaeology. The inclusion of results stemming from research at the Injury Biomechanics Research Center at The Ohio State University is particularly compelling for the use of histological analysis in biomechanics and trauma analysis, and reveals a complex relationship between functional adaptation, bone biomechanics, and skeletal trauma for human pediatric bone.

In Chapter 5, *Biomechanical Foundations of Histological Analysis in Limb Bones*, John Skedros expands upon biomechanical and mechanical adaptation discussion covered in Chapters 2–4, by identifying correlations between structure, material, function, and load history. In this chapter, Skedros proposes that bone's adaptations for its load history can best be interpreted using a systematic approach that assesses both structural and material characteristics that considers load-complexity category, and shear resistance-priority hypotheses, modeling and remodeling working in concert, and the mechanical relationships of osteons, osteon morphotypes, and predominant collagen fiber orientation (CFO).

The next five chapters of the book focus on the application of histological methods to explore aspects of the biological profile, such as estimation of age and evaluation of pathological conditions that affect the skeleton, or to determine if remains are human or non-human. These chapters discuss how analyses are used to answer questions in forensic and bioarchaeological contexts.

Teeth also provide a record of their growth in their histological structures and are a valuable resource for skeletal analyses. In Chapter 6, *Histological Features of Dental Hard Tissues: Methodologies and Utilities in Forensic Anthropology and Bioarchaeology*, Chris Aris discusses the application of histological methodology to dental hard tissues. More specifically, he focuses on how histological analysis can be applied in the fields of bioarchaeology

(and osteoarchaeology) and forensic anthropology. Included is a review of methods of analysis applicable to the three main hard dental tissues (enamel, dentine, and cementum) typically used in anthropological and forensic research and practice. Finally, the chapter highlights potential developments, and how they could impact the application of dental hard tissue histology for skeletal remains.

For those having to deal with extremely fragmented remains, the question of whether the bone is of human origin is often critical. In many cases, when diagnostic landmarks are not present, the fragmentation precludes the use of macroscopic methods to differentiate human bone from non-human bone. In such cases, histological analysis is a potential means to differentiate human from non-human bones. Chapter 7, *Human versus Non-Human: Bone Microstructure in Species Differentiation and Experimental Research*, by Victoria Dominguez, looks at the differentiation of human from non-human skeletal remains based upon bone microstructure from a broad perspective. The chapter begins with a short summary of the evolutionary history and origins of skeletal tissues as currently understood, followed by an introduction to bone microstructure in the context of human and non-human growth and development, including an overview of existing methods and related considerations for distinguishing between human and non-human bone at the microstructural level. The chapter concludes with a brief discussion of how bone microstructure can enhance the selection of appropriate non-human models for experimental research.

In Chapter 8, *Age-at-Death Estimation from Bone Microstructure*, Gocha, Mavrouda, and Goldstein describe and evaluate the utility of bone microstructure to estimate age-at-death from human skeletal remains and underscore the merits of histological age-at-death estimating methods. A chronological survey of some of the major developments in methods for age estimation through histological investigation of cortical bone microstructure is utilized to offer a temporal context to advances in the field as they arose, e.g., population-specific histological age-at-death estimation equations, offering less complicated and invasive methods, methods applicable to sub adult bone, application of three-dimensional data for age estimation, validation of histomorphometric definitions, use of novel technological, and more sophisticated statistical methodologies.

In Chapter 9, *The Histopathological Analysis of Human Skeletal Remains*, Assis, Gómez García-Donas, and de Boer provides a comprehensive overview of the benefits, limitations, and challenges for the histopathological analysis of dry bone, and offers a realistic assessment of the contribution of histology in the diagnostic process. Following a discussion of how histology should be incorporated in "the broader diagnostic work-up" is an overview of relevant bone diseases, and how histology may aid in their diagnosis, including various types of metabolic bone disease, infections, tumors, and trauma.

Histological methods can be effectively applied to bone derived from a broad range of contexts. In Chapter 10, *Histological Analyses of Human Bone from Archaeological Contexts*, Pfeiffer and Pinto discuss the application and benefits of histological analysis of human bone in archaeological contexts. The chapter focuses on research and methods based upon transmitted light microscopy. Whether determining if fragmentary remains are human, providing an age at death estimate, evaluating the extent of diagenesis during time since death, or evaluating skeletal health and disease, the authors demonstrate that the histological evaluation of bone provides useful information for bioarchaeological and paleopathological studies, as well as modern forensic casework. They also note the role that histological analysis can play in other types of analyses that require adequate preservation of bone tissue, such as assessing bone sample quality preliminary to ancient DNA or isotopic analyses. The

authors make the important point that histological study of archaeologically derived material can contribute to research questions arising in forensic and biomedical fields. Given the potential for histological analysis in the broader scope of skeletal analysis, the authors propose that histology of normal cortical and cancellous human bone should be included in osteological training.

The final three chapters of the book discuss current research trends in bone histology, with a focus on technological advances in imaging and methods. Reviews of four well-documented skeletal collections developed specifically for bone histological and imaging research are discussed, as well as the importance of such collections for future research.

In Chapter 11, *Skeletal Collections for Histological and Imaging Research*, Andronowski and colleagues discuss skeletal collections that are amenable to histological and imaging research, including profiles of specific bone collections including backgrounds, ethical considerations, bone procurement processes, demographic compositions, and existing imaging and histological data available. In addition, they illustrate the emergence of digital repositories that allow sharing of data retrieved from skeletal collection reference banks and encourage national and international collaboration among researchers interested in human skeletal biology, biological anthropology, and related fields.

Chapter 12, *Three-Dimensional Microstructural Imaging of Bone: Technological Developments and Anthropological Applications*, Cooper and colleagues provide an overview of the development of 3D analytical techniques for the study of bone microstructure and their applications in biological anthropology, including an in-depth review of anthropological applications of 3D micro-CT and synchrotron radiation (SR) micro-CT, discussion of associated methodological issues, and suggested future uses of this technology in the field.

Chapter 13, *Visualization and Interpretation of Cortical Porosity*, by Mary E. Cole, provides an overview of the morphometry of vascular pores of cortical bone. It describes cellular processes that form the cortical pore network, summarizes microscopic and radiographic imaging techniques for visualizing pore morphometry in bone tissue, and concludes with a broader discussion of specific aspects of pore morphometry and interpretations of their possible mechanical origins and physiological modifications with age.

This volume has assembled authors with extensive experience and expertise in various aspects of hard tissue biology. Its intended goal is to provide readers with an overview of the current state of research and potential applications of histological analysis in biological anthropology, forensic anthropology, and skeletal biology. It is our hope that the contents provide a useful resource for students, researchers, and practitioners in these and related disciplines.

Editors

Sam D. Stout received his PhD in physical anthropology from Washington University in St. Louis in 1976 and is currently Professor Emeritus of anthropology at The Ohio State University. He is a skeletal biologist specializing in microstructural (histological) analysis of bone. His research has application in several disciplines, including bone biology, bioarchaeology, paleopathology, and forensic anthropology. During his career, he has been called upon to provide forensic analyses of human remains for local, state, and federal agencies, including histological age estimations of historically important skeletal remains, such as Francisco Pizarro, Janaab Pakal (Mayan Ruler of Palenque), and the "Sundance Kid." He is a Fellow of the American Academy of Forensic Sciences, and American Association for the Advancement of Science.

Christian Crowder, PhD, D-ABFA, received his BA from Texas A&M University, MA from the University of Texas at Arlington, and PhD in Biological Anthropology from the University of Toronto. Dr. Crowder is board certified in Forensic Anthropology by the American Board of Forensic Anthropology and has published numerous scientific articles and book chapters covering topics such as bone histology, skeletal trauma, and mass fatality response. He is the Chief of the Human Identification and Anthropology Laboratories for the Tarrant County Medical Examiner's Office in Fort Worth, TX. He has previously worked as a forensic anthropologist for international, federal, county, and city agencies. Over his career he has worked for the United Nations International Criminal Tribunal for the former Yugoslavia, the Department of Defense POW/MIA Accounting Agency (previously known as the Joint POW/MIA Accounting Command Central Identification Laboratory), the Office of Chief Medical Examiner – New York City, the Office of the Armed Forces Medical Examiner, the Harris County Institute of Forensic Sciences, and the Southwestern Institute of Forensic Sciences. In addition to his practitioner duties, Dr. Crowder is adjunct faculty at Pace University in New York City and at the University of Texas at Arlington.

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Bone Remodeling and Its Histomorphological Products

1

MARY E. COLE, CHRISTIAN CROWDER, AND SAM D. STOUT

1.1 Introduction

Skeletal analyses can be approached at several hierarchical levels. These range from organ-level macroscopic analyses of whole bones, to microscopic analyses of bone tissue structure, to nanoscale analyses of the tissue's collagen and mineral components, to molecular analyses of bone cell signaling pathways. Intermediate at the micro-scale is the histological analysis of bone tissue, which Harold Frost (Frost, 1986) described as the level of "skeletal intermediary organization," representing the collaborative activity of cells. The mineralized structures of this microscopic anatomy, or histomorphology,[1] are the observable and measurable products of bone cell activity over the lifespan. An anthropological perspective interprets the morphometry of these tissue structures as representing life history, including the cellular activity related to growth, aging, trauma, and pathology (Stout et al., 2019). Bone tissue microstructure is also an important component of bone quality, or the structural and material properties that contribute to bone strength (Paschalis et al., 2011). In order to interpret the biological information encrypted in the histomorphology of bone, it is essential to understand the basic biology underlying the creation of histomorphological structures.

Bone metabolism, whether for growth, adaptation, or homeostasis, involves two basic kinds of cells: bone-forming osteoblasts and their derivatives, and bone-resorbing osteoclasts. The normal activity of these same cells is associated with two distinct physiological processes referred to as modeling and remodeling. Bone modeling describes the independent activity of bone cells to either form bone (osteoblasts) or resorb bone (osteoclasts) on a surface (Frost, 2003; Hughes et al., 2020). Bone modeling can occur on both periosteal (external) and endosteal (internal) surfaces (Szulc & Seeman, 2009). Bone modeling is essentially restricted to the growing skeleton, where it works in concert with growth to adapt bones to their changing biomechanical environment by adjusting the amount and spatial distribution of bone tissue. Exceptions where modeling occurs in the adult skeleton are fracture repair and certain cases of pathological mechanical loadings (Peck & Stout, 2009). Testosterone also promotes bone formation responsible for periosteal expansion with age, particularly in males, in response to the loss in bone strength that follows age-associated endosteal resorption (Feik et al., 2000; Martin, 1993). Chapter 2 provides an in-depth discussion of the bone modeling process. Bone remodeling refers to the coordinated or "coupled" action of bone-resorbing osteoclasts and bone-forming osteoblasts at the same tissue location, focally replacing old or damaged bone (Frost, 2003; Hughes et al., 2020). A second type of remodeling that is stochastic and non-site-dependent is also proposed (Burr, 2002). This bone turnover occurs throughout the lifespan and is the predominant process in the adult skeleton responsible for its characteristic histomorphological features.

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This chapter will focus on bone remodeling in adult humans with an anthropological perspective, suitable for application to bioarchaeology, forensic anthropology, paleopathology, and paleontology. Anthropologically related analyses typically focus on the histomorphometric products of cortical bone remodeling, which are well defined in bone cross-sections. Cortical bone is compact bone, forming the shafts of long bones and the thin shells surrounding flat, short, and irregular bones (Eriksen, 2010). Although cortical bone appears solid macroscopically, it is microscopically perforated with pore systems, which house vascular structures (cortical pores) and osteocytes (lacunar-canalicular networks) (Andronowski & Cole, 2021). Cortical bone surrounds cancellous bone, which is located inside the marrow cavity and composed of trabecular struts and plates, giving it a "spongy" appearance (Eriksen, 2010).

The histological analysis of skeletal remains is made possible because remodeling occurs throughout life and produces discrete, definable, and quantifiable microscopic features such as osteons (Andronowski & Cole, 2021; Stout et al., 2019). The application of some methods to other animals is possible, but problematic because of the highly variable expression of cortical remodeling due to biomechanical and physiological differences (Schaffler & Burr, 1984). See Chapter 7 for the use of histomorphology to distinguish between human and nonhuman skeletal remains. Most notably, in addition to age at death estimation, histomorphometry can provide insights into bone remodeling history that can be used to estimate remodeling rates, creating what can be called "paleophysiology."

The purpose of this chapter is to provide a broad overview of the theoretical foundations, cellular activities, microstructural products, and histomorphometric interpretations of bone remodeling. The first section of this chapter will discuss principles and models of bone functional adaptation. The second section will describe the mechanotransduction of mechanical stimuli into bone remodeling. The third section will provide a brief overview of bone cell activity during each phase of bone remodeling. The fourth section presents considerations for interpreting remodeling activity from histomorphometry, including methodological guidelines, identifying osteon types, sources of intra- and inter-skeletal variation, techniques for static and dynamic histomorphometry, and method reliability.

1.2 The Role of Remodeling in Bone Functional Adaptation

Mechanical loading alters the composition and function of cells in structural tissues of the body, ultimately shaping tissue development, maintenance, and repair. Mechanobiology studies the cellular mechanisms that transduce these mechanical forces into biological signaling cascades (Wang & Thampatty, 2006). More broadly, bone functional adaptation describes how the mechanical environment modifies bone shape and structure over the lifespan (Ruff et al., 2006).

1.2.1 Early Concepts of Bone Functional Adaptation

Skeletal adaptation to mechanical loading was recognized in the early 16th century by Galileo, who noted that bone width scales with load-bearing and not just with size (Skerry, 2006). The idea was not popularized until the 19th century, when orthopedic surgeon Julius Wolff introduced "the law of bone transformation" (Wolff, 1892). Wolff was influenced by anatomist Georg Hermann von Meyer (von Meyer, 1867) and engineer Karl Culmann

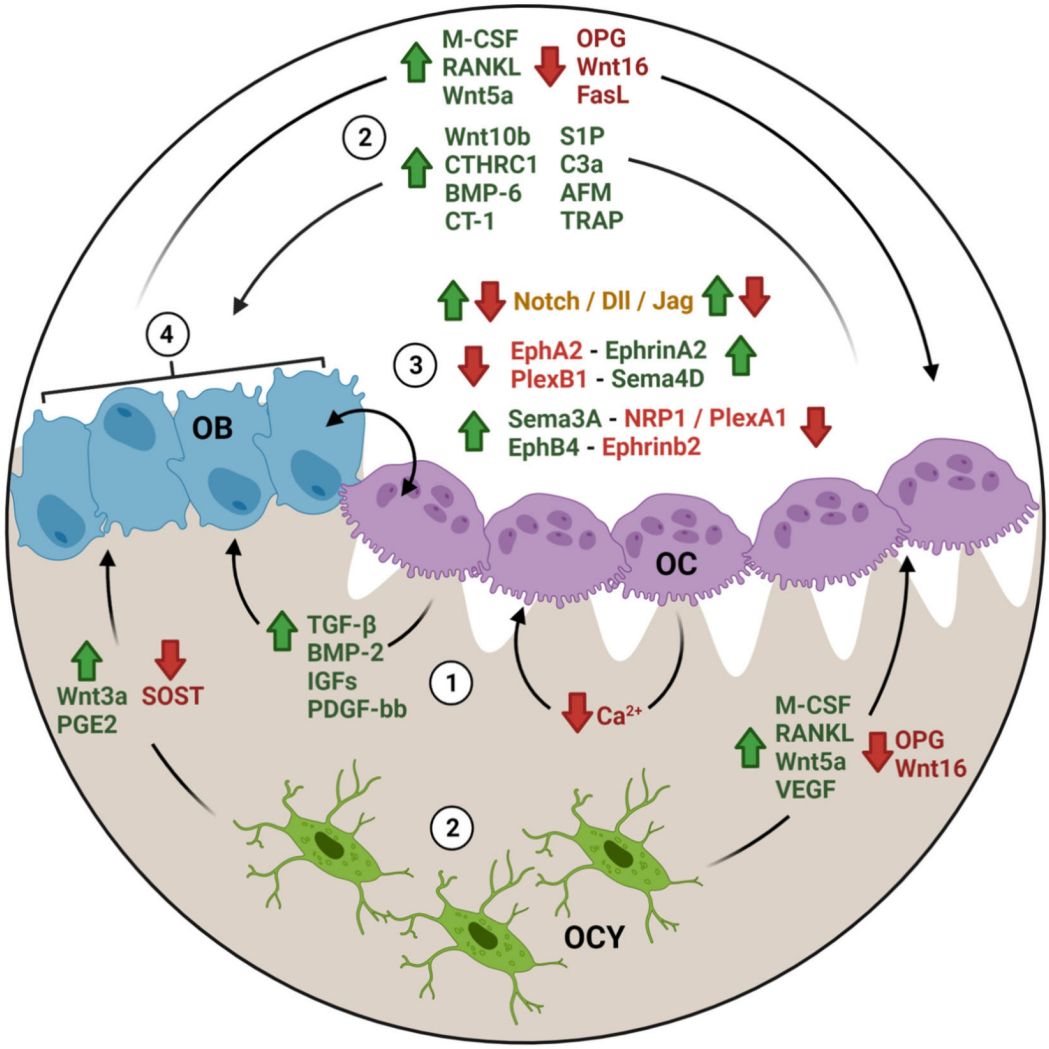

Figure 1.3 Four classes of factors that couple bone resorption to bone formation are illustrated in this figure: (1) Matrix-derived signals released from resorbed bone slow osteoclast function and promote osteoblast differentiation. (2) Factors secreted by osteoclasts and osteoblast-lineage cells promote or inhibit differentiation of the opposing cell type. (3) Membrane-expressed factors allow bi-directional signaling between osteoclasts and reversal cells, promoting differentiation of one cell type while repressing the other. (4) Topographical changes in reversal cell size and shape signal the critical cell density required to initiate bone formation. Figure created with BioRender.com.

and mature osteoblasts also progress the remodeling cycle through cell signaling pathways that "couple" osteoclast activity to osteoblast activity. Sims and Martin (2020) identify four main classes of coupling factors (Figure 1.3):

1) **Matrix-derived signals** released from resorbed bone slow osteoclast function and trigger osteoblastogenesis. Growth factors released from the bone matrix promote osteoblast progenitor replication, migration, and differentiation, including transforming growth factor β (TGF-β), bone morphogenetic protein 2 (BMP-2),

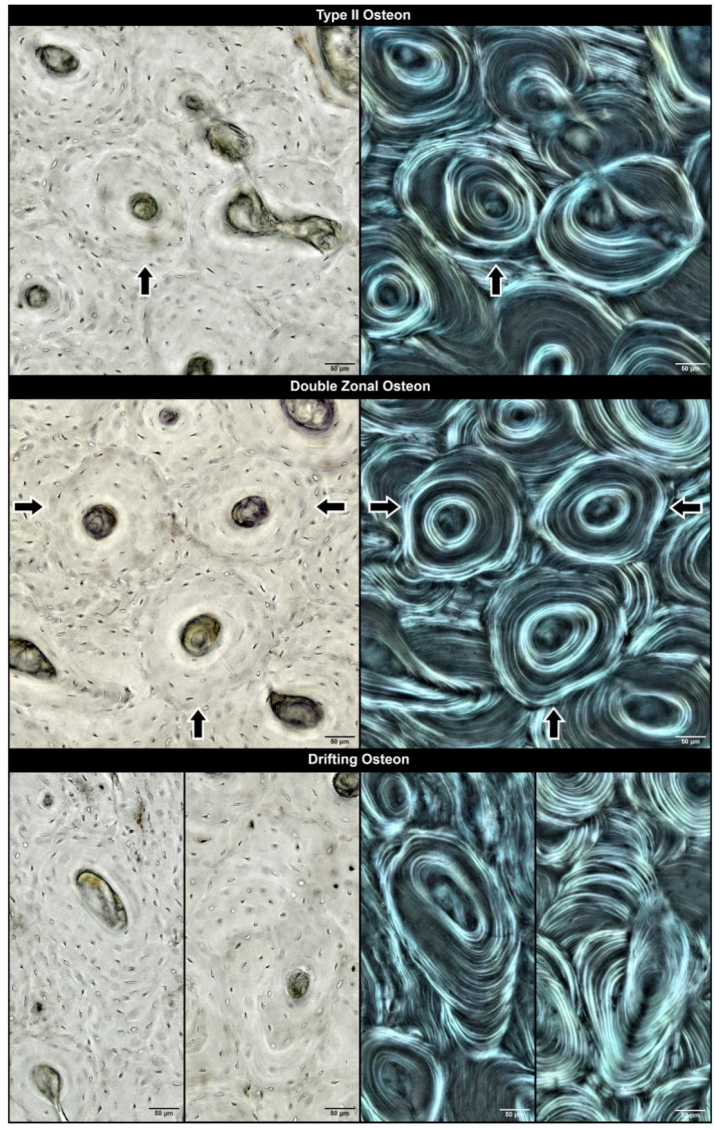

Figure 1.6 Microscopic images taken with brightfield (left) and circularly polarized light (right) show additional types of secondary osteons, including a Type II osteon with an offset, internal cement line (top), a double-zonal osteon with a hyper-mineralized ring within its concentric lamellae (middle), and two examples of drifting osteons with tails of semicircular lamellae (bottom).

observed with backscattered electrons in scanning electron microscopy, or even with linearly polarized light microscopy (Raguin & Streeter, 2018). Importantly for distinguishing these types with polarized light microscopy, the lamellae of double-zonal osteons are aligned, as they represent an arrest in the same remodeling cycle. In contrast, the lamellae and cement lines of type II osteons should be slightly offset between the internal and surrounding osteons, as they represent separate remodeling cycles.

zero fractures or incomplete fractures if they did fail, likely due to their complex material composition. Material properties are likely contributing to fracture types, which explains the relationships observed with age and lack of relationships with sex since rib material properties are known to be strongly associated with age but not sex (Albert et al., 2021; Katzenberger et al., 2020).

Bone type can also influence fracture behavior. Liu et al. (2000) found that post-failure, primary lamellar bone tends to break cleanly while lamellar bone containing osteon accumulations remains attached, providing an additional adaptive explanation for secondary lamellar bone function. Lynn and Fairgrieve (2009) found lamellar bone separation to be typical for fracture surfaces under dynamic loading conditions. They also found that osteon pullout is more likely to occur in fleshed than unfleshed bone, providing useful information for understanding fracture context. Osteon pullout, an interesting adaptive process that debonds the osteon at its interface, allows for the fatigue life and toughness of the bone to be extended. Osteon pullout has been shown to be affected by load type and vary by location, but how this phenomenon affects bone integrity requires further investigation (Hiller et al., 2003). Relationships between bone microstructure, specifically cortical porosity, and fracture type have been preliminarily investigated in human tibiae (Cole et al., 2023). This study utilized Pore Extractor 2D (Cole et al., 2021) to facilitate computer-assisted identification of cortical borders and pore spaces in cross-sections at the 66% site of tibiae that were impacted at the 50% site at 5 m/s in a lateral-medial direction. The quantification of pore morphometry included percent porosity, pore density, and mean pore size and shape descriptors. Exemplars of tibia cross-sections and cortical porosity data are provided in Figure 4.7. The results of this study demonstrated trends of variation in cortical area and

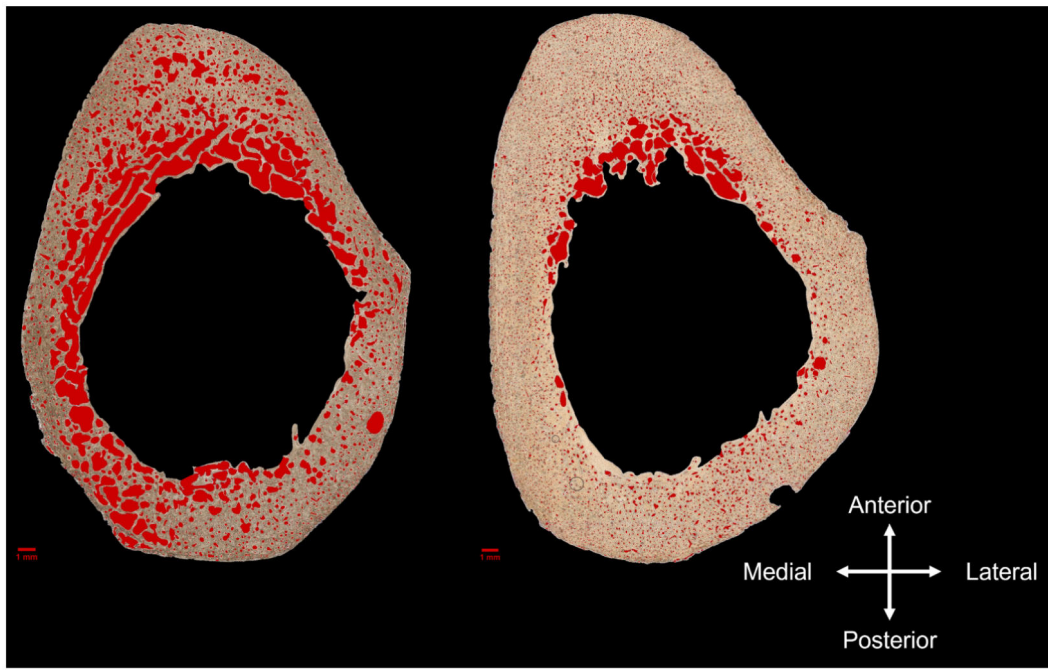

Figure 4.7 Exemplar images of variable cortical porosity in tibia cross-sections. Cortical pores shown in red. Left image demonstrates a tibia cross-section with a higher percent porosity, while the right image is a tibia cross-section with a lower percent porosity.

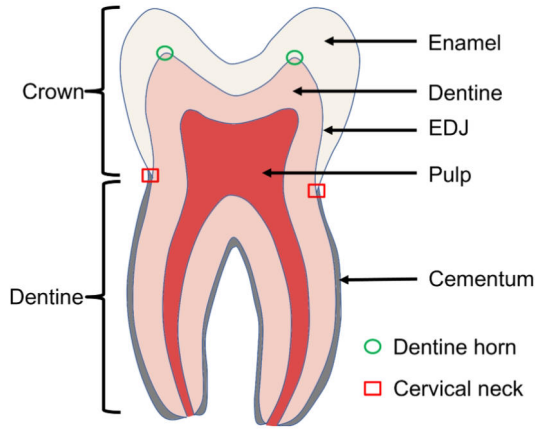

Figure 6.1 Diagram a longitudinal cross section of a molar tooth highlighting the crown and root regions as well as showing the primary tissues (hard tissues: enamel, dentine, and cementum; soft tissues: pulp).

the chapter is to provide an overview of all these factors, detailed explanations of research analyses and methodologies can be found in the literature cited throughout.

6.2 The Processes of Dental Histology

In order to understand the microstructure of teeth it is helpful to have a basic knowledge of how teeth are typically sectioned for examination. While methods can vary according to the context of the sectioning, they near-universally involve the key preparatory steps of: exfoliating (if necessary) teeth from alveolar bone (i.e. removing them from the socket), cleaning them, and embedding them in resin (e.g. Weale et al., 2023). Dental histological methods also involve comparable cutting and cleaning steps including: two cuts made using a slow speed wafering blade, mounting on glass slides, cleaning using alcohols, and cover slipping using ultra-thin glass. It is only recently that the processes of dental histology have become the focus of dedicated research (Aris, 2020). This recent work has highlighted the importance of ethical and preservative steps that can be included when sectioning teeth which include: conducting micro-CT scans where possible, producing resin casts of teeth before cutting/embedding, removing portions of roots for alternative future research before embedding, and digitising finished cross sections. After taking these steps final cross section will typically be 100–150 μm (0.01 cm) thick. By taking all the aforementioned steps dental histology allows for the viewing of microscopic internal structures of all three dental hard tissues (enamel, dentine, and cementum), the study of cell-sized features, and the development of methods using these features in research and casework. While this chapter will not go into detail on any of these steps specifically, reviews of each can be found in recent publications (Aris, 2020 and Weale et al., 2023).

6.3 Enamel: Growth Processes and Associated Histological Features

While teeth are harder than bone in general, hardness also varies within a tooth between its different parts. Enamel is by far the hardest of all these parts and the pale-white outer shell and is directly involved in breaking down food when chewing. Enamel's hardness

Retzius periodicity is a useful tool for age-at-death and enamel cap formation time estimations (discussed in more detail later).

Until recently the exact cause of Retzius line formation was unknown. However, recent findings suggest that these lines form as a result of genetic and hormonal clocks causing oscillating biorhythms (Havers-Halberg Oscillation) which reflect like ripples in enamel growth (see Mahoney et al. 2022 for a detailed review). While this biorhythm has been studied extensively, it has not always been within the field of dental histology. However, the work which has been done using histological methods has used ground thin sections to link variation in Retzius periodicity to life history and body mass (e.g. Mahoney et al., 2017).

Within a tooth's earliest forming enamel at the tip of the enamel cap (cuspal or occlusal enamel) Retzius lines form like a stack of cones within the enamel and are thus not visible from the outside. In contrast, Retzius lines forming at the sides of the enamel cap (lateral enamel) reach the outer surface of enamel (see Figure 6.2). Where a line reaches the outer lateral enamel it forms as a slight ringed bevelling on the surface – these are called 'perikymata' (singular: perikyma; see Figure 6.3; Hillson, 1996). Perikymata relate to dental histology in that they can be used where such methods are not possible. In particular, as perikymata are external manifestations of Retzius lines, the periodicity of the two is the same. Thus, when viewed under reflected light the number of perikymata can be counted on a single tooth, average human Retzius periodicity be considered, and then an estimated range for the time the tooth's lateral enamel took to form be calculated (Guatelli-Steinberg and Reid, 2008).

Alongside the numerous cross striations and Retzius lines, there are two other kinds of notable linear developmental structures within enamel – the neonatal line (NNL) and accentuated striae (colloquially known as Wilson bands; Rose et al., 1978). The NNL is a prominent stria, appearing thicker and darker than other enamel striae, that forms in enamel as a result of birth, and roughly 7–10 days after the day of birth (Adserias-Garriga, & Visnapuu, 2019; Aka et al., 2015). As a result of this timing neonatal lines only form in deciduous (milk/baby) teeth and permanent (adult) first molars (Hillson, 1996; Figure 6.4). The cause of neonatal line formation is believed to be the result of the birth process disturbing ameloblast secretion

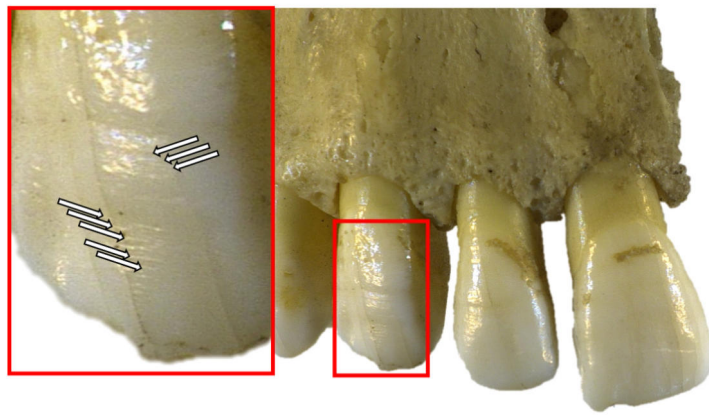

Figure 6.3 Image of right anterior permanent incisors and canine in situ within the maxilla. White arrows on superimposition point to surface perikymata lines. Photos courtesy of Megan Smith-Hands.

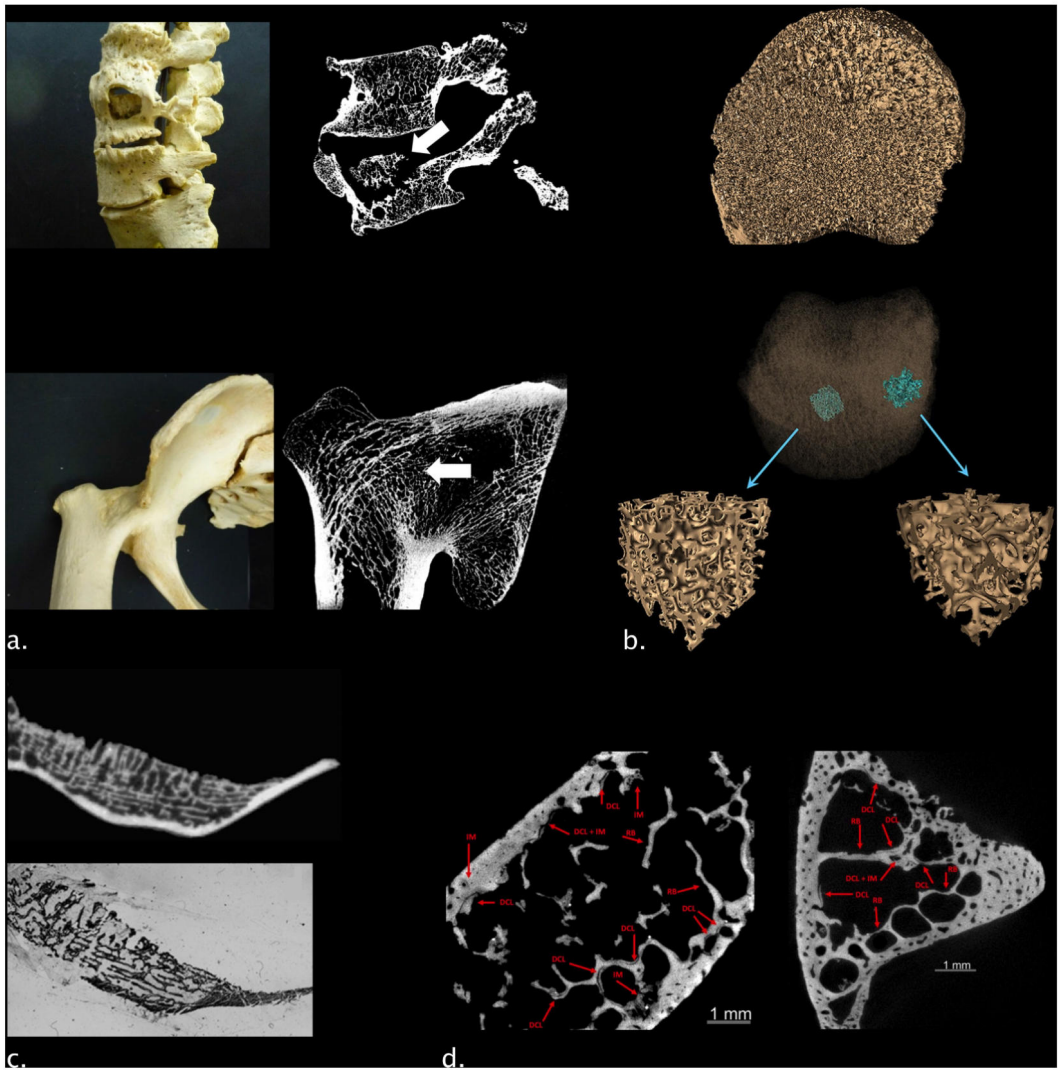

Figure 12.8 (a) Comparison of tuberculosis lesions between dry bone and micro-CT. top: A\a. sequester inside L3 vertebra, surrounded by a defective zone and a sclerotic zone; bottom: an ankylotic right hip joint showing trabecular defect (Vekszler et al., 2022). (b) 3D rendering of early signs of vertebral trabecular microstructural change (Coqueugniot et al., 2015). (c) micro-CT image (top) showing comparable microstructural details compared to histological thin sections (bottom) in *cribra orbitalia* lesions (Saint-Martin et al., 2015). (d) Features exhibiting defective mineralization seen in vitamin D deficient bone: defective areas adjacent to cement line (DCL), improper mineralization (IM), and resorptive bays (RB) (voxel size=6 µm, Welsh et al., 2020).

classify the presence and severity of the lesions (Fraberger et al., 2021) reflecting a blending of qualitative and quantitative applications of micro-CT.

Detailed evaluation of noninfectious inflammatory responses such as porosity, new bone formation, and erosive lesions can also benefit from micro-CT analysis. Dittmar and colleagues utilized micro-CT to evaluate the scooped-out, lytic lesion characteristic of gout, an inflammatory arthritis often associated with diets rich in alcohol and purine that could be linked to socioeconomic status (Dittmar et al., 2021). Cranial porotic lesions are a common