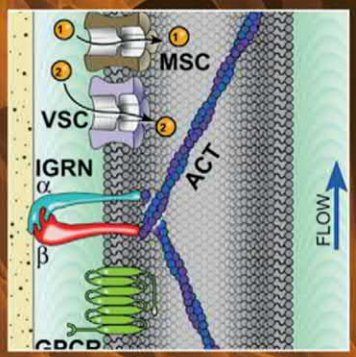
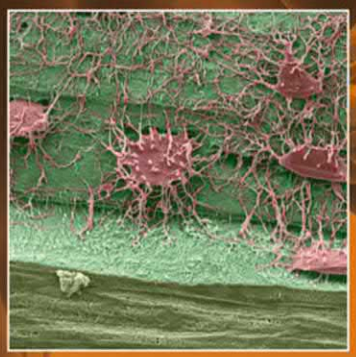
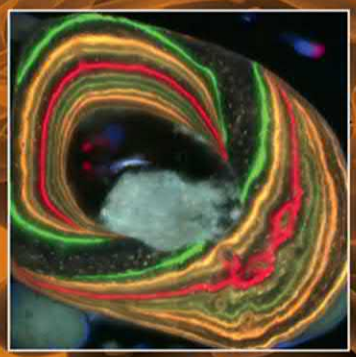
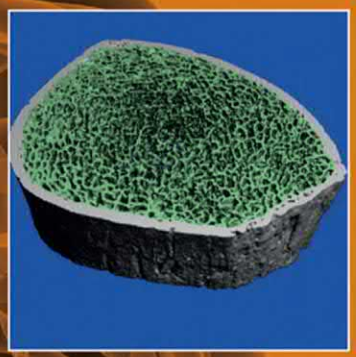
Basic and Applied

Bone Biology

Second Edition



Edited by
David B. Burr
Matthew R. Allen

BASIC AND APPLIED BONE BIOLOGY

SECOND EDITION

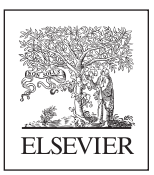
Edited by

DAVID B. BURR

Department of Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, IN, United States;
Department of Biomedical Engineering, Indiana University-Purdue University-Indianapolis, Indianapolis, IN, United States

MATTHEW R. ALLEN

Department of Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, IN, United States;
Department of Biomedical Engineering, Indiana University-Purdue University-Indianapolis, Indianapolis, IN, United States;
Department of Medicine-Nephrology, Indiana University School of Medicine, Indianapolis, IN, United States;
Roudebush Veterans Administration Medical Center, Indianapolis, IN, United States



ACADEMIC PRESS

An imprint of Elsevier

Academic Press is an imprint of Elsevier
125 London Wall, London EC2Y 5AS, United Kingdom
525 B Street, Suite 1650, San Diego, CA 92101, United States
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, United Kingdom

Notices
Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

ISBN: 978-0-12-813259-3

Publisher: Stacy Masucci
Acquisition Editor: Tari Broderick
Editorial Project Manager: Karen Miller
Production Project Manager: Kiruthika Govindaraju
Cover Designer: Miles Hitchen

Typeset by TNQ Technologies

This book is dedicated to the wonderful teachers I have had over many years—Denny, Bruce, Harold, Eric, Mitch, and Charles, among many others—who have so patiently taught me about the beauties and intricacies of our skeleton.

And to my wife, Lisa, and son, Erik, who have tolerated and supported my obsession with bone.

David B. Burr

This book is dedicated to Kristine, Sophie, Gus, and Faye who provide me with a daily reminder that there is so much more to life than bone biology. And to my mom who has influenced my life more than she will ever know.

Matthew R. Allen

List of Contributors

*Alexandra Aguilar-Pérez Department of Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, IN, United States

*Matthew R. Allen Department of Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, IN, United States; Department of Medicine-Nephrology, Indiana University School of Medicine, Indianapolis, IN, United States; Department of Biomedical Engineering, Indiana University-Purdue University-Indianapolis, Indianapolis, IN, United States; Roudebush Veterans Administration Medical Center, Indianapolis, IN, United States

Mary F. Barbe Department of Anatomy and Cell Biology, School of Medicine, Temple University, Philadelphia, PA, United States

*Teresita Bellido Roudebush Veterans Administration Medical Center, Indianapolis, IN, United States; Department of Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, IN, United States

Nicoletta Bivi Director-Assay Development, Laboratory of Experimental Medicine, Eli LIlly and Company, Indianapolis, IN, United States

*Andrea Bonetto Department of Surgery, Indiana University School of Medicine, Indianapolis, IN, United States

*Lynda F. Bonewald Department of Anatomy and Cell Biology and Orthopaedic Surgery, Indiana University School of Medicine, Indianapolis, IN, United States

*Angela Bruzzaniti Department of Oral Biology, Indiana University School of Dentistry and Department of Anatomy & Cell Biology, Indiana University School of Medicine, Indianapolis, IN, United States

*David B. Burr Department of Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, IN, United States; Department of Biomedical Engineering, Indiana University-Purdue University-Indianapolis, Indianapolis, IN, United States

Laura M. Calvi Department of Medicine-Endocrinology, University of Rochester Medical Center, Rochester, NY, United States

Julia F. Charles Department of Orthopaedics, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, United States

*Robert H. Choplin Department of Radiology and Imaging Sciences, Indiana University School of Medicine, Indianapolis, IN, United States

Timothy J. Corbin Department of Transgenics and Reproductive Technologies, Stowers Institute for Medical Research, Kansas City, MO, United States

Robin Daly Institute for Physical Activity and Nutrition, Deakin University, Melbourne, VIC, Australia

*Linda A. DiMeglio Department of Pediatrics, Division of Pediatric Endocrinology and Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, IN, United States

*Robyn K. Fuchs Department of Health and Rehabilitation Sciences, Indiana University School of Medicine, Indianapolis, IN, United States

*Theresa A. Guise Department of Medicine, Indiana University School of Medicine, Indianapolis, IN, United States

Christopher J. Hernandez Sibley School of Mechanical and Aerospace Engineering, Cornell University, Ithaca, NY, United States; Meinig School of Biomedical Engineering, Cornell University, Ithaca, NY, United States; Hospital for Special Surgery, New York, NY, United States

*Kathleen M. Hill Gallant Department of Nutrition Science, Purdue University, West Lafayette, IN, United States; Indiana University School of Medicine, Indianapolis, IN, United States

Mary Beth Humphrey Department of Medicine, University of Oklahoma Health Sciences Center and the Oklahoma City Veterans Administration, Oklahoma City, OK, United States

*Erik A. Imel Department of Pediatrics, Division of Pediatric Endocrinology and Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, IN, United States; Department of Medicine, Indiana University School of Medicine, Indianapolis, IN, United States

*Melissa A. Kacena Department of Orthopaedic Surgery, Indiana University School of Medicine, Indianapolis, IN, United States

*Are members of the Indiana Center for Musculoskeletal Health, Indiana University School of Medicine, Indianapolis, IN, United States

Dongbing Lai Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, United States

*__Jiliang Li__ Department of Biology and Center for Developmental and Regenerative Biology, Indiana University-Purdue University, Indianapolis, IN, United States

Bruce H. Mitlak Radius Health, Waltham, MA, United States

*__Sharon M. Moe__ Division of Nephrology, Indiana University School of Medicine, Rodebush Veterans Administration Medical Center, Indianapolis, IN, United States

Katherine J. Motyl Center for Molecular Medicine, Maine Medical Center Research Institute, Scarborough, ME, United States

Mary C. Nakamura Department of Medicine, University of California at San Francisco, and San Francisco Veterans Administration Health Care System, San Francisco, CA, United States

Thomas L. Nickolas Department of Medicine-Nephrology, Columbia University Medical Center, New York, NY, United States

*__Munro Peacock__ Department of Medicine, Indiana School of Medicine, Indianapolis, IN, United States

Roger Phipps Department of Pharmacology, Husson University School of Pharmacy, Bangor, ME, United States

*__Lilian I. Plotkin__ Department of Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, IN, United States

*__Alexander G. Robling__ Department of Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, IN, United States

*__G. David Roodman__ Department of Medicine, Indiana University School of Medicine, Indianapolis, IN, United States; Richard L. Roudebush VA Medical Center, Indianapolis, IN, United States

Tae-Hwi Schwantes-An Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, United States

Viral N. Shah Barbara Davis Center for Diabetes, School of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, United States

David L. Stocum Department of Biology and Center for Developmental and Regenerative Biology, Indiana University-Purdue University, Indianapolis, IN, United States

*__Joseph M. Wallace__ Department of Biomedical Engineering, Indiana University-Purdue University, Indianapolis, IN, United States

*__Connie M. Weaver__ Department of Nutrition Science, Purdue University, West Lafayette, IN, United States

*__Kenneth E. White__ Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, United States

Biographies

Dr. Burr is a University-Distinguished Professor of Anatomy and Cell Biology at Indiana University School of Medicine, and Professor of Biomedical Engineering at IUPUI. He joined the Indiana University School of Medicine faculty in 1990 as Chair of the Department of Anatomy (1990–2010), following faculty positions at the University of Kansas and West Virginia University Medical Schools. He served as President of the American Association of Anatomists (2007–09) and the Orthopaedic Research Society (2008–09), and was the Director of the Sun Valley Workshop on Musculoskeletal Biology for nearly 15 years (2004–17). He is a Fellow of both the American Association of Anatomists (AAA) and of the Orthopaedic Research Society (ORS). He has been the recipient of the Borelli Award from the American Society of Biomechanics (2008), the Gideon A. Rodan Award for Excellence in Mentorship from the American Society for Bone and Mineral Research (ASBMR), and the Henry Gray Scientific Achievement Award from the AAA. He serves as Editor-in-Chief for *Current Osteoporosis Reports*, Editor for *Bone*, and Associate Editor of the *Journal of Musculoskeletal and Neuronal Interactions*. He is the author of more than 250 research articles in the peer-reviewed literature; 56 book chapters and reviews; and 5 books on the structure, function, and mechanics of bone.

Dr. Allen is a Professor of Anatomy and Cell Biology, Orthopaedic Surgery, and Medicine-Nephrology at Indiana University School of Medicine (ISUM), Biomedical Engineering at Indiana University-Purdue University-Indianapolis, and a nonclinical scientist at Roudebush VA Medical Center. He also holds an Assistant Dean position in Faculty Affairs | Professional Development | Diversity at IUSM. His research career, and interest in bone biology, began at Alma College during a summer research fellowship and continued during his years as a PhD student at Texas A&M (in Kinesiology) and postdoctoral fellow at IU School of Medicine. His research focuses on understanding how interventions can be maximized to strengthen the skeleton. He serves as Editor-in-Chief for *Clinical Reviews in Bone and Mineral Metabolism* and is on the Editorial Board for the *Journal of Bone and Mineral Research, BONE, Osteoporosis International, Journal of Orthopaedic Research, JBMR-Plus and Bone Reports.* He has authored more than 130 original research articles and 25 book chapters and reviews.

Preface (First Edition)

More than 10 years ago when we began to teach our graduate-level Basic Bone Biology course at Indiana University (IU), there were several excellent reference works available, primarily targeted to researchers working in a wide range of areas in skeletal biology. These included (and still include) *Principles of Bone Biology*, edited by John Bilezikian, Lawrence Raisz, and John Martin, which has since been expanded to two volumes; "Big Red" (*Osteoporosis*), the excellent and very complete reference edited by Bob Marcus, David Feldman, and Jennifer Kelsey; and the more succinct *Primer of Metabolic Bone Diseases*, updated and republished every few years by the American Society of Bone and Mineral Research. These are still available, and still excellent, but they do not serve well as textbooks for a bone biology course either because they are too extensive, too expensive, or do not cover relevant topics in sufficient depth. Therefore, we have chosen over the years to use primary reference materials—mostly, recent papers published in the peer-reviewed literature—for our course. From a didactic standpoint, this is an acceptable approach, and even a desirable one, especially for a graduate course in which the goal is to teach the student how to read and evaluate the literature. However, it became clear over time that this was not a sufficient surrogate for a true textbook.

As the skeletal biology group at IU grew over the years, we incorporated topic experts to deliver lectures in their area of expertise. We soon realized that the course and content experts provided the foundation for building a textbook on basic and applied skeletal biology. As we discussed this idea with our colleagues here at IU and up the road at Purdue University, there was universal support and enthusiasm. Discussion with colleagues outside of our group made it clear that, beyond our own requirements, there was a need and a desire by the academic community for such a text. Writing this textbook began as something of a selfish idea—we needed it for our course—but we truly hope that it will be welcomed and used by others who find it appropriate for their own courses, or as a more modest reference than existing books on a wide range of topics in skeletal biology.

Basic and Applied Bone Biology covers those topics that we feel are relevant to a modern course in skeletal biology. The book is organized, like bone, in a somewhat hierarchical manner. The first section begins with the basic construction of bone, including its cellular structure and dynamics and the basic physiological processes that bone uses to grow and adapt itself over a lifetime. This is succeeded by several chapters related to the technical aspects used to assess bone in health and disease—various imaging modalities; biomechanical measurements useful for assessing bone properties; histomorphometric techniques to evaluate the dynamics of bone modeling and remodeling; and genetic approaches used to tease out the roles of specific genes, proteins, and epigenetic influences in the basic metabolic functions of bone. These early chapters provide the foundation for the next several chapters on skeletal adaptation, highlighting mechanically induced adaption of bone, fracture healing, and adaptation of the oral cavity associated with orthodontics and implants. Following this, the text transitions (gradually we hope) into areas that are more clinically related, the applied aspects referred to in the title. These chapters address growth and development, metabolic and hormonal processes, and how these are related to health and disease. The text ends with a chapter on pharmaceutical treatments for osteoporosis, which we hope incorporates both the clinical elements of treatment and the biological reasons for, and effects of, these treatments.

Skeletal biology is, by its nature, interdisciplinary. The course that we teach at IU typically includes students in the basic medical sciences, general biology, the dental sciences, several engineering subspecialties, foods and nutrition, kinesiology, and rehabilitation sciences. We have written this textbook to cover a range of topics that we feel would be relevant to these groups and have attempted to write various chapters in a way that will be understandable to those students whose particular expertise and interest may not be in the area covered by a given chapter. We have also attempted to write the chapters so that they will be suitable for students at various levels of study, including undergraduate, graduate, and even postgraduate. We realize that the danger of this is that some chapters may be too superficial for students who are more expert in the area covered by that chapter. However, the textbook is meant to be supplemented by additional readings that delve into specific topics in greater depth for those who wish to specialize in that area. To this end, we have included a list of 10–15 suggested readings at the end of each chapter that can serve as a starting point for supplementary reading

and discussion. Further, we have incorporated study questions at the end of each chapter. We have resisted the temptation to include answers to these questions. They are intended to be used for discussion (although they could also be used for testing), and there may not be a single "correct" answer. We hope that they will permit further exploration of the chapter topic, at the level appropriate for the student.

Finally, we have not only had a lot of fun putting this text together but have also learned a lot in areas that are not within our own expertise. We sincerely hope that it serves the same purpose for you.

David B. Burr, PhD
Matthew R. Allen, PhD

February 16, 2013

Preface (Second Edition)

Our understanding of the musculoskeletal system continues to expand. Since conceptualizing the first edition of *Basic and Applied Bone Biology* several years ago, there has been a rapid growth in knowledge about how bone interacts with other organ systems. When presented with the opportunity to revise this textbook in 2017, we saw an opportunity to tap into the comprehensive expertise of the expanding collection of investigators in the Indiana Center for Musculoskeletal Health at Indiana University School of Medicine, as well as that of our close colleagues from other institutions, to cover these emerging areas in musculoskeletal biology.

This second edition of *Basic and Applied Bone Biology* takes the same fundamental approach to organization as did the previous edition, moving from basic cell biology to clinically focused topics. In addition to updates in all chapters and some reorganization of content between chapters, this new edition is highlighted by the addition of seven new chapters. In the opening section, *Bone biology and physiology,* we have added a chapter on bone marrow and the stem cell niche to overview the complex interplay of cells that are generated and housed within bone. An entire new section, *The Interaction of bone with other organ systems*, covers bones' connection to muscle, the immune system, the nervous system, the microbiome, and the kidney. Finally, the *Skeletal disease and treatment* section has been expanded to cover topics of skeletal interplay with cancer and diabetes.

Our guiding principle for this revision, as with the original edition, was to provide a resource for the next generation musculoskeletal researcher. Whether you engage with the text as part of a structured course or less formally on your own, we hope the text gives you a framework of musculoskeletal biology and provides you with a foundation on which to make the next innovative leap in the field.

David B. Burr, PhD
Matthew R. Allen, PhD

July 10, 2018

PART I

BASIC BONE BIOLOGY AND PHYSIOLOGY

CHAPTER

1

Bone Morphology and Organization

David B. Burr[1,2]

[1]Department of Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, IN, United States; [2]Department of Biomedical Engineering, Indiana University-Purdue University-Indianapolis, Indianapolis, IN, United States

THE FUNCTIONS OF BONE

Bone is multifunctional, playing roles in mechanical support and protection, mineral homeostasis, and hematopoiesis. In recent years, it has become clear that bone also serves an important endocrine function.

The mechanical functions of bone are by far the most widely recognized and studied. Both trabecular and cortical bones serve this function, although the nature of this function is partly specific to each. The dense cortical bone comprises most of the bone mass and takes on most of the role for load bearing. Although the more porous cancellous bone also supports load, one of its important functions is to redirect stresses to the stronger cortical shell. The mechanical function of bone extends beyond simple load bearing, which requires a certain degree of strength and stiffness. Because of its organization as a multiscale material, it is also highly adapted to avoid fractures caused by repetitive loading at physiologic levels, i.e., failure in fatigue.

Bone also serves a protective function, especially in those vital areas such as the torso and head where injury can be fatal. In these locations, the bone microstructure is not different from that of bone in other locations, but it is organized in a manner that can absorb maximum energy with minimum trauma to the bone itself. For instance, the cranial vault is constructed of two thin plates of dense bone that sandwich porous cancellous bone (the porous appearance of this bone is why it is sometimes called *spongy bone*). Ribs are also constructed in this way, but with less dense cancellous bone. In the case of the ribs, the inherent curvature of the bone also increases its ability to absorb impact energy. Developmentally, bones that serve a protective function (e.g., calvarium and ribs) are formed, at least in part, through intramembranous ossification rather than through endochondral ossification (see Chapter 5) and are part of the axial skeleton.

It is not widely realized that bone is a blood-forming (hematopoietic) organ, but regions composed largely of *spongy* bone such as the iliac crest, vertebrae, and proximal femur are good sources of red blood cells throughout life. The marrow cavity within the bone is an important site of red marrow, indicative of hematopoiesis, during growth and development, but it is largely composed of yellow fat in adults. White fat and brown fat are also found in the human body, and while these are acted on by osteocalcin, which is produced by osteoblasts, these types of fat are not actually found within the bone marrow itself. White fat stores energy and secretes adipokines, and too much of it is associated with diabetes and other metabolic conditions that affect bone. Brown fat, on the other hand, burns lipid droplets and creates heat. Brown fat cells have large amounts of energy-producing mitochondria, which store iron. Young children have large amounts of brown fat, especially around the upper spine and across the shoulders. This keeps them warm, but it also provide a large iron reservoir that is important for their rapid metabolism and skeletal development. It is now known that brown fat is present in adults as well, but the amount of it declines with aging and may decline more in those who are prone to obesity. Yellow marrow fat originates from the same precursor cells that differentiate to become bone-forming osteoblasts. It provides an energy store and may contribute to lipid metabolism by regulating triglycerides. Because of the large surface area, regions with a lot of cancellous bone are also responsible for rapid turnover of bone tissue and play an important role in the long-term control of calcium balance.

Bone turnover can be sensitive to changes in energy metabolism that occur as a function of aging, hormone deficiency, or the production of skeletal hormones, and this provides the means for long-term exchange of calcium

Basic and Applied Bone Biology, Second Edition
https://doi.org/10.1016/B978-0-12-813259-3.00001-4

and phosphate (as well as other minerals such as iron and magnesium). Although calcium provides bone with its stiffness and much of its strength, calcium ions in the mineral phase are also important for enzyme reactions, blood clotting, muscle contraction, and the transmission of nerve impulses. Both cortical and cancellous bones are sites of long-term storage and of the rapid exchange of ions within the mineral phase. The release of minerals through bone remodeling is a relatively lengthy process requiring timescales of days to weeks, and replacing these minerals completely takes even longer. However, the extensive surface area represented by the many osteocyte lacunae and canalicular channels provides a significant opportunity for short-term exchange to meet immediate demands. By way of example, the surface area of osteocyte canaliculi is estimated to be about 120 times greater than the surface area of all the cancellous bone in the body. The exchange of mineral that occurs at these surfaces is the source of the halo that can sometimes be observed around osteocyte lacunae, and which can appear either hypermineralized or hypomineralized depending on the content of labile calcium at this surface.

Bone has been identified as an endocrine organ that helps to mediate phosphate metabolism and energy metabolism by secreting two hormones: fibroblast growth factor 23 (FGF23) and osteocalcin. Most of the body's FGF23 is produced by osteocytes, the most abundant cell type in bone. It causes a reduction in renal reabsorption of phosphate and a decrease in serum levels of 1,25-dihydroxyvitamin D_3. Working with other hormones, bone helps to coordinate processes in the kidney and intestine that regulate its own mineralization. There is now also some evidence that the undercarboxylated form of osteocalcin, which is released from the bone matrix during resorption, helps to regulate pancreatic beta-cell proliferation and enhance insulin secretion. It also acts on adipocytes outside of bone to produce adiponectin, which can reduce insulin resistance. This has the dual effect of increasing glucose utilization and reducing fat in the body cavities. Regulation of the body's energy stores by bone may also be mediated by leptin, acting through the autonomic nervous system and hypothalamus. Thus bone, through its several hormones, helps to coordinate processes in the bone marrow, brain, kidney, and pancreas that affect skeletal tissue mineralization, fat deposition, and glucose metabolism.

BONE IS ORGANIZED AS A MULTISCALE MATERIAL

To achieve these functional goals, bone is organized in a hierarchical, fractal-like manner, from nanometer- to millimeter-sized structures (Fig. 1.1). This contributes to an unusual combination of both high stiffness and

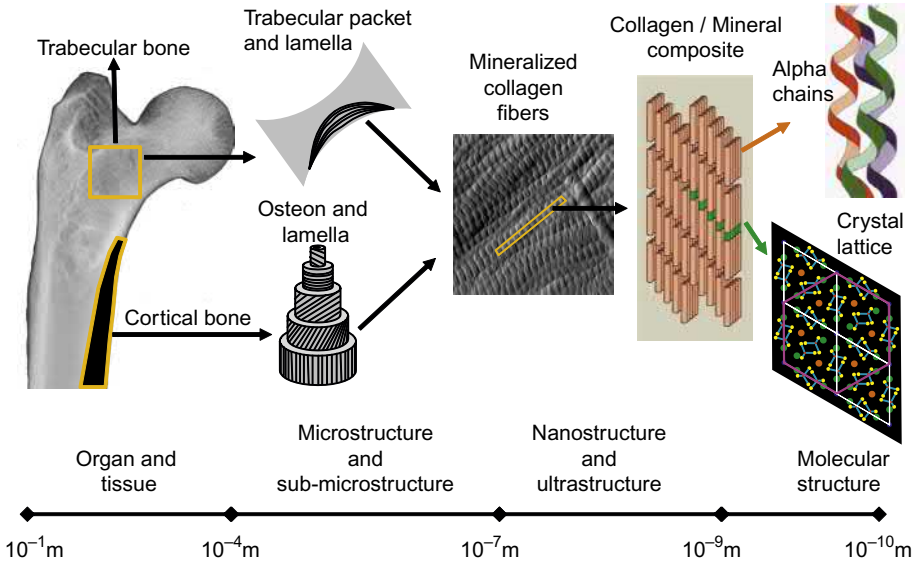


FIGURE 1.1 The hierarchical organization of bone. At the macroscopic level, bone is seen as a composite, with dense cortical bone forming an outside shell and cancellous (spongy, trabecular) bone within the marrow cavity. The cancellous bone serves to attenuate loads and to direct forces to the stronger cortical bone. At the microscopic level, cortical bone is composed of many secondary haversian systems, or osteons, that are the product of bone resorption and replacement with human new bone. These osteons are composed of a central canal carrying a blood vessel, nerves, and lymphatics surrounded by layers of concentric lamellae. Trabecular bone is also lamellar, but its structure comprises a combination of lamellae that run approximately parallel to the trabecular surface and the remnants of older remodeled bone that can appear osteonal in some cases. At the ultra- and nanostructural levels, bone is a composite of collagen fibers with plates of mineral interspersed within the collagen fibrils (intrafibrillar) and between the collagen fibers themselves (interfibrillar). Together, these can form a cross-fibrillar phase in which the crystals can expand beyond the dimensions of a single collagen fibril. The collagen fibrils are composed of molecules forming a triple helix composed of two α_1 chains and a single α_2 chain. *Part of figure courtesy of Beck, et al.* J. Struct. Biol. *1998;122:17–29.*

great toughness (these are often inversely related, see Chapter 7), as well as to its mechanical role in support and movement of the body. At the nanostructural level, bone is composed of organic and mineral components, mainly consisting of a matrix of cross-linked type I collagen mineralized with nanocrystalline carbonated apatite. The collagen and mineral combine to form a composite material, with mineral providing stiffness to the structure and collagen providing resilience and ductility. This composite not only protects the brittle mineral (hydroxyapatite) from breaking but also doubles its load carrying capacity while distributing the forces around discontinuities in bone and reducing stress in the bone matrix. At a microscopic (micrometer [10^{-6} m]) level, the individual collagen fibers with interspersed mineral are organized in different ways, depending specifically on the rate, location, and substrate (if any) on which it is formed. At this microstructural level, the organization of bone tissue is very much related to its functional needs: rapid formation for stabilization (in fracture healing) or rapid growth during development; or slower formation to adapt to changing mechanical needs or to replace preexisting bone to provide repair of damaged regions and maintain their unique mechanical properties. At this level, bone can be denser (cortical or compact bone) or rather porous (cancellous, trabecular, or spongy bone), depending on the specific mechanical or biological needs and its location.

BONE COMPOSITION

Approximately 65% of bone by weight is composed of mineral (primarily carbonated apatite), but as a living tissue, its organic component, mostly type I collagen, contributes about 20%–25% to its composition (Fig. 1.2).

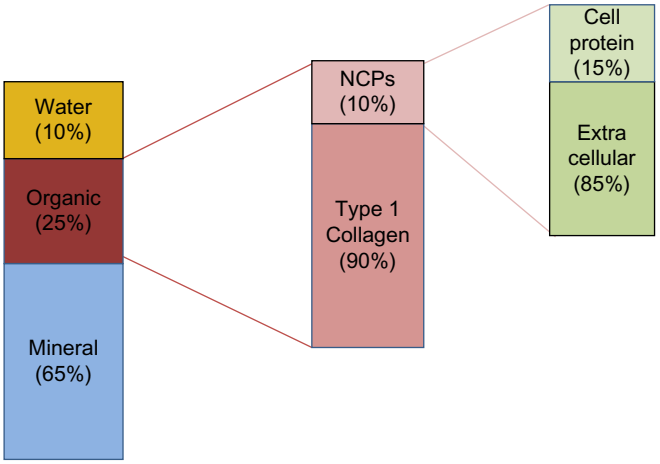


FIGURE 1.2 Bone is composed of an organic matrix, mineral, and water. Most of the organic matrix is type I collagen, but noncollagenous proteins (NCPs) that contribute to mineralization and adhesion are also present. Most of this is extracellular, although a small amount of protein is also found within the cells.

The remainder (approximately 10%) is composed of water that is bound to the collagen–mineral composite and unbound water that is free to flow through canalicular and vascular channels in bone. Unbound water can be redistributed as the bone undergoes loading and probably contributes to the signals detected by cells, informing them of loading conditions (see Chapter 11). Water is exchanged on a nearly 1:1 basis with mineral, so as bone becomes more mineralized, water content declines, and vice versa. This is important to its mechanical behavior; more highly mineralized bone is stiffer because it has more mineral, and also because it has less water. In addition, although it is stiffer, drier bone tends to be more brittle, and therefore break more easily.

About 90% of the organic portion of bone is type I collagen, with smaller amounts of types III and V collagen also found in the zone surrounding the bone cells. The remaining 10% is made up of noncollagenous proteins (NCPs) which play a vital role in regulating collagen formation and fibril size, mineralization, cell attachment, and microcrack resistance. Of this small amount of NCPs, about 85% is extracellular and the remainder is found within bone cells.

THE NANOSCALE ORGANIZATION OF BONE

Collagen

At a fundamental level, bone is composed of collagen fibers interspersed with plates of mineral both within and between the fibrils. Individual collagen molecules are formed from two α1 chains and a single α2 chain that assemble into a triple helix (Fig. 1.3A). Each chain is about 1000 amino acids in length, and the helical center portion of collagen molecules comprise repeating units of a Gly-X-Y triplet. The periodic repetition of glycine residues is essential to the formation of the triple helix structure. While almost all amino acids are present in collagen, X and Y groups are often occupied by proline and hydroxyproline residues. Both these amino acids form a ring with the main backbone of the chain, resulting in improved helical rigidity (Box 1.1).

Hydroxyproline is particularly critical, and unique, to collagen as its hydroxyl group is essential for hydrogen bonding with water molecules. This interaction is so critical because the stability of the triple helix is maintained by a sheath of water molecules attracted by hydroxyproline. During the intracellular production stage, the nonhelical registration peptides (N-propeptide and C-propeptide) at both ends of the molecule secure the chains together by sulfur cross-links. The triple helix with its terminal propeptides is known as the procollagen molecule. Following the exocytosis of molecules, these propeptide

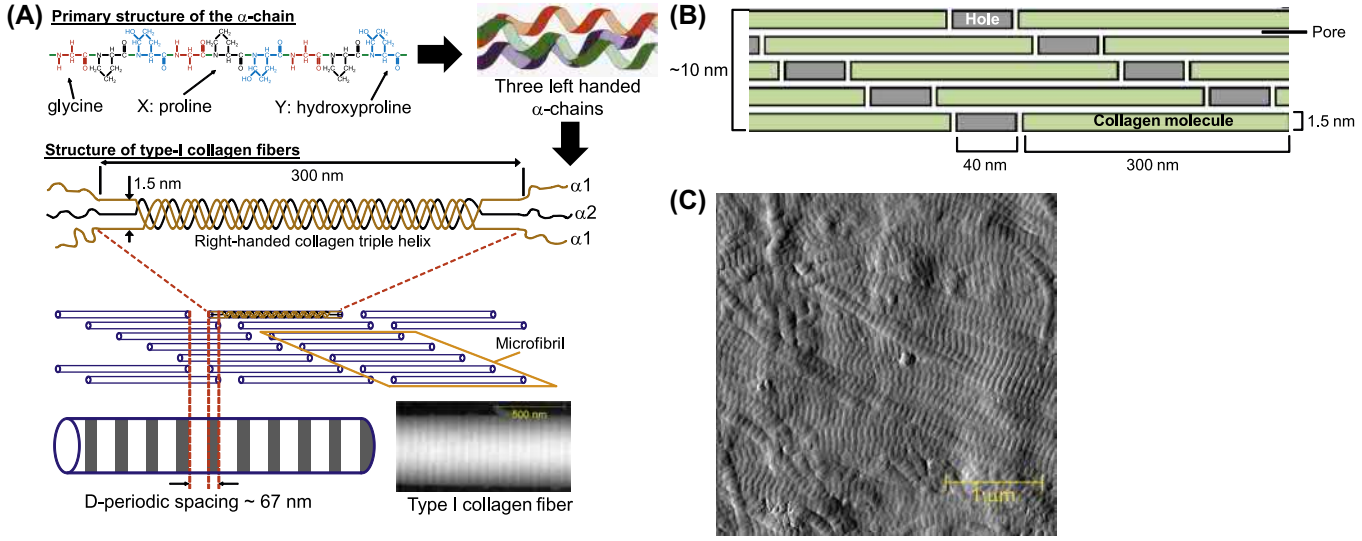


FIGURE 1.3 Collagen fibrils are constructed from many collagen molecules. (A and B) Each collagen molecule is approximately 300 nm long and 1.5 nm thick. The molecules are stacked in a quarter-staggered array such that there are 67 nm hole zones between the ends of the molecules and spaces between the laterally contiguous molecules known as pores. (C) The hole and overlap zones give the collagen fibril its characteristic banded appearance when viewed with atomic force microscopy. Both holes and pores enable the deposition of mineral during primary and secondary mineralization processes. The diameter of the entire collagen fiber can vary; its thickness is regulated by the action of noncollagenous proteins such as decorin. *Part of figure courtesy of Beck et al. 1998.* J. Struct. Biol. *122:17–29.*

BOX 1.1

OSTEOGENESIS IMPERFECTA

Osteogenesis imperfecta (OI) is a set of heritable conditions of variable severity usually caused by mutations in the collagen I genes, but in some types, other genes such as CRTAP or P3H1 are involved. More than 200 mutations have been described affecting various aspects of collagen formation and cross-linking. In severe cases, an increase in the hydroxylation of lysine leads to a more gradual formation of the collagen triple helix. OI leads to skeletal fragility, and it is sometimes termed "brittle bone disease" because of the multiple fractures (sometimes prior to birth) that can occur. Eight known forms of OI have been described, with type I, the most mild, and type II, the most severe. The incidence of the condition is about 5–7 out of 100,000 people, with two-thirds of these cases afflicted with the milder type I and type IV forms. OI is sometimes characterized as an osteoporosis because it is associated with low bone mass, thin cortices, and architectural deterioration of trabeculae, but the changes to the bone matrix caused by the collagen mutations exacerbates the fragility that accompanies the low bone mass and architectural deficits. Because it is a collagen mutation, OI also affects nonmineralized tissues that harbor the COL1A1 or COL1A2 genes (such as the eyes and skin) and is associated with short stature, hearing loss, respiratory problems, and a disorder of tooth development.

There are several mouse models of OI, each with a slightly different genotype and phenotype. The most common of these are the oim/oim and Brittle IV (Brtl) mice. The oim mouse is characterized by a mutation in the COL1A2 gene associated with α1(I) homotrimers and nonfunctional proα2(I) chains. This leads to reduced collagen content and fiber diameter, and fewer divalent collagen cross-links, either because of the reduced collagen content or because of a more rapid transformation to more mature trivalent cross-links. The Brtl IV mouse is a model of type IV OI characterized by a substitution of a cysteine for a glycine in the triple helix of one of the Col1α1 alleles. Like the oim mouse, it is associated with reduced collagen fiber diameter, but unlike the oim, it also affects mineral crystalline organization and is not associated with alteration in collagen cross-linking. Both oim and Brtl animals have significantly lower mechanical properties, even after accounting for bone mass, illustrating the role collagen plays in mechanical integrity. Because of the heterogeneity of causes for OI, any animal model is not likely to recapitulate the human condition entirely. Choice of an appropriate animal model should also consider what type of OI is under study.

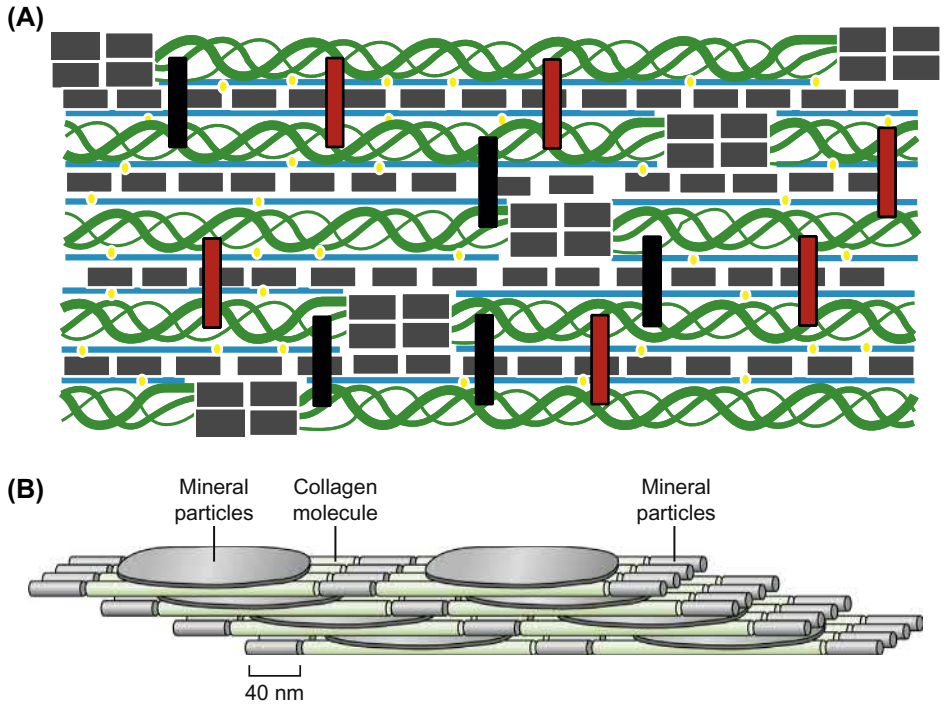


FIGURE 1.4 (A) Collagen molecules (*green helix*) are cross-linked within the fibril by bonds that are formed through enzymatic processes, and by those formed without the need for an enzymatic reaction. Enzymatically formed cross-links (*black bars*), such as pyridinoline or deoxypyridinoline, form near the ends of the molecules, the C- and N-termini. Nonenzymatically formed bonds (*red bars*), such as pentosidine, are randomly located between the molecules. Mineral (*gray blocks*) is deposited in the hole and pore zones between the collagen fibrils. Water (*blue lines*) and hydrogen bonds contribute to the bonding of mineral and collagen within the fibril. (B) Hole and pore zones between the molecules contain plates of bone mineral (hydroxyapatite). Water is bound to collagen in these spaces, and this alters the distribution of load sharing between the collagen and bone mineral deposited in this location.

regions are cleaved enzymatically, leaving nonhelical domains at both ends of the molecule, termed the *N-* or *C-telopeptides* (at the N-terminus or C-terminus, respectively). Cleavage of the registration peptides forms the mature collagen molecule, composed of the helical triple helix region and the nonhelical terminal N- and C-telopeptides.

Lateral and longitudinal aggregation of collagen molecules is essential to extend from the nanoscale to the microscale. In this assembly, five molecules form a microfibril in a semihexagonal arrangement. Microfibrils aggregate laterally and longitudinally to make up fibers that are eventually about 150 nm in diameter and 10 μm in length. Electron microscopy images of collagen fibers reveal an approximately 67 nm banding pattern, called the D-banding pattern, which represents the space between the ends of contiguous collagen molecules, and the overlap between the end regions of laterally contiguous molecules (Fig. 1.3B). The mean diameter of collagen fibrils and their spacing is less in osteoporotic bone than in healthy bone, which may increase the bone's fragility.

Collagen fibrils are connected by different kinds of cross-links that can have profound effects on the material properties of the tissue and ultimately on the mechanical behavior of the whole bone (Fig. 1.4). These can be broadly grouped into those formed through enzymatic processes and those formed through processes of nonenzymatic glycation, which create advanced glycation end products (AGEs).

Enzymatically Mediated Collagen Cross-Linking

Pyridinoline and deoxypyridinoline are two mature cross-links of collagen that are derived from an enzymatic pathway initiated by the enzyme lysyl oxidase. Pyridinoline is the maturation product of two hydroxylysyl (Hyl) residues from the telopeptide with a Hyl from the α-helix, whereas the deoxypyridinoline analog contains a lysine residue from the α-helix. These trivalent cross-links are very stable. The content of these *mature* cross-links in human bone collagen increases sharply up to the age of about 10–15 years and thereafter remains constant or possibly declines slightly, although the number of pyridinoline and deoxypyridinoline cross-links can change with treatments that alter bone turnover. An increased pyridinoline:deoxypyridinoline ratio has been related to increased compressive strength and stiffness in bone but probably has no effect on toughness or ductility.

Nonenzymatically Mediated Collagen Cross-Linking

There are several cross-links of collagen that result from the nonenzymatic condensation of arginine, lysine, and ribose. Pentosidine, a fluorescent AGE, constitutes the smallest fraction of these nonenzymatically glycated cross-links, but it is often used as a marker for their total content because it is one of the only AGEs that can be accurately quantified. Other AGEs include Nε-carboxymethyllysine (CML), furosine, imidazolone, and vesperlysine. Recently, CML, which is nonfluorescent, has been shown to be present in bones at levels 40–100 times greater than pentosidine. As AGE formation occurs over a period of years, proteins with long half-lives, such as collagen, can accumulate substantial amounts of AGEs with age; for example, pentosidine accumulation triples over the last three decades of life; CML accumulation may increase by 5–10 fold over the same period. In addition, AGEs have been shown to reduce collagen fibril diameter. Because they can be formed in the presence of sugars (such as glucose or ribose), they are accumulated by individuals with diabetes mellitus (Chapter 23); this is one contributor to the increased bone fragility found in these individuals.

Accumulation of AGEs in the extracellular matrix of bone also regulates the proliferation and differentiation of bone-forming cells, the osteoblasts, through interaction with the AGE-specific receptor (RAGE). Binding to RAGE activates NF-κB in osteoblasts and stimulates the production of cytokines. The AGE–RAGE binding interaction also upregulates the production of reactive oxygen species, which elevates inflammation in the bone microenvironment and leads to bone loss. Accumulation of AGEs in collagen can impair osteoblast proliferation and differentiation, reduce osteocalcin secretion, and cause disruptions in cell–matrix interaction and cell adhesion that ultimately affect bone formation.

AGEs also may regulate both osteoclastogenesis and osteoclast activity. Osteoclastic resorption is slowed in the presence of AGEs, in part, perhaps, because the solubility of collagen is reduced. These pathways—AGE regulation of osteoclast differentiation and activity, as well as effects on matrix solubility—may contribute to normal or even elevated bone mass that is found in people with type 2 diabetes who have high concentrations of AGEs in their bone. However, the presence of AGEs makes the bone material (tissue) brittle and thus more susceptible to fracture even though there is more bone mass.

Collagen Orientation

Historically, collagen in bone has been reported to be regularly organized, with collagen fiber bundles arranged parallel to each other in adjacent sheets (lamellae), either perpendicular to each other or arranged alternately in adjacent lamellae. This stemmed from the different microscopic appearance of bone under polarized light (Fig. 1.5). It is likely that this is partly a function of the optics and plane of section, rather than the way that collagen bundles are arranged. Under cross-polarized light, collagen fiber bundles that are oriented transverse to the plane of viewing appear light, or birefringent, whereas those that are oriented parallel to the plane (i.e., longitudinally) are dark. This is because transversely oriented fiber bundles rotate the plane of polarized light with respect to the viewing plane, whereas those that run longitudinally do not. Alternately arranged, or intermediate, collagen fiber bundles are interpreted as representing a combination of fiber arrangements in successive lamellae. In reality, there are many variations on this theme, and it is likely that collagen fiber bundles even within a lamella are arranged in many different orientations, with the predominant orientation being responsible for what is observed either microscopically or by X-ray diffraction.

The collagen in these layers has been shown to be preferentially oriented with respect to the predominant stress in the bone. Longitudinally oriented fibers are predominantly found in portions of bone that are under tension (i.e., being pulled further apart), whereas transversely oriented fibers are more abundant in regions that are usually under compression (i.e., being pushed closer together). This has been shown by mapping the numbers of light or dark osteons across sections of bone in which the primary loading directions are known. This has also been shown experimentally, by altering the direction of loading and observing the collagen fiber direction in the newly formed bone. Both approaches suggest that there is a relationship between collagen fiber orientation and the predominant direction of loading.

For reasons to do with optics and light transmission, bone collagen may only appear in polarized light to be oriented preferentially in these directions. In reality, bone collagen may be organized in a *twisted plywood* configuration that is continuously rotated through 180 degrees cycles (Fig. 1.5B). In this scheme, the collagen fibers gradually, rather than abruptly, change orientation from one successive lamella to another. Under polarized light, this would make the bone appear lamellar, with differing light and dark areas. It is as if one took a piece of plywood in which the fibers in successive plies were perpendicular to each other, twisted it, and then examined the orientation of the individual plies *end on*. The fibers would then appear as arches, rather than as discrete and oriented fibers. Because the fiber orientation repeats in this model, the tissue-level structure appears lamellar, thus giving bone its characteristic laminar appearance under the microscope.

Whether the collagen is twisted or not, how it becomes oriented in the directions it does is something of a mystery. It has been suggested that the orientation of

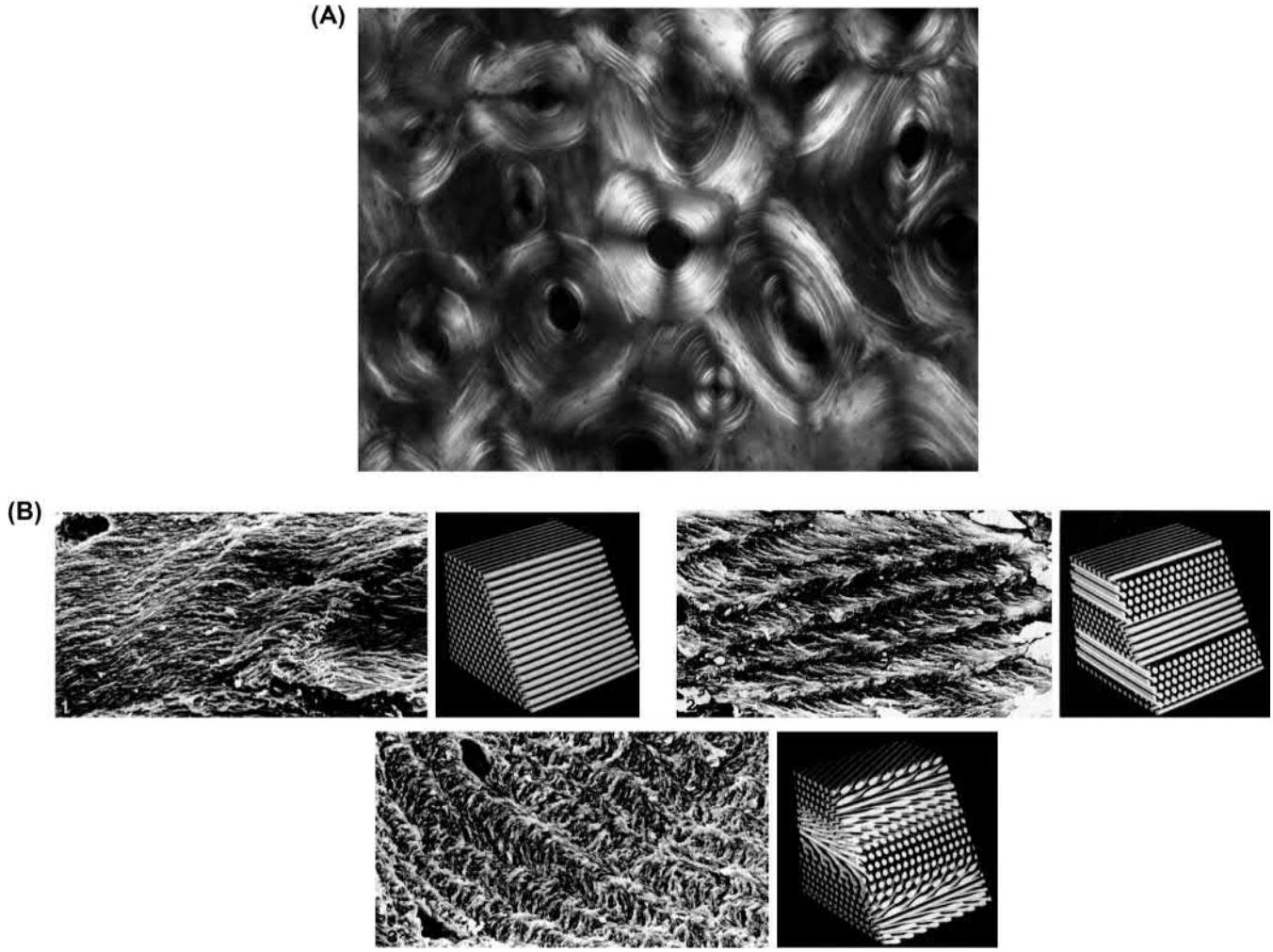


FIGURE 1.5 (A) Variations in collagen orientation can be viewed using polarized light. Fibers that are oriented transversely to the direction of light appear bright, whereas those oriented along the path of the light are dark. In a cross section of cortical bone, dark osteons are composed of longitudinally oriented fibers. Some osteons on the right side of this image would also be characterized as alternately fibered. (B) Electron microscopy images of parallel and alternately fibered collagen bundles in bone are shown along with a schematic representation (top two images). Collagen in bone may in reality be organized in a helicoidal arrangement in which collagen orientation changes only slightly from lamella to lamella. The bottom image shows this effect in three dimensions. *Panel (B) reproduced with permission from Ruggeri et al. In: Motta PM, editor. Recent advances in microscopy of cells, tissue, and organs. Rome: Antonio Delfino Editore, 1997.*

the osteoblast depositing it determines the collagen orientation and that the mineral is simply deposited in the spaces between collagen fibrils. An alternative suggestion is that collagen is deposited without any preferred orientation and that the deposition of the mineral, which is charged, causes both the collagen and the mineral to become oriented in directions that are dependent on the mechanical environment. Which of these ideas is correct is still under debate.

Bone Mineral

Bone mineral is composed of highly substituted, poorly crystalline carbonated apatite mineral, which nucleates within the gap regions between the ends of the collagen fibrils, also known as hole zones, as well as along the pores that run longitudinally between the fibrils (Fig. 1.4). Mineral is initially deposited as an amorphous calcium phosphate, along with large amounts of calcium carbonate. As bone tissue matures, the carbonate content is reduced, and mineral crystals grow laterally, becoming more plate-like, and orient themselves parallel to one another and to the collagen fibrils. The long axis of the mineral plate, or c-axis, aligns with the longitudinal axis of the bone. The average size of the mineral crystals in bone tends to span a wide range, but the majority (98%) has a thickness less than 10 nm. Eventually, the plates coalesce with other crystals to become larger polycrystalline aggregates that can become greater than the width of the collagen fibrils. As bone ages, the mineral crystals also can enlarge due to ion substitutions and changes in mineral stoichiometry. Therefore, the measured average size of mineral crystals is highly dependent on tissue age. However, it can be difficult to distinguish between smaller crystals with many

imperfections and larger crystals with a few imperfections; both may demonstrate similar crystalline properties.

More soluble carbonate can exist in a labile form on the surface of the crystallites, but it can also substitute for the phosphate and hydroxyl groups in carbonated apatite. These substitutions within the mineral allow it to be more easily resorbed. Such substitutions distort the shape and size of the crystals and reduce the stability of the mineral lattice. During episodes of acid load, systemic bicarbonate (HCO_3^-) is consumed to buffer the blood pH. The HCO_3^- deficiency is offset by carbonate and phosphate ions present in bone's mineral reservoir. Thus, in chronic acidosis, the bone's mineral reservoir not only helps to maintain acid–base homeostasis but also results in bone loss. In addition, different cations (e.g., magnesium, sodium, and strontium) can substitute for the calcium ions, and fluoride can substitute for the hydroxyl group in the apatite lattice; in some cases, the mechanical properties of bone are altered and in others the activity of osteoblasts and osteoclasts is affected. At one time, sodium fluoride (NaF) was considered a promising anabolic therapy for osteoporosis. There is evidence that NaF stimulates osteoprogenitor cells and preosteoblasts, promoting direct bone formation without the need for prior resorption. Moreover, fluoroapatite (the mineral with fluoride substituted for the hydroxyl groups) is more resistant to resorption than is the carbonated apatite. However, the substitution of fluoride into the mineral crystal increases the brittleness of bone and may therefore hasten rather than delay fracture. Although this may or may not occur with other ion substitutions in the mineral crystal, it is instructive in showing that hydroxyapatite is finely adapted to the bone's specific mechanical needs.

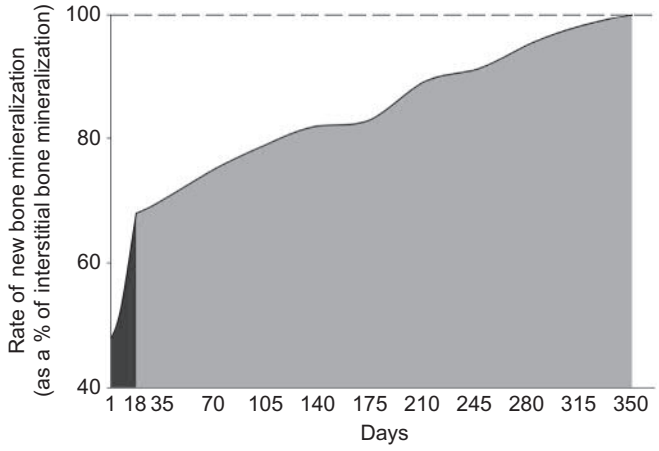


FIGURE 1.6 Primary mineralization of bone occurs within the first 3 weeks after the osteoid is deposited (black colored region). Secondary mineralization occurs in part through a slower growth and maturation of the crystals and can require a year or more to complete (gray colored region).

There are two sequential and continuous phases to the deposition of mineral in bone: an initial and rapid increase in the number of mineral crystals due to heterogeneous nucleation (primary mineralization) and a slower growth and maturation of those crystals to an eventual size of about 40 nm × 3 nm × 7.5 nm. During primary mineralization, mineral is rapidly deposited within the collagen framework, achieving 65%–70% of its total mineralization within about 3 weeks after the initial deposition of collagen. During the secondary phase of mineralization, the bone matrix continues to accumulate mineral at a slower, more progressive rate until the amount of mineral reaches a physiologic limit (Fig. 1.6). Estimates for the completion of secondary mineralization range from a few months to many years.

Noncollagenous Extracellular Matrix Proteins

There are numerous NCPs in bone that regulate and direct the construction and maintenance of the extracellular matrix. Although these proteins only account for about 2% of the bone by weight, they play vital roles in embryogenesis and development, regulate the formation and size of collagen fibrils, control mineralization, and provide conduits for cellular signaling and attachment. They can be divided into several large classes (Table 1.1), including

1. Proteoglycans (heparin sulfate, hyaluronan, small leucine-rich proteoglycans [SLRPs], and versican);
2. Glycoproteins (alkaline phosphatase [ALP], fibronectin, thrombospondin [TSP1 and 2], and vitronectin);
3. Proteins of the small integrin-binding ligand N-linked glycoprotein (SIBLING) family that are associated with bone mineralization (dentin matrix acidic phosphoprotein 1 [DMP-1], matrix extracellular phosphoglycoprotein [MEPE], osteopontin, sialoproteins);
4. Osteocalcin (or bone Gla protein); and
5. Osteonectin (also known as secreted protein acidic and rich in cysteine [SPARC]).

Proteoglycans and Glycosaminoglycans

Proteoglycans are a broad class of molecules defined by a core protein covalently bonded to a variable number of sulfated glycosaminoglycan side chains. Proteoglycans range widely in size, although those in bone tend to be in the smaller range. Proteoglycans in bone help to regulate mineralization by affecting apatite nucleation and growth. Hyaluronan and its receptor, CD44, work together to direct skeletal development. Hyaluronan is a long chain of nonsulfated glycosaminoglycan. It is found mostly in the periosteum and along the endocortical surfaces of bone, but it is also present around all major cell types, including

TABLE 1.1 Noncollagenous Proteins in Bone

PROTEOGLYCANS AND GLYCOSAMINOGLYCANS	
Heparan sulfate	Produced by osteoclasts and osteoblasts
	Plays important roles in cell–cell interactions
Hyaluronan	Nonsulfated glycosaminoglycan
	Hyaluronan in periosteum, endosteum, and around cells
	CD44 is the cell surface hyaluronan receptor and plays a role in development
Small leucine-rich proteoglycans	Provide structural organization in bone
Biglycan	Found in pericellular location undergoing morphological delineation Upregulated in osteoblasts and may act as shear sensors when found in osteocytes Binds collagen and TGF-β
Decorin	First appears in preosteoblasts and is downregulated in more terminal osteoblastic cells Binds to collagen and TGF-β and may regulate fibril diameter Inhibits cell attachment to fibronectin
Fibromodulin	Binds to distinct regions of collagen fibers Binds TGF-β
Osteoadherin	Contains RGD sequence Function unknown
Versican	CS-containing PG found in osteoid
	May capture space destined to become bone
GLYCOPROTEINS	
Alkaline phosphatase	Potential Ca^{2+} carrier
	Hydrolyzes inhibitors of mineral deposition such as pyrophosphates
	Loss of function leads to hypophosphatasia
	Bone formation marker
	Nonspecific and bone-specific forms (bone-specific alkaline phosphatase [BSAP])
Fibronectin	Produced during early stages of bone formation
	Binds cells in an RGD-independent manner
	May be involved in proliferation
Thrombospondin	Role in development—found in early stages of bone formation (MSCs and chondrocytes during cartilage development)
	Antiangiogenic
Vitronectin	Involved in cell attachment and spreading; shows specificity for osteopontin
SIBLING FAMILY OF GLYCOPROTEINS	
Bone sialoprotein	Limited pattern of expression
	Marks late stage of differentiation and early stage of mineralization
Dentin matrix acidic phosphoprotein 1 (DMP-1)	Expressed by osteocytes and osteoblasts
	Has affinity for hydroxyapatite and the N-terminus of type I collagen
	Regulates mineralization
Matrix extracellular phosphoglycoprotein	Expressed by osteocytes and osteoblasts
	Regulates mineralization
	Negative regulator of osteoblast activity
Osteopontin	Secreted by bone cells in early stages of osteogenesis
	Promotes adhesion of different tissues (cement line and periodontal ligament)
	Inhibits mineral formation and crystal growth

Continued

TABLE 1.1 Noncollagenous Proteins in Bone—cont'd

OTHER IMPORTANT NONCOLLAGENOUS PROTEINS	
Osteocalcin	Enhances calcium binding, controls mineral deposition
	Expressed by osteoblasts and osteocytes
	Bone remodeling marker
	Overexpressed in cancer and some autoimmune diseases
Osteonectin	Binds to collagen, HA, and vitronectin
	Located at sites of mineral deposition (possible nucleator)
	May play a role in osteoblast proliferation

Ca^{2+}, calcium ion; *CS*, chondroitin sulfate; *HA*, hyaluronic acid; *MSC*, mesenchymal stem cell; *OPG*, osteoprotegerin; *PG*, proteoglycan; *RGD*, Arg-Gly-Asp.

osteocytes, within the bone matrix. Versican is a chondroitin sulfate–containing proteoglycan that is important for cartilage formation and is therefore found in the developing skeleton. However, it is also found in osteoid in adult bone, where it may inhibit or regulate mineralization. Heparan sulfate is produced by osteoblasts and osteoclasts and plays a role in cell–cell communication. It binds FGF and can act as a coreceptor on this protein. It also binds and modulates the activities of transforming growth factor beta (TGF-β) and osteoprotegerin/tumor necrosis factor receptor superfamily member 11B (OPG), both important signaling molecules during the process of bone remodeling and repair.

SLRPs are small proteoglycan molecules that are involved in constructing the collagenous matrix, controlling the aggregation and size of collagen fibrils, and possibly assisting in collagen–mineral interactions. Perhaps the most important SLRPs are decorin and biglycan: both maintain osteoblast numbers, but they perform that function at different stages of osteoblast development. Decorin is expressed early during cell differentiation, by preosteoblasts, and is downregulated during terminal differentiation of osteoblasts. Biglycan, on the other hand, can induce apoptosis in osteoblast progenitor cells and is upregulated in mature osteoblasts. It is also found in osteocytes and in the pericellular regions of the matrix and may act as a sensor of shear stress in this location. Both decorin and biglycan function in a complementary manner to maintain osteoblast number. In addition, both bind to collagen and to TGF-β and can therefore modulate growth factor activity.

Glycoproteins

There are a number of glycoproteins in bone, and some of their functions are not completely understood. However, several are critical to the regulation of bone mineralization. ALP is used as a biomarker for bone formation because it hydrolyzes pyrophosphates, which inhibit mineral deposition by binding to mineral crystals. Neutralizing the pyrophosphates in bone allows normal crystal growth and leads to normal mineralization. ALP is produced by many different organs in addition to bone (e.g., kidney and liver). Therefore, alterations in ALP levels are not necessarily an accurate indicator of the activity of mineralization processes in bone. However, bone-specific alkaline phosphatase can also be measured and represents a widely used and beneficial marker of bone formation and mineralization. Low levels or loss of function of ALP results in a condition known as hypophosphatasia, which causes hypercalcemia and can lead to death in children. TSP1 and 2 are antiangiogenic NCPs that are important during the early stages of bone formation and are found in mesenchymal stem cells and chondrocytes during cartilage development. TSP2 is a promoter of the mineralization process and increases in osteoid undergoing mineralization. Fibronectin and vitronectin are two other glycoproteins that bind to cells. The former may be important in the early stages of bone formation and cell proliferation. In contrast, vitronectin regulates cell attachment and spreading. It is found in the osteoclast plasma membrane and may collaborate with osteopontin in attaching osteoclasts to the mineral matrix.

SIBLING Proteins

The SIBLING family of phosphoproteins includes bone sialoprotein (BSP), DMP-1, MEPE, and osteopontin. All of these phosphoproteins play a role in bone mineralization. Osteopontin is secreted by osteoblasts in the early stages of osteogenesis. It inhibits mineral formation and crystal growth, and it is found locally in regions of lower mineralization, such as the cement line in bone and the periodontal ligament surrounding the teeth. It may also act to provide a scaffold between tissues with different matrix composition and to provide cohesion between them. It has been suggested that this is the bone *glue* that provides fiber matrix bonding, as well as crack bridging in the case of microcrack formation. Osteopontin also binds to osteoclasts and promotes the adherence of the osteoclast to the mineral in bone during the resorption process. DMP-1 is expressed by osteocytes and osteoblasts. It has a high affinity for hydroxyapatite and the N-telopeptide region of type I collagen and functions to locally regulate the mineralization process.

It has been implicated in DNA binding, gene regulation, and integrin binding. The absence of DMP-1 causes elevated FGF23 and results in hypophosphatemic rickets. It is unknown whether DMP-1 plays a role in the differentiation of osteoblasts to osteocytes. MEPE is another protein of the SIBLING family that regulates bone mineralization locally. It is found predominantly in odontoblasts and osteocytes, where it is highly expressed during the mineralization process. MEPE is highly expressed in tissues undergoing rapid mineralization, for example in the woven bone of a fracture callus, as well as in endochondral and intramembranous ossification. Animal studies suggest it is a negative regulator of osteoblast activity; the absence of MEPE results in a high bone mass and resistance to bone loss.

Osteocalcin

Osteocalcin enhances calcium binding and controls mineral deposition. It is expressed by osteoblasts and osteocytes. For this reason, it is used as a marker of bone formation, although it may also function to regulate osteoclasts and their precursors. Mice in which osteocalcin is absent have severe osteopetrosis. Therefore, osteocalcin can be more accurately viewed as a marker of bone remodeling, and its level increases with the remodeling rate even in those cases, such as postmenopausal osteoporosis, in which there is a severe imbalance between formation and resorption.

Osteonectin

Osteonectin is located at sites of mineral deposition, where it binds to hydroxyapatite, collagen, and vitronectin, and may promote nucleation of new mineral crystals. It may also play a role in osteoblast proliferation and its absence results in osteopenia or low bone mass. It binds to several different growth factors (FGF2, platelet-derived growth factor [PDGF], and vascular endothelial growth factor [VEGF]) and may regulate their activity.

THE MICROSTRUCTURAL ORGANIZATION OF BONE

At the microstructural level, bone can be organized in a variety of different ways, determined by its function and the manner in which it is deposited. Most, but not all, bone is to some degree lamellar, meaning that collagen and mineral exist in discrete sheets that can be visualized under the microscope. The lamellae create circumferential bands of bone, each 3–7 μm thick, which give the appearance of tree rings, each separated by an interlamellar layer approximately 1 μm thick. The lamellae may be arranged around the endocortical (wall of the marrow cavity) or the periosteal (outer border of the bone) circumference of the bone (circumferential lamellae), within individual trabeculae, or concentrically around individual vascular channels (concentric lamellae; Fig. 1.7).

Woven Bone

Woven bone is rapidly formed and highly disorganized and is therefore not arranged in a lamellar pattern (Fig. 1.8). Its rapid formation is the result of a large cell:bone

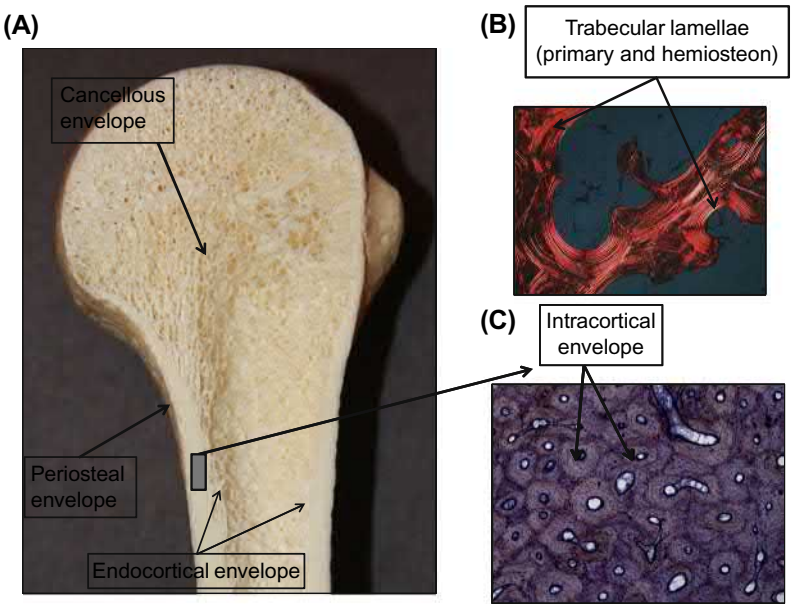


FIGURE 1.7 (A) Macroscopically, bone appears as either porous cancellous bone or denser cortical bone. This structure creates four different kinds of surfaces, called *envelopes*, on which bone cells can act. (B) Trabeculae in the cancellous bone compartment consist mostly of primary lamellae. However, remodeled areas (areas in which bone has been resorbed and reformed) can also form *hemiosteons*, similar to half osteons. (C) The intracortical envelope in humans is packed with secondary osteons, or haversian systems.

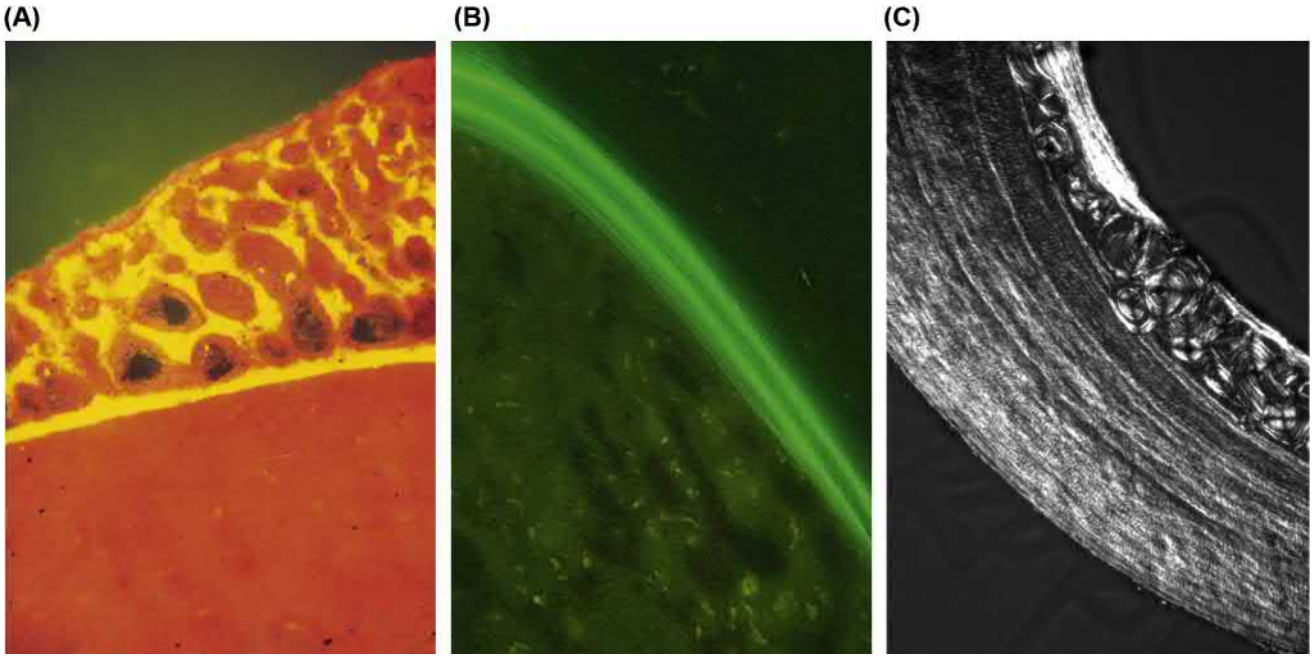


FIGURE 1.8 (A) Woven bone is laid down rapidly, and the collagen fibers within it lack any preferred organization. This is demonstrated by the diffuse tetracycline labeling (yellow) found among the pores within it. (B) Lamellar bone is deposited in sheets in a more organized fashion than woven bone. (C) The lamellar structure can be seen using polarized light microscopy.

volume ratio. It is usually, but not always, deposited de novo without any previous hard tissue or cartilage model (anlage). It is composed of small and randomly arranged type I collagen fibers that are rapidly mineralized, probably resulting in a tissue that is more highly mineralized than lamellar bone. Because it forms so quickly, it initially presents as a lattice structure, with large pores present within the mineralized structure. This is primarily a repair tissue, forming the callus that bridges the gap during fracture healing to provide stability for the bone during the healing process. It also occurs in response to inflammation, such as in osteomyelitis. However, woven bone is also formed in nonpathologic situations when mechanical loads are much higher than usual or are presented in a way to which the bone is not fully adapted and is found in the region of the growth plate during endochondral ossification during normal skeletal development.

Primary Bone

There are three types of primary bone that are differentiated by their microscopic organization: primary lamellar bone, plexiform (or laminar) bone, and primary osteons. They are morphologically distinct and impart different mechanical and physiologic properties to satisfy their different functions. They are united by the commonality that they must be deposited directly onto a substrate of either bone or cartilage (or calcified cartilage), without resorption of preexisting bone.

Primary Lamellar Bone

Primary lamellar bone (Fig. 1.8) is the principal type of bone formed on the periosteal surface. It is characterized by a series of parallel laminar sheets. It can become quite dense and has few vascular canals. Therefore, it is very strong and provides a primarily mechanical function. However, it is also deposited on the surfaces of the marrow cavity and on trabeculae within the marrow, where it can be quite labile. It may turn over rapidly and be replaced and may therefore serve to support calcium metabolism.

Plexiform Bone

Plexiform bone (Fig. 1.9) sometimes called fibrolamellar bone, is generally not found in humans (although it has been reported to occur around the time of the major growth spurts) but is found in many animals, especially those that grow rapidly (e.g., cows and sheep). It is a combination of nonlamellar bone, which forms a core substrate, and primary lamellar bone, which is deposited on the surface of the substrate. The nonlamellar portion forms de novo within the fibrous periosteum as buds of fine-fibered bone composed of small and randomly oriented collagen fibrils (Fig. 1.10). These buds of bone unite with adjacent buds to form a bridge of bone separated from the surface of the preexisting bone by a space that includes vascular elements. Plexiform bone derives its name from this interconnecting vascular plexus. The

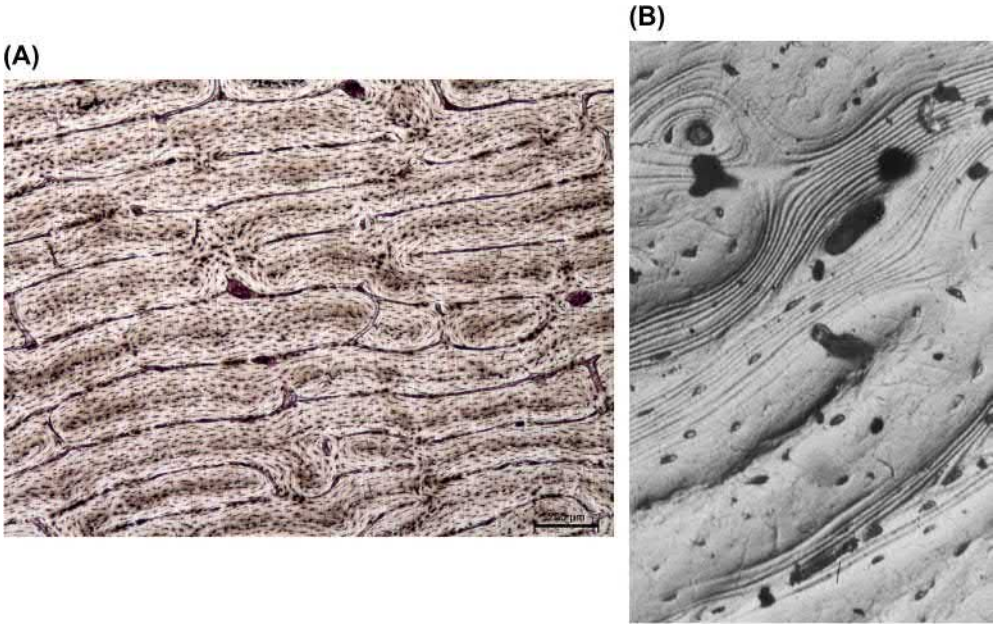


FIGURE 1.9 Plexiform bone is composed of lamellar bone laid down on a woven bone core. (A) This gives it its *bricks and mortar* appearance which can be best visualized using reflected light microscopy. (B) Backscattered electron microscopy. *Courtesy Dr. Mitchell Schaffler.*

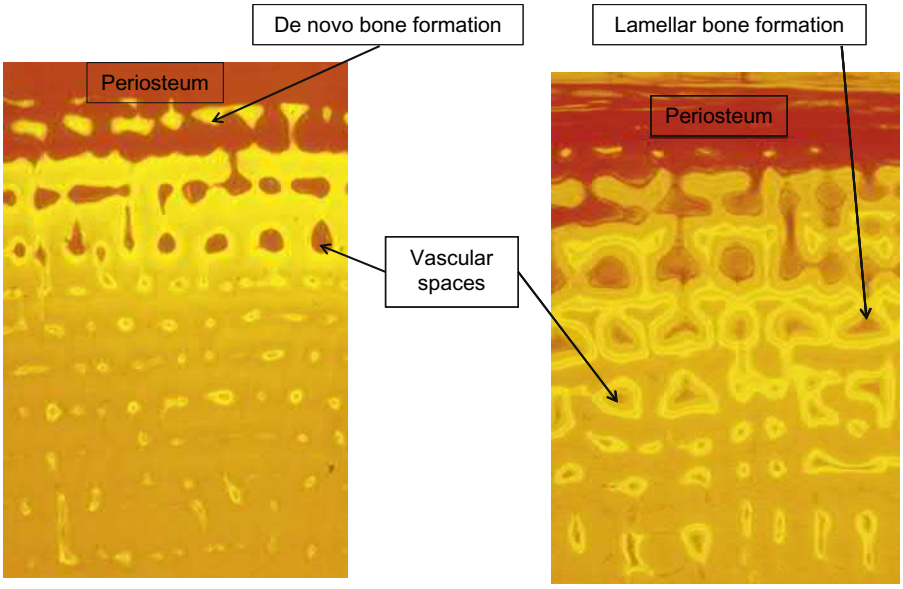


FIGURE 1.10 The formation of plexiform bone. Bone formation begins with intramembranous ossification within the periosteal membrane. Vascular spaces surrounding these bone cores are filled by lamellar apposition.

initial bridging also provides a way to rapidly increase bone strength, as small amounts of bone on the outer surface will contribute significantly to its strength (see Chapter 7). The bridges of bone provide several surfaces on which lamellae can be deposited and are one of the reasons that the bone can form so rapidly. As the lamellae form on the surface of the nonlamellar bridges, they gradually fill in these vascular spaces, while retaining smaller spaces for the vessels of approximately 25–50 μm in diameter.

Primary Osteons

Primary osteons are formed by infilling of enlarged vascular channels, usually found within well-organized lamellar bone. The osteonal lamellae are concentrically deposited on the surface of the canal until only a

small vascular canal remains. Bone cells are arranged in several circular layers (resembling a solar system) around the vascular canal. Primary osteons are only about 50–100 μm in diameter or smaller, typically have fewer than 10 lamellae, and do not have a well-defined boundary separating them from the rest of the existing matrix. It has been suggested that the existence of primary osteons is related to body size and rapid growth; the presence of primary osteons in rapidly growing deer antler supports this notion.

Secondary Bone

Primary bone is new bone made in a space where bone has not previously existed, although it may be formed on an existing bone surface. When bone is the product of resorption of previously deposited bone followed by deposition of new bone in its place, it is called *secondary bone*. This distinction is important because the apposition of primary bone, which requires only formation, is different from that of secondary bone, which requires a coordinated (coupled) series of processes to resorb and replace the bone that was already there. This is one way of repairing microscopic damage, which is constantly being created. The result of this process of resorption and replacement is a *secondary osteon* or *haversian system* (Fig. 1.11). Secondary osteons form longitudinally arrayed fibers embedded in a matrix composed of interstitial lamellae but separated from the matrix by a ductile interface, the cement line. The secondary osteon is distinguished from a primary osteon in being larger (100–250 μm in diameter), having more concentric lamellae (approximately 20–25 lamellae), and having a cement line at its outer boundary. The number and size of the osteons varies with age in predictable ways, becoming more numerous but smaller as we grow older. As with primary osteons, the lamellae surround a central haversian canal (approximately 50 μm in diameter) that carries a neurovascular bundle. Secondary osteons are about 1–10 mm long, running at an average angle of 11–17 degrees with respect to the long axis of the bone. However, the orientation of any individual osteon may be quite variable, as the vascular spaces they contain branch extensively. Moreover, haversian vessels are connected in a vascular plexus by other vessels that run between them in a more or less transverse direction. The canals in which these vessels run are called Volkmann's canals. The vessels in these canals also connect the haversian capillaries with the marrow vasculature, and with the vascular plexus in the periosteal membrane.

The cement line, or reversal line, represents a remnant of the reversal phase of bone remodeling, i.e., the point at which osteoclastic bone resorption stops and bone formation begins. It clearly demarcates the secondary osteon from its surrounding bone matrix (Fig. 1.11). Cement lines are mechanically important structures that serve as fiber reinforcements to the bone tissue. It is well established using histologic, birefringence, and electron microscopic techniques that the cement line is collagen deficient. There is some debate about whether cement lines are highly mineralized or deficient in mineral, but in either case, their mechanical function in preventing or deflecting crack growth would be the same. In addition to mineral and collagen, the cement line also contains high levels of certain kinds of NCPs, such as glycosaminoglycans and osteopontin. This makes a great deal of sense, as osteopontin plays a role in osteoclast adhesion during resorption and the cement line is where the osteoclasts stop resorbing.

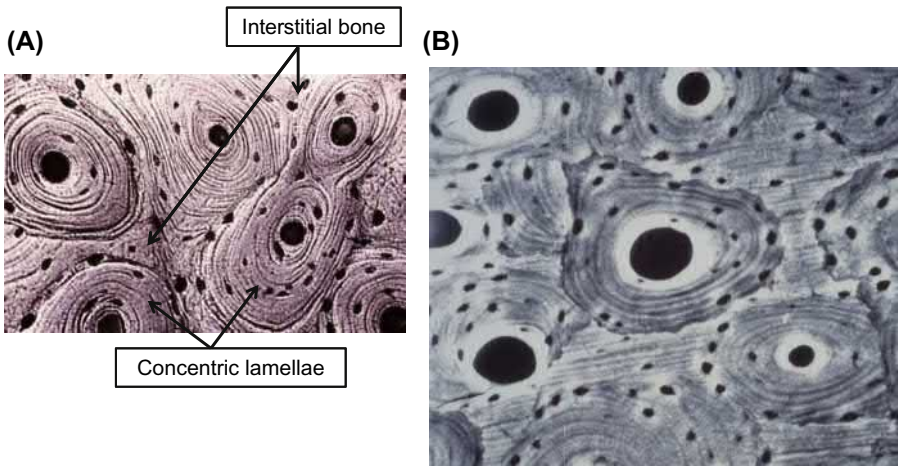


FIGURE 1.11 Secondary bone is formed by removal of preexisting bone and replacement with new bone. (A) In the cortex, this results in a secondary osteon, or haversian system, that surrounds a canal transmitting vessels and nerves associated with the vessels. (B) The osteon is bounded by a cement line that separates it from interstitial bone. *Courtesy Dr. Mitchell Schaffler.*