PERSPECTIVE

Advancing bone biology: The mutual promotion of biology and pioneering technologies

Liping Tong,[1] Andre J. van Wijnen,[2] Huaiyu Wang,[3,*] and Di Chen[1,4,*]

[1]Research Center for Computer-aided Drug Discovery, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen 518055, China

[2]Department of Biochemisty, University of Vermont, Burlington 05405, USA

[3]Center for Human Tissues and Organs Degeneration, Shenzhen Institute of Advanced Technology Chinese Academy of Sciences, Shenzhen 518055, China

[4]Faculty of Pharmaceutical Sciences, Shenzhen University of Advanced Technology, Shenzhen 518055, China

*Correspondence: hy.wang1@siat.ac.cn (H. W.); di.chen@siat.ac.cn (D.C.)

Received: February 29, 2024; Accepted: July 9, 2024; Published Online: August 1, 2024; https://doi.org/10.59717/j.xinn-life.2024.100078

Citation: Tong L., Wijnen A., Wang H., et al., (2024). Advancing bone biology: The mutual promotion of biology and pioneering technologies. The Innovation Life **2(3)**: 100078.

Bone is a mineralized tissue that provides fundamental support for body posture and biomechanical forces. Bones have also been meticulously used by humans as fine tools for at least over 25,000 years, perpetually captivating our curiosity and driving exploration into its mysteries. In this perspective, we revisit the milestones in the history of bone biology since the 17th century, and highlighted the transformative impact of pioneering technologies. From macroanatomical observations to microscopic cellular analyses and molecular signaling pathway explorations, each milestone has heralded groundbreaking discoveries. These include the elucidation of Wolff's Law, the regulatory mechanisms of calcium metabolism (via osteotropic parathyroid hormone analogs and calcitonin), as well as the revelation of critical cell interactions and signaling pathways in bone remodeling. These foundational insights deepen our understanding of bone as an enigmatic and underexplored tissue, but also yield significant clinical advancements. Bone biology stands poised for an explosive expansion of knowledge as we usher in the era of systems biology, propelled by cutting-edge multi-omics approaches, including single cell RNA sequencing (scRNA-seq) and spatial transcriptomics, as well as artificial intelligence. Therefore, challenges and future directions are discussed to navigate the complexities of integrating emerging technologies and to foster interdisciplinary collaborations that will propel bone biology research towards innovative breakthroughs and holistic solutions.

Bone, a versatile and strong mineralized tissue, has been worked by humans for at least 25,000 years. Throughout history, the quest to unravel the mysteries of bone has remained unabated, evidenced by ancient Greek and Egyptian frescoes depicting our ancestors' fascination with these enigmatic structures. In the 17th century, Anton van Leeuwenhoek, the father of micromorphology, revolutionized our comprehension of bone microstructures by describing the presence of tiny cavities and small channels within the bone using optical microscopy. Building upon Leeuwenhoek's pioneering work, Heinrich Müller (19th German anatomist) described microscopic features like canaliculi and bone matrix through microscopy and improved staining techniques. Concurrently, German naturalist Lorenz Oken presented the bone development and evolution through comparative anatomy studies. These efforts laid foundational stones for modern bone histology. Simultaneously, biochemical advancements shed light on the organic and inorganic constituents of bone. Collagen and hydroxyapatite were identified as the major organic and inorganic structural components of bones and teeth, deepened our fundamental understanding of bone architecture but also catalyzed advancements in the development of inorganic biomaterials for bone and tooth repair.

As the 20th century unfolded, the research interesting of bone research evolved from a static anatomical perspective to a dynamic physiological process. This era witnessed rapid advancements driven by innovative techniques rooted in classical physics, which broadened the horizons of biological research. For instance, the adoption of accelerated electron beams over traditional light sources elevated resolution from micron scale (~μm) to the nanoscale (~nm). Electron microscopy thus became invaluable for observing the ultrastructure of organelles and biological macromolecules. Additionally, technologies such as X-ray diffraction technology, near-infrared spectroscopy, isotope labelling, and nuclear magnetic resonance (NMR) spectroscopy were employed to determine crystal structures and chemical bonds of biomacromolecules and to trace biochemical reaction process. These technologies provide us with "eyes" to observe the dynamic process of life activities at the molecular and atomic levels.

With these tools, the field generated fundamental models for bone architecture, enabling a shift from descriptive anatomy to the exploration of cellular interactions and functional regulations. Four most relevant cell types to bone, namely osteoblasts, osteoclasts, osteocytes and osteogenic cells (also termed as mesenchymal stem cells) were identified.[1] In addition, bone remodeling was characterized as a dynamic metabolic process where osteoclastic resorption and osteoblastic bone formation are coupled in a spatiotemporal manner. In-depth research revealed the involvement of signaling pathways such as RANKL/RANK/osteoprotegerin (OPG), which represents a molecular cascade that regulates bone resorption by stimulating or attenuating osteoclast activity via TNF-α related proteins. Additionally, studies have highlighted the roles of morphogenic signaling pathways like Wnt/β-catenin and BMP/Smad in bone formation.[2] Disruptions in bone homeostasis lead to clinical conditions like osteoporosis and osteopetrosis. Fortunately, both physiological and pharmaceutical strategies can regulate bone remodeling, offering potential treatments for these skeletal diseases.

Mechanical forces, bisphosphonates, parathyroid hormones (PTH and PTHrP/PTHLH) and sclerostin (SOST) are pivotal regulatory factors in bone development, function and regeneration. The elucidation of their regulatory effects on bones represents major milestones in anabolic bone biology research, leading to widely adopted interventions for treating bone metabolic imbalances. Wolff's law, formulated by German anatomist Julius Wolff, describes how bones adapt to mechanical loading, providing a fundamental principle that guides clinical applications in orthopedics, rehabilitation, and sports medicine. Early progress in catabolic bone biology was marked by the development of anti-resorptive bisphosphonates, which attenuate bone resorption by osteoclasts and bind to hydroxyapatite at bone resorption sites. PTH, a polypeptide secreted by the parathyroid glands, regulates serum ionized calcium homeostasis by influencing calcium reabsorption and release in bones and kidneys. PTH and its therapeutic analogs, such as teriparatide, exert their effects through the PTH/PTHrP receptor (PTH1R), activating multiple intracellular signaling pathways, including the cyclic adenosine monophosphate (cAMP), phospholipase C (PLC), β-protein kinase C (PKC), and cAMP-PKA. Teriparatide, a PTH-analog has been advanced to the clinic and is licensed for treating osteoporosis in postmenopausal women at high risk of fracture.[3] SOST, a glycoprotein primarily secreted by osteocytes, supports bone remodeling in response to mechanical stimulation. As an inhibitor of Wnt signaling pathways, SOST binds to LRP5/6 receptors on the surface of osteoblasts, preventing the activation of the Wnt/β-catenin signaling pathway. This inhibition decreases the proliferation and activity of osteoblasts, leading to reduced bone formation. Romosozumab (brand name Evenity), an anti-sclerostin monoclonal antibody, has been approved by the FDA for the treatment of osteoporosis.[3] In addition to the aforementioned interventions, the role and clinical application potential of new key regulatory signaling pathways and their targeted drugs, biomaterials, and metal ions in bone metabolic balance are being deeply explored. The combined success of strategies that attenuate bone resorption or stimulate bone formation offers promising prospects for restoring the dynamic balance of bone homeostasis in patients.

In the mid-20th century, a monumental revolution in the field of molecular biology inaugurated a new era in biological research with the publication of the double helix DNA structure by James Watson and Francis Crick, inspired by the X-ray-diffraction pattern of DNA.[4] This discovery profoundly altered the

trajectory of modern molecular biology and led to the "central dogma" of biology on the relationships between DNA, RNA and proteins. This foundational theory spawned a range of technologies for "reading", "copying" and "applying" genetic information, including DNA sequencing by Sanger, Maxam and Gilbert, recombinant technology for the production of protein therapeutics, and rapid *in vitro* DNA amplification by polymerase chain reaction. While these breakthrough technologies had major impact on all biological sciences, these techniques also opened new opportunities for further exploration of the genetic basis of bone development, skeletal diseases, and individual variations in bone health.

With advances in high throughput DNA sequencing, genetic mutations associated with skeletal diseases, such as osteogenesis imperfecta, fibrous dysplasia, and multiple epiphyseal dysplasia are being discovered. Causal genes identified by molecular and cellular approaches are being validated using functional studies with advanced mouse genetics. Rapidly evolving gene editing techniques, spanning from traditional genetic engineering to the latest "CRISPR-Cas9" equip bone biologists now with the capability to "rewrite" genetic information. These techniques not only advance our understanding of the genetic underpinnings of bone diseases but also facilitate the establishment of cross-species research models to explore the mechanisms and interventions of human bone diseases. Mouse models with mutations in collagen genes (*Col1a1*, *Col1a2*) and the *FGFR3* gene have been designed to mimic osteogenesis imperfecta and achondroplasia, respectively. Introducing mutations in *PHEX* or *FGF23* genes result in mice presenting phenotypes resembling X-linked hypophosphatemia or hypophosphatemic rickets. Editing the *SOST* gene (knockout or knockdown) demonstrates increased bone formation, confirming sclerostin's regulatory role in bone metabolism and serving as an invaluable drug screening platform. To mitigate the lethality associated with systemic editing of vital genes, scientists have devised conditional gene editing technologies utilizing the Cre-LoxP and Flp-FRT systems. These innovative techniques enable gene expression within target bones and cell populations, facilitating activation or deactivation at specific developmental stages or in response to particular environmental cues. Through the application of conditional gene recombination, mutation and editing methods, researchers have pinpointed pivotal genes (e.g., *Runx2*, *Sp7/Osterix*, *Sox9*) and key signaling pathways (e.g., Wnt/β-catenin, BMP, Hedgehog, Notch) that mediate bone development, mineralization, and remodeling.

Entering the third millenium, the integration of bioinformatics and multi-omics analysis, which encompasses genomics, transcriptomics, proteomics, and metabolomics, allows for the analysis of large-scale omics data and provides a holistic view of bone biology. Biomarkers associated with bone health and diseases, such as CTX-I, PINP, Dkk-1, and Sclerostin for osteoporosis, as well as COMP and MMPs for osteoarthritis, have been identified as tools for early diagnosis. Furthermore, utilizing multi-omics to screen patient samples allows for the selection of therapeutic targets and tailoring personalized interventions. In musculoskeletal regenerative medicine and orthopedic surgery, biomechanical principles and engineering techniques are applied to develop novel biomaterials and implants. Tissue engineering approaches are optimized for critical size bone-defects and bone reconstruction using bioprinted scaffolds with advanced biopolymers or nanoparticles for the delivery of growth factors. Collectively, these approaches promote bone tissue regeneration and integration.

Recently, application of artificial intelligence (AI) has dramatically increased across various aspects of bone biology research and clinical practice. AI algorithms and automatic image analysis are used to analyze medical images, assisting healthcare professionals to outline the fractures, tumors, osteoporosis, and other abnormalities, thus enhancing diagnostic precision and efficiency. Additionally, AI-driven virtual screening enables surgeons to plan intervention strategies, aiding in the precise removal of bone-related cancers or necrotic tissue and the accurate placement of artificial prostheses. Furthermore, AI-based biomechanical modeling and simulation techniques offer predictive capabilities regarding bone strength, fracture risk, and orthopedic implant performance under diverse loading conditions. This facilitates the optimization of orthopedic device design, refinement of surgical procedures and comprehensive rehabilitation planning, ultimately enhancing treatment outcomes.

Undoubtedly, the breakthroughs in multi-disciplinary basic principles and ensuing waves of technological innovation have driven the progress of bone biology research over the past century. The trend of integrating diverse technologies and perspectives is poised to continue in the foreseeable future. Powerful spatiotemporal analysis tools based on high throughput DNA sequencing permit analysis of the cellular heterogeneity, lineage relationships, and molecular mechanisms governing bone development, homeostasis, and diseases with precise spatiotemporal controls, including scRNA-seq, spatial transcriptomics (e.g., Visium 10×, Nanostring), and multi-spectral imaging of proteins (Codex) in histological sections. High resolution scRNA-seq permits in silico cell sorting and enables investigators to identify diverse cell populations, providing direct insights into the cellular composition and organization of bone tissue, as well as the dynamic changes across different life stages and at different individual levels. However, with the accumulation of massive data, the challenge now is how to manage, screen, verify and utilize this precious information effectively. Urgent efforts are required to establish standardized protocols, data inclusion criteria, screening processes, and validate through independent experiments, ensuing that critical data are highlighted and shared globally.

The focus of bioinformatic analysis should shift towards comprehensive utilization of the collected data to uncover the underlying laws governing complex biological processes in bone, transcending observations on a case-by-case basis. Clinical applications, such as biomechanics and PTH analogs, are not solely derived from the observation of their effects but rather from an understanding of their regulatory laws on bone remodeling. Thus, the revelation of internal laws, rather than mere observation, holds immense value for clinical application and translation. Integration of scRNA-seq with multi-omics analyses (e.g., bulk RNA-seq, HiC, ChIP-seq, ATAC-seq, proteomics) holds promise for identifying key genes, pathways and regulatory elements which may shed the light on the intricate molecular networks and underlying laws. Applying machine learning algorithms and developing computational models is a feasible way to assist exploration of new physiological and molecular rules in bone biology. The realm of model building and training remains relatively unexplored until now, posing significant challenge for future investigations.[5]

There is no doubt that the current understanding of the multifaceted role of bone in the body falls short of its true significance. As a functionally diverse organ, bone serves as more than just a structural framework, it acts as a crucial endocrine organ and a reservoir for essential minerals, hematopoietic stem cells, and immune cells. Its indispensable role in regulating mineral metabolism, neural signal transmission, immune function, and other processes underscores the need for further exploration in the following key areas:

(1) Identification of novel biomarkers, therapeutic targets, and intervention strategies: Although the molecular mechanisms underlying common orthopedic diseases have been extensively studied, the identification of biomarkers for early diagnosis remains a pressing need. Additionally, elucidating molecular signaling pathways involved in bone diseases and remodeling is vital for discovering therapeutic targets and advancing drug development.

Use of controllable biomaterials and personalized tissue engineering therapies presents a promising approach managing bone diseases. Designing and precisely regulating tissue engineering systems that align with different stages of bone regeneration, along with tracking degradable biomaterials status and loaded drug metabolism through *in vivo* imaging, offers exciting potential. Lifestyle interventions, proven effective in optimizing bone health, warrant increased attention, highlighting the importance of understanding how diet, exercise, and other lifestyle factors influence bone metabolism. Moreover, rare bone disorders pose unique challenges due to their scarcity, necessitating innovative approaches to study their pathological mechanisms and develop targeted therapies within limited clinical resources.

(2) Incomplete understanding of bone-metabolism interactions: While the impact of systemic metabolism on skeletal metabolism has been well-documented, the reciprocal influence of bone physiology on energy metabolism requires further investigation. Bones not only regulate mineral ion concentrations critical for enzymatic reactions but also participate in energy metabolism through secreted factors, such as osteocalcin, impacting insulin secretion, sensitivity, and glucose metabolism.[6] Although this perspective is still controversial, there is a pressing need to elucidate the precise mechanisms involved and to identify additional factors released by bone cells which

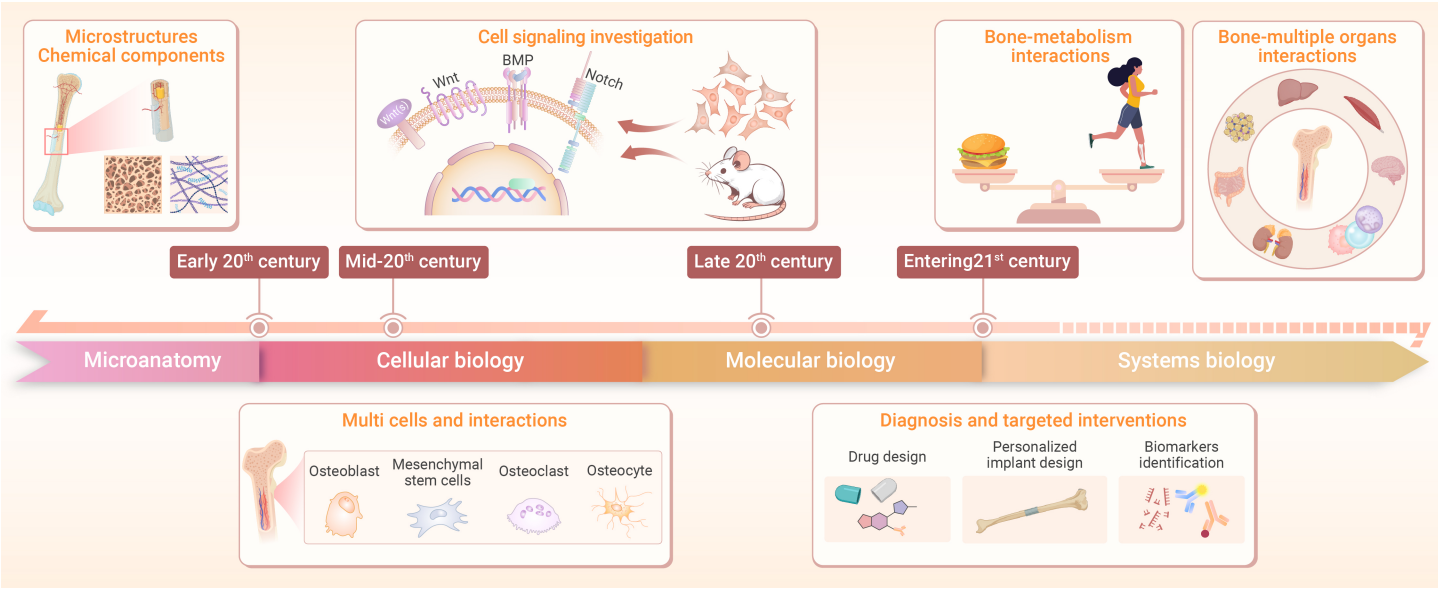

Figure 1. The development and future directions of bone biology.

is now recognized as "osteokines".[7] Additionally, adipocytes and osteoblasts derived from a common precursor, mesenchymal stem cells, which located in bone marrow. The regulation of adipocyte differentiation by the bone microenvironment and its implications for lipid metabolism remain incompletely understood.

(3) Limited understanding of bone-immune interactions: Although the role of local osteo-immunity in bone metabolism and bone remodeling is recognized, the reciprocal regulation between the immune system and the bone microenvironment warrants deeper exploration. Bone marrow serves as a niche for immune cell storage and activation, giving rise to various immune cell lineages, including lymphocytes, myeloid cells, and granulocytes, and shaping their destinies. For example, memory T cells can localize to bone tissue and contribute to immune surveillance and responses to pathogens;[8] cancer cells exploit the bone marrow as a refuge from the immune surveillance, evading anticancer therapies. However, the mechanisms underlying bone-immune cell crosstalk and its implications for immune surveillance, pathogen responses, and cancer metastasis remain poorly understood.

(4) Challenges in studying bone-multiple organs interactions: Emerging research suggests intricate interactions between bone and other organs including liver, kidney, brain, gut, thyroid gland and adrenal glands to modulate metabolism, immune responses, interoception adjustment and physiological processes throughout the body.[9] Notably, bone-nervous system interactions are particularly fascinating, particularly in pain perception, sensory feedback, and the impact of neurological conditions on bone health. Neurological conditions such as Parkinson's disease and multiple sclerosis can impact bone health through various mechanisms, including reduced physical activity, altered hormonal signaling; on the contrary, active physical exercise can delay aging and prevent the occurrence of Alzheimer's.[10] Moreover, the communication and reconstruction of neurovascular networks play a critical and inevitable role in bone healing and repair. However, the precise mechanisms by which the nervous system and its released neurotrophic factors and neurotransmitters orchestrate the repair process spatiotemporally remain elusive. Increasing evidences point to the existence of bidirectional communications, forming the "bone-brain axis" whereby osteokines influence the cognitive function. Collectively, these exciting phenomena need further investigation. Even though there are challenges posed by the complexity of these interactions, advanced technologies at our disposal and sophisticated new in silico tools, provide optimism that we may continue to increase our understanding of the physiology and pathophysiology of bone. As our ancient ancestors sculpted bones as tools and curious objects to support their daily lives, we can leverage our current informatic and problem solving power to gain insights into the mystery of bones in a new era of humanity.

REFERENCES

1. Rosy, S. and Paulus, R. (2018). Bone development and growth. Haisheng, Y. (ed). Osteogenesis and bone regeneration (IntechOpen), pp: 1-20. DOI: 10.5772/intechopen.82452.
2. Yan, Y., Tang, D.Z., Chen, M., et al. (2009). Axin2 controls bone remodeling through the β-catenin-bmp signaling pathway in adult mice. J. Cell Sci. **122**: 3566–3578. DOI: 10.1242/jcs.051904.
3. Reid, I.R. and Billington, E.O. (2022). Drug therapy for osteoporosis in older adults. Lancet **399**: 1080–1092. DOI: 10.1016/S0140-6736(21)02646-5.
4. Watson, J.D. and Crick, F.H.C. (1953). Molecular structure of nucleic acids - a structure for deoxyribose nucleic acid. Nature **171**: 737–738. DOI: 10.1038/171737a0.
5. Zhang, H. and Zhu, J. K. (2023). Improving the scientific cycle. The Innovation Life **1**: 100002. DOI: 10.59717/j.xinn-life.2023.100002.
6. Jin, F., Liu, M., Zhang, D., et al. (2023). Translational perspective on bone-derived cytokines in inter-organ communications. The Innovation **4**: 100365. DOI: 10.1016/j.xinn.2022.100365.
7. Liang, W., Wei, T., Hu, L., et al. (2024). An integrated multi-omics analysis reveals osteokines involved in global regulation. Cell Metab. **36**: 1144–1163. DOI: 10.1016/j.cmet.2024.03.006.
8. Wu, Z.X., Lin, X.X., Yuan, G.X., et al. (2024). Innate lymphoid cells: New players in osteoimmunology. Eur. J. Immunol. **54**. DOI: 10.1002/eji.202350381.
9. Lv, X., Gao, F., and Cao, X. (2022). Skeletal interoception in bone homeostasis and pain. Cell Metab. **34**: 1914–1931. DOI: 10.1016/j.cmet.2022.09.025.
10. De Miguel, Z., Khoury, N., Betley, M.J., et al. (2021). Exercise plasma boosts memory and dampens brain inflammation via clusterin. Nature **600**: 494–499. DOI: 10.1038/s41586-021-04183-x.

FUNDING AND ACKNOWLEDGMENTS

This work was financially supported by the National Key Research and Development Program of China (2023YFC2415800), National Natural Science Foundation of China (NSFC) grants (82172397, 82030067, 82161160342 and 82250710174). We also acknowledge the Shenzhen Science and Technology Research Funding (JCYJ20220818101414032) and Youth Innovation Promotion Association of Chinese Academy of Sciences (No. 2020353). The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

AUTHOR CONTRIBUTIONS

Liping Tong and Di Chen conceived the idea. Liping Tong prepared the manuscript with input from all authors. Andre J. van Wijnen, Huaiyu Wang and Di Chen involved in the manuscript revision. All authors read and commented on the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.