Review

Modeling the Clockwork of Bone: A Narrative Review of Experimental Approaches to Circadian Rhythm in Bone Metabolism

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Modeling the Clockwork of Bone: A Narrative Review of Experimental Approaches to Circadian Rhythm in Bone Metabolism

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Abstract

Circadian rhythms are fundamental regulators of bone remodeling, orchestrating the coordinated actions of osteoblasts, osteocytes, and osteoclasts. Recent studies have highlighted how core clock genes, such as *Bmal1, Clock, Per1/2, and Cry1/2,* exhibit rhythmic expression in bone tissue and modulate key markers of bone formation and resorption. Disruptions in circadian regulations, whether caused by environmental factors or genetic alterations, have been linked to osteoporosis, impaired fracture healing, and increased risk of bone fragility. This review provides a comprehensive evaluation of current experimental models used to study circadian regulation in skeletal biology, including in vivo, ex vivo, and in vitro approaches. We summarize their respective advantages and limitations and outline the molecular and cellular markers employed to assess circadian function in bone cells. We also discuss the emerging co-culture models and human-relevant platforms, for their potential to bridge the gaps between mechanistic research and translational applications. By comparing model characteristics and highlighting integrated research strategies, this review aims to advance circadian bone research and inform future investigations into potential temporal aspects of skeletal health.

Keywords: circadian rhythm; bone remodeling; in vivo model; ex vivo model; in vitro model; osteoblasts; osteoclasts

1. Introduction

The circadian rhythm is a highly conserved biological timing system that coordinates physiological activities across multiple organ systems, including the cardiovascular system and nervous system[1–3]. It influences a wide range of processes, including metabolism [4–6], immune responses [7], and has been implicated as a critical factor in bone biology [8,9]. Recent discoveries have highlighted its active role in bone remodeling via osteoblasts and osteoclasts [10–12].

It has been acknowledged that circadian homeostasis is integral to skeletal maintenance [11]. Disruptions of these rhythms, arising from night shifts [13], jet lag, aging, or mutations in clock genes, have been associated with impaired skeletal health [10,12]. A study by Bukowska-Damska et al. showed that night shift workers exhibit lower bone mineral density and heightened susceptibility to fractures [14].

At the cellular level, irregular circadian signaling may pose a potential threat to osteoblast–osteoclast cycles, tipping the balance towards bone resorption. Molecularly, genes such as *Bmal1*, *Npas2*, *Per*, and *Cry* show rhythmic expression in these cells [15], influencing key markers for bone homeostasis including: receptor activator of nuclear factor kappa-ligand (RANKL), Osteoprotegerin (OPG), C- and N-terminal Telopeptide of type I collagen (CTX and NTX), procollagen type I N- and C-terminal propeptide (PINP and PICP), alkaline phosphatase (ALP), and tartrate-resistant acid phosphatase (TRAP) —each of which reflects the shifting states of bone formation and breakdown—

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for review see [16]. Misalignment of these pathways may impair temporal regulation of bone remodeling and contribute to the development of skeletal disorders such as osteoporosis, as shown in Table 1, Figures 1 and 2.

Table 1. Summary of the circadian rhythm of bone markers.

Marker	Function	Effect under Disruption	Species	Ref.
Bmal1	Core clock gene (activator)	↓ Expression, impaired bone mass	Mouse / Human	[15,17–19]
Clock	Core clock gene (activator)	↓ Expression, impaired bone mass	Mouse / Human	[15,17–19]
Per1/2	Negative loop regulator	Phase shift / ↓ amplitude	Mouse	[15,17–19]
Cry1/2	Negative loop regulators	Phase shift / ↓ amplitude	Mouse	[15,17,19]
RANKL	Promotes osteoclastogenesis	↑ Expression, increased bone loss	Mouse / Human	[16,19]
OPG	Inhibits RANKL	↓ Expression, enhanced bone resorption	Mouse / Human	[16,19,20]
ALP	Osteoblast activity marker	↓ Activity	Human	[16]
CTSK	Osteoclast activity marker	↑ Activity	Mouse	[19]
TRAP	Osteoclast activity marker	↑ Activity	Human	[16]
CTX	Bone resorption marker (serum)	Unclear	Human	[16,20–22]
PINP	Bone formation marker (serum)	Unclear	Human	[16,21,22]

Despite a growing body of research, the mechanisms by which circadian rhythms regulate bone biology remain incompletely defined, partly due to limitations in current experimental approaches. While animal models offer systemic insights, they are resource-intensive and often lack temporal precision. Meanwhile, conventional mono-culture in vitro systems fail to recapitulate the complex cellular interplay that underlies bone physiology.

Emerging co-culture models of osteoblasts and osteoclasts present a promising alternative—for review see [23–26]. These systems allow researchers to observe temporal gene expression and dynamic interactions under controlled conditions, providing a more integrated perspective on circadian regulation in bone. This review summarizes key methodologies for investigating circadian control in skeletal systems, with emphasis on comparing in vitro co-culture platforms, ex vivo molecular markers, and in vivo model systems.

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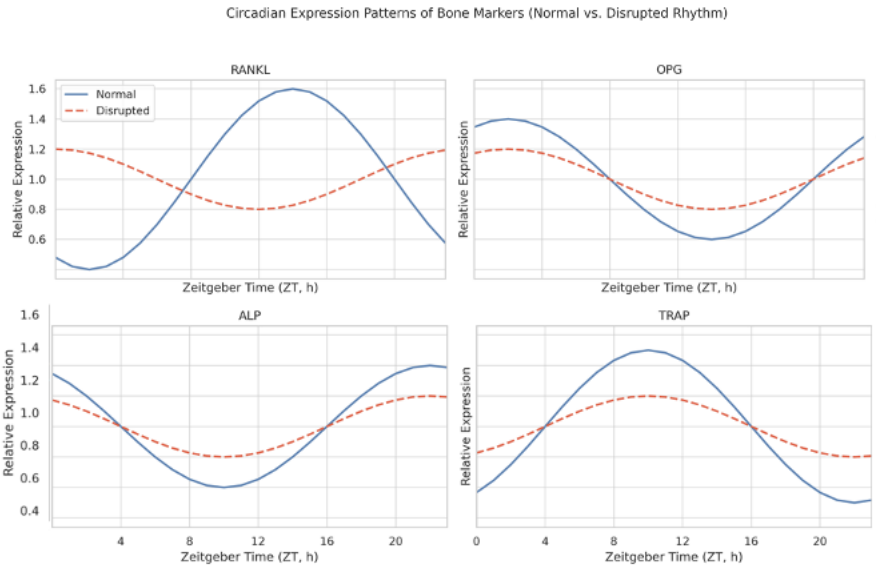

Figure 1. Conceptual summary of circadian expression patterns of bone markers. The figure presents the 24-hour Zeitgeber Time (ZT) oscillatory patterns of essential bone remodeling markers in the human body under physiological (blue) and disrupted (red, dashed) circadian conditions. This figure is adapted from the results of Dovio et al. [27] and Diemar et al. [16]. In normal states, markers including RANKL, OPG, ALP, and TRAP follow robust and well-synchronized rhythmic profiles. Conversely, circadian misalignment of the bone markers is characterized by a reduction in oscillatory amplitude, phase shifts, and an overall blunting of rhythmicity, indicating impaired temporal regulation of bone remodeling processes.

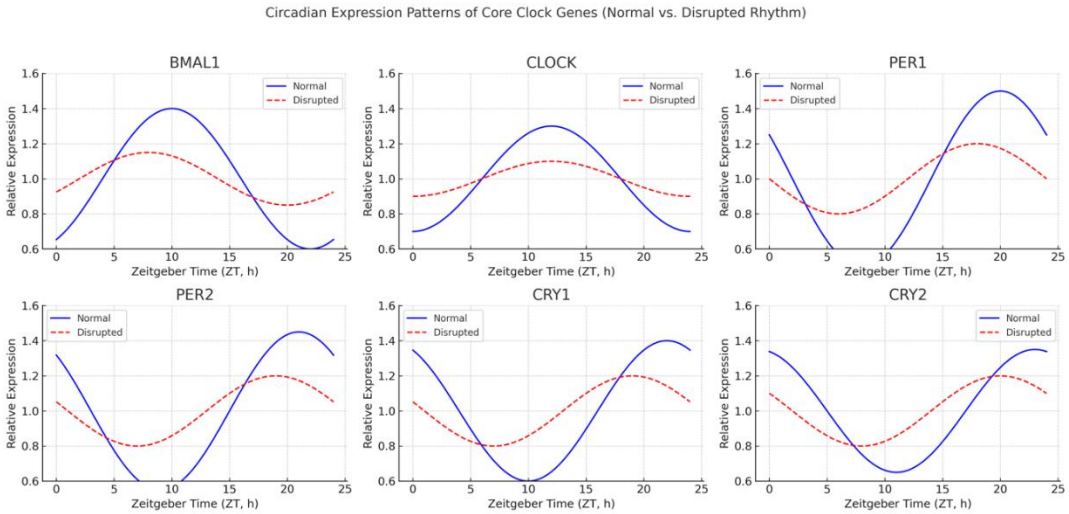

Figure 2. Conceptual summary of circadian expression patterns of core clock genes. The figure illustrates the circadian expression profiles of core clock genes (*BMAL1, CLOCK, PER1, PER2, CRY1, and CRY2*) in mouse across a 24-hour Zeitgeber Time (ZT) cycle under normal (blue) and disrupted (red, dashed) conditions. This figure is adapted from the results of Schilperoort et al. [19] and Zvonic et al. [15]. Normal rhythms exhibit robust and well-phased oscillations, while circadian disruption results in phase shifts, reduced amplitude, or blunted oscillations, potentially affecting downstream regulation of bone remodeling.

2. Search Strategy

A comprehensive literature search was performed using the PubMed database to identify relevant publications on the interplay between circadian rhythms and bone biology. The search was restricted to the past 20 years (from July 2005 to July 2025) to capture recent advancements. The

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keywords applied were "circadian rhythm", "bone", and "model", and all article types, including original research and reviews, were considered.

The initial search yielded 165 records. During the screening process, studies that focused solely on one or two of the three core topics (i.e., circadian rhythm, bone, or model systems) were excluded. Only studies explicitly addressing all three topics simultaneously were retained. After this refinement, a total of 60 articles were included in the final analysis, comprising 48 original research articles and 12 review papers. The research models of circadian rhythm can be divided into three categories: in vivo models (51.67%), ex vivo models (31.67%), and in vitro models (16.67%).

Duplicate entries were removed, and only English-language articles with accessible full texts were included in the review. The identified manuscripts were assigned to the three categories: "in vitro", "ex vivo", and "in vivo", based on the used model system.

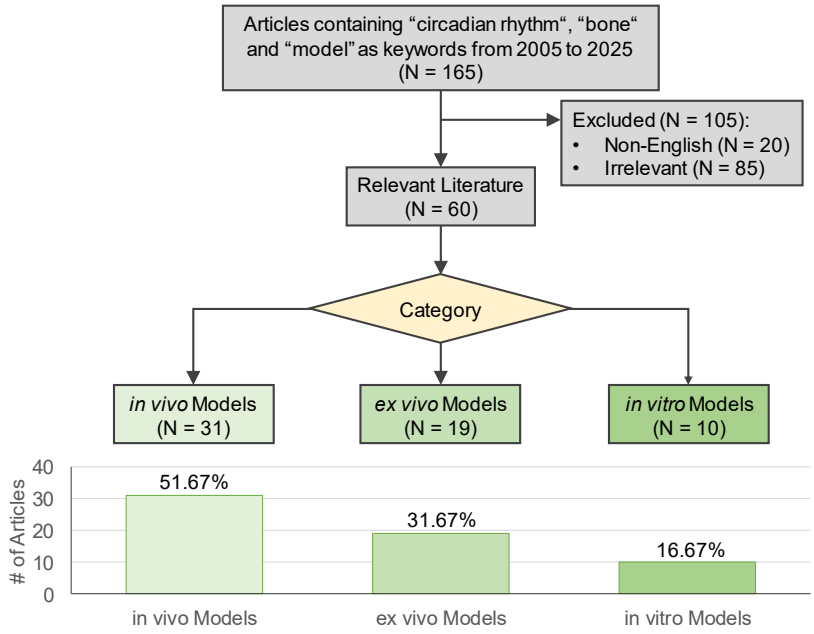

Figure 3. Research strategy. The graph shows the proportion of in vivo (31 studies, 51.67%), ex vivo (19 studies, 31.67%), and in vitro (10 studies, 16.67%) models identified in the literature. In vivo models dominate due to their ability to preserve systemic physiological regulation, whereas ex vivo models provide a balance between tissue-level complexity and experimental control. In contrast, in vitro models, although less common, are valued for their high controllability and suitability for molecular-level investigations.

3. Model Systems

3.1. In Vivo Model Systems

In vivo models are indispensable for exploring the interplay between circadian rhythms and bone metabolism. By preserving the physiological context of a living organism, these models enable the study of bone remodeling processes under the influence of systemic factors such as hormonal signaling, immune responses, neural regulation, and mechanical loading. Rodents, particularly mice, are commonly employed in this field due to their genetic tractability and physiological similarities to the human circadian system. These murine models typically fall into two categories: (1) environmental models, where light-dark cycles are strategically altered to disrupt circadian rhythms, and (2) genetic models, where key clock genes (e.g., *Bmal1, Clock, Per*) are selectively deleted to directly evaluate their roles in bone cellular activity.

In rhythm disruption models, jet lag-like light shift protocols are frequently employed, such as shifting the light-dark cycle by eight hours every three days [28] or exposure to weekly alternating or continuously altered bright or dim light for 12 hours [19,29]. While the continuous switch of the

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"normal" bright-dim cycle to a dim-bright cycle seemed to only deteriorate inflammation-associated bone degradation [29], weekly alternating bright-dim cycles resulted in marked bone degradation [19,29]. This effect may even be transferred to the offspring, when the jet lag phases are applied during gestation [28]. Similar effects can be observed in models for periodontal disease, where circadian disruption has also been linked to alveolar bone loss and altered macrophage responses, potentially exacerbating periodontal bone deterioration [30–33].

To counter these effects, therapeutic interventions have been explored, notably with melatonin receiving particular attention as a hormone closely linked to circadian regulation. In experimental models, melatonin has been reported to restore rhythmic stability and attenuate bone degeneration, suggesting potential utility as an adjunctive strategy for conditions such as osteoporosis—for review see [34,35].

Genetic knockout models provide deeper insight into the molecular basis of skeletal health. For example, removal of *BMAL1*, a core clock gene, has been shown to interfere with osteoblast maturation [36], resulting in reduced bone formation and an osteoporosis-like phenotype—for review see [37]. These findings underscore the critical role of circadian regulation in maintaining bone integrity and suggest that clock-related genes may represent potential targets for future investigations into metabolic bone disorders.

In addition to mouse models, researchers have employed other species to investigate circadian effects on bone metabolism, including laying hens. Dietary phosphate feeding regimens showed circadian effects on eggshell deposition and thus the eggshell quality [38]. Simultaneously, medullary bone samples collected showed inverse regulation of bone metabolism (eggshell strengthening led to bone weakening), as detected by osteoblast and osteoclast function [38]. This approach revealed rhythmic fluctuations in bone cell activity within a physiologically relevant context of mineral mobilization for eggshell production, mineral balance, and skeletal biology.

A major strength of the described in vivo systems is their capacity to preserve endogenous circadian synchronization across multiple tissues. This feature enables the study of complex intercellular and inter-organ interactions and facilitates modeling of bone-related pathologies such as osteoporosis, diabetes, and periodontitis, and is therefore commonly employed in this context [6,31,33,35,39,40]. Advanced genetic tools, including transgenic reporter lines (e.g., *Per1:Luc* mice), further enhance the precision of these investigations by enabling real-time monitoring of clock gene activity in bone cells [17,41].

Rhythmic bone cell function is typically assessed by measuring serum biomarkers of bone formation and resorption, as well as regulatory markers [16,21,42].

- Bone formation markers: PINP, PICP, osteocalcin (OCN), bone-specific ALP.
- Bone resorption markers: CTX, NTX, CAII, TRAP, cathepsin K.
- **Bone metabolism regulatory markers**: RANKL, OPG, fibroblast growth factor 23, and Leptin.

Additionally, expression levels of core circadian genes (*Bmal1, Clock, Per1/2, Cry1/2*) can be quantified in bone tissue using methods such as qRT-PCR (quantitative reverse-transcription polymerase-chain reaction), in situ hybridization, or bioluminescence imaging. Hormones with circadian variation, such as melatonin, are also monitored through timed blood sampling to evaluate endocrine influences on bone remodeling—for review see [34,35,43].

Despite their advantages, in vivo models also face several challenges. Their complexity can introduce biological variability and confounding factors, making it difficult to isolate specific circadian effects. Moreover, these studies are resource-intensive and must comply with rigorous ethical standards. Collecting samples at multiple time points, which is essential for circadian analysis, is technically demanding and may itself disrupt physiological rhythms. Compared to in vitro systems, in vivo models offer limited mechanistic resolution but superior physiological relevance (Figure 4). Ultimately, in vivo models serve as a cornerstone of circadian research in bone biology, providing an integrated perspective on skeletal dynamics within the context of the whole organism.

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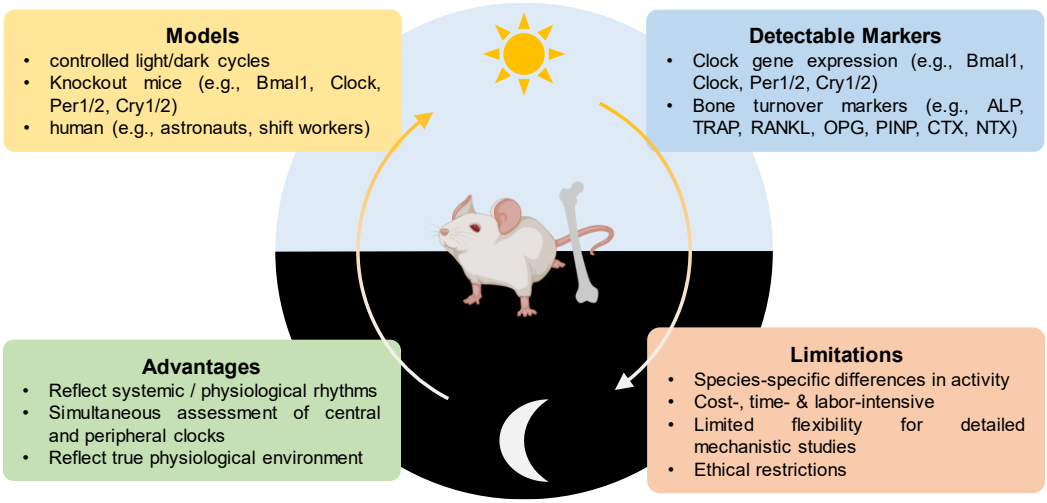

Figure 4. Visualization of in vivo models. In vivo models are instrumental in elucidating the dynamic interplay between circadian rhythms and bone remodeling processes. These models typically involve rodents, especially mice, maintained under controlled light–dark cycles or genetically engineered to lack key circadian genes such as *Bmal1, Clock, Per1/2, and Cry1/2*. Such setups allow researchers to investigate the systemic and tissue-specific effects of circadian disruption on skeletal physiology. However, animal models also have their limitations, such as high costs and ethical concerns. Image was created with the help of BioRender icons (https://app.biorender.com/illustrations/69ca989269f3c9dfd83ad093).

3.2. Ex Vivo Model Systems

Ex vivo models represent an intermediate platform between in vivo and in vitro systems for investigating circadian regulation in bone biology. These models typically involve the extraction of intact tissues such as bone slices, periodontal segments, or intervertebral discs from animal or human sources at defined circadian time points, followed by short-term culture under controlled environmental conditions [44]. This strategy allows the investigation of intrinsic rhythmic activity in bone cells outside the organism, while maintaining native tissue architecture and intercellular interactions.

Although recent literature reports on circadian studies using transgenic *Per1: Luc* mice are limited, this model remains one of the most widely adopted ex vivo systems for circadian research. In *Per1: Luc* mice and *Per2: Luc* mice, the real-time expression of the core clock gene *Per1* and *Per2* can be visualized through bioluminescence imaging in bone explants [17,45,46]. These studies have demonstrated that peripheral tissues, including bone, retain autonomous circadian oscillations ex vivo for several days, allowing time-resolved analysis of clock gene expression and bone cell function.

Beyond luminescent reporters, ex vivo systems allow the quantification of various circadian genes and the detection of extracellular calcium matrix and bone tissue. Clock genes such as *Bmal1, Clock, Per1/2, NR1D1,* and *Cry1/2,* and some osteoblasts and osteoclast markers are commonly assessed using qRT-PCR, enzyme-linked immunosorbent assay (ELISA), Western blot, or dot blot techniques [47,48]. Specific bone markers secreted into the culture supernatant may also be detected by enzyme-linked immunosorbent assay or dot blot [48]. Extracellular calcium matrix and bone tissue morphology are also routinely assessed by (immuno-)histological stainings, scanning electron microscopy, or Raman spectroscopy [45,47,49,50].

In addition to classical ex vivo methodologies, ex vivo models frequently incorporate transgenic mice or rats as cell or tissue source, offering valuable translational insights into the potential clinical consequences of circadian misalignment on bone physiology. One study showed that surgical procedures were performed on the hind limbs of transgenic mice to induce femoral fractures, followed by external fixation of the fracture site. After a defined period of stabilization, the fractured femoral tissue was excised and subsequently cultured in vitro. The expression of circadian rhythm–related genes in the bone tissue was first assessed under baseline culture conditions. Thereafter,

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stimulating factors such as parathyroid hormone (PTH) were introduced, and gene expression was re-evaluated to determine the regulatory effects of these stimuli on circadian gene expression in bone. The results showed that PTH may have a potential role in promoting fracture healing [45].

In another ex vivo experiment, the role of *Rev-erbα* in growth plate cartilage was investigated. Metatarsal tissue was isolated from mice and cultured under controlled conditions, after which a *Rev-erbα* antagonist was introduced into the culture medium. Subsequent analyses assessed bone tissue proliferation, differentiation, and mineralization. The findings demonstrated that inhibition of *Rev-erbα* suppressed growth plate development and longitudinal elongation of metatarsals, primarily through upregulation of the mitogen-activated protein kinase (MAPK)—extracellular signal-regulated kinase 1&2 (ERK1/2) signaling pathway [47].

Ex vivo systems offer distinct advantages for circadian research in bone: they reduce the systemic variability inherent in whole-animal models, permit high-resolution temporal sampling, and preserve native tissue architecture and cell–cell interactions that are absent in traditional monolayer cultures. Nonetheless, these models are constrained by the lack of systemic regulatory inputs, such as hormonal and neural signals, and by the limited viability of tissue outside the organism, which restricts long-term rhythmic analysis [51]. Despite these challenges, ex vivo platforms remain a useful tool to elucidate the molecular basis of circadian control in bone, particularly in combination with genetic manipulation and advanced imaging techniques [46,49] (Figure 5).

Taken together, evidence from ex vivo experiments, clinical observations, and translational research strongly supports the notion that circadian rhythms are integral to maintaining skeletal homeostasis. Disruption of these rhythms, whether through experimental manipulation or environmental factors, can lead to measurable alterations in bone turnover and compromised structural integrity.

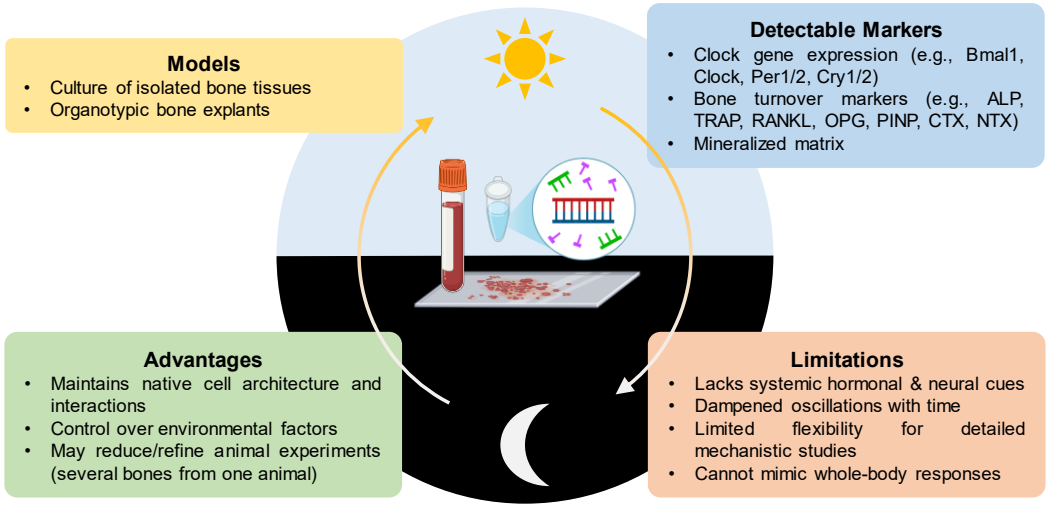

Figure 5. Visualization of ex vivo models. Ex vivo models are useful for studying circadian regulation in bone tissues while preserving some native structure and cell–cell interactions. These models typically involve culturing bone or bone marrow-derived samples to monitor time-dependent changes in clock genes (e.g., *Bmal1, Clock, Per1/2, Cry1/2*) and bone markers like PICP, PINP, ALP, CTX, TRAP, OPG, RANKL, OCN, and NTX. Common methods include qRT-PCR, ELISA, Western blot or dot blot, luciferase assays, and immunostaining. While these systems offer precise control over environmental factors, the absence of systemic cues may limit sustained circadian rhythms compared to in vivo models. Image was created with the help of BioRender icons (https://app.biorender.com/illustrations/69ca989269f3c9dfd83ad093).

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3.3. In Vitro Model Systems

In vitro systems provide a flexible and controlled framework for exploring the molecular and cellular dynamics of circadian rhythms in bone biology. These models typically involve culturing isolated bone cell types such as osteoblasts, osteoclasts, or mesenchymal stromal cells (MSCs) under well-defined laboratory conditions. This setup enables researchers to examine intrinsic circadian oscillations and functional responses without interference from systemic physiological factors. In vitro approaches are commonly categorized into mono-culture and co-culture formats, each providing specific strengths for dissecting particular aspects of bone cell rhythmicity.

3.3.1. Simulating Circadian Rhythms In Vitro

To mimic circadian fluctuations in vitro, cells must be synchronized using external cues known as zeitgebers. Commonly employed zeitgebers include serum starvation followed by serum shock (e.g., 50% fetal bovine serum for 2 hours) [52] or PTH [45]. Once synchronized, the oscillatory expression of core clock genes such as *Bmal1, Clock, Per1/2,* and *Cry1/2* can be tracked over 24 to 72 hours using techniques like qRT-PCR, ELISA, Western blot or dot blotting, or bioluminescence-based real-time reporting in luciferase-tagged cell lines or primary cultures [53].

3.3.2. Functional Insights into Circadian Gene Regulation

In vitro platforms are instrumental in uncovering how circadian genes influence bone cell behavior. For example, knockdown or gene editing of *Bmal1* or *Clock* in osteoblasts has been shown to influence cell apoptosis, cell proliferation, differentiation, and matrix mineralization. These effects are often mediated through pathways such as Wnt, Sirt1, MAPK, and ERK1/2 [54–57]. In osteoclast precursors derived from *Bmal1*-deficient mice, studies have reported an increase in bone loss phenotype [58], partly due to the altered expression of key osteoclast markers such as TRAP and carbonic anhydrase II (CAII) [17]. These findings underscore the pivotal role of circadian genes in regulating osteoclastogenesis [18,59,60].

One noteworthy investigation employed human periodontal ligament fibroblast (PDLF)-like cells cultured under mechanical stress, supplemented with 10% fetal calf serum and varying concentrations of melatonin, a hormone known to modulate circadian rhythms. The study revealed that PDLFs differentiated into osteoclast-like cells, which was suggested to be mediated by melatonin-induced activation of the core clock gene *Bmal1* [61]. Similarly, studies using in vitro cell models have reported that mechanical stress affects the circadian rhythm of skeletal muscle (C2C12 myoblasts) by reducing *Per*/*Cry* gene expression and enhancing *Clock*/*Bmal1* gene expression [62], suggesting that in vitro models may be partly affected by external cues, such as mechanical stimulation.

Co-culture models, which more accurately replicate the in vivo bone microenvironment, enable direct evaluation of osteoblast and osteoclast activity under circadian regulation. These systems utilize a range of functional assays, including resazurin-based metabolic assays, ALP, CAII, and TRAP activity measurements, and Alizarin Red or von Kossa staining for calcium deposition [25,26,63,64]. Collectively, these techniques facilitate dynamic and real-time assessment of how circadian disruption or synchronization influences bone cell viability and functional behavior [60].

In vitro models offer several key advantages that make them particularly useful for circadian research in bone biology. Their experimental versatility, cost-effectiveness, and capacity for precise control over individual variables render them well-suited for mechanistic studies and pharmacological evaluations. The described in vitro systems are particularly effective for evaluating circadian modulators such as melatonin and *REV-ERB* agonists and changes in gene expression, cellular behavior, and signaling pathways relevant to skeletal function [11,59,60]. However, these models also present notable limitations. They lack the physiological complexity of the in vivo environment, including endocrine rhythms, neural regulation, vascular networks, and mechanical loading, all of which are indispensable elements in the accurate replication of the circadian dynamics

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in bone tissue (Figure 6). Additionally, immortalized cell lines often display attenuated or incomplete oscillations of core clock genes, which can compromise their translational relevance [65].

Despite these constraints, in vitro systems remain indispensable for uncovering causal relationships between circadian genes and bone cell activity. In combination with in vivo and ex vivo approaches, they provide important mechanistic insights into how circadian regulation controls skeletal homeostasis and can contribute to the development of chronotherapeutic strategies for the treatment of bone diseases.

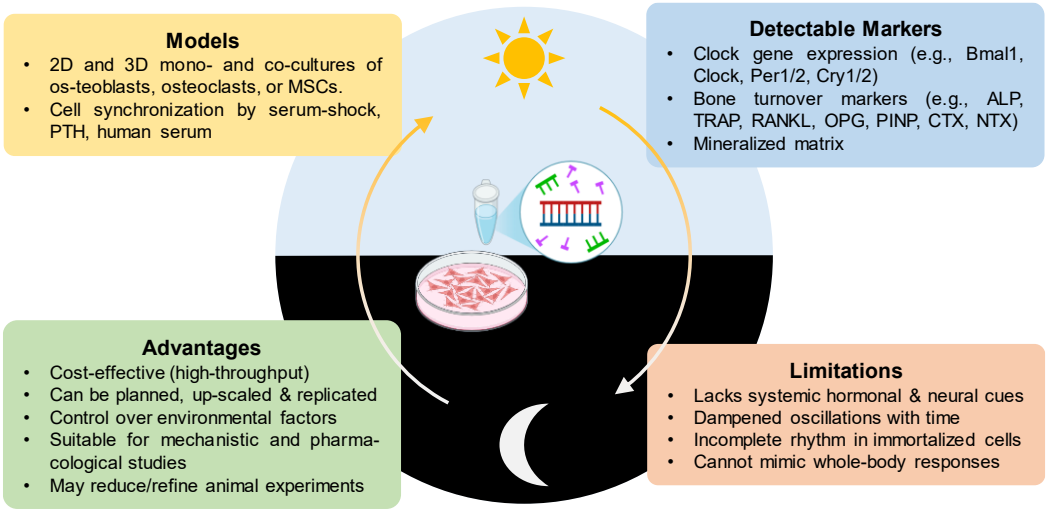

Figure 6. Visualization of in vitro models. In vitro models rely on cultured osteoblasts, osteoclasts, or their co-cultures to investigate circadian regulation at the cellular level. Serum shock is commonly used to synchronize rhythms, allowing analysis of clock genes (*Bmal1*, *Clock*, *Per1/2*, *Cry1/2*) and functional markers such as mitochondrial activity, ALP, TRAP, CAII, and Alizarin Red. These systems are low-cost, scalable, and highly controllable, ideal for mechanistic and drug screening studies. However, they lack systemic inputs, and circadian rhythms may weaken over time, especially in immortalized cell lines. Image was created with the help of BioRender icons (https://app.biorender.com/illustrations/69ca989269f3c9dfd83ad093).

4. Discussion

Investigating circadian rhythms in bone biology requires a strategic balance between cost-efficiency, experimental control, methodological feasibility, translational relevance, and data reliability. The three primary model systems in vitro, ex vivo, and in vivo each offer distinct advantages and limitations, depending on the specific research objectives.

From a cost perspective, in vitro models are the most economical. They require minimal space, equipment, and reagents, and are particularly suitable for high-throughput screening of molecular pathways and pharmacological interventions [66]. Ex vivo models occupy an intermediate option, utilizing animal-derived tissues without incurring the long-term costs associated with maintaining live animals. In contrast, in vivo studies, particularly those involving transgenic mouse lines, are significantly more resource-intensive, especially when extended time courses or large cohorts are required [67].

Experimental control and plannability also vary across model types. In vitro systems offer the highest degree of flexibility and reproducibility, with tightly regulated environmental conditions and straightforward synchronization protocols [45,52,63]. Ex vivo models provide moderate control while preserving native tissue architecture and complexity; however, the circadian rhythm of isolated tissue culture will weaken after a period of time in vitro [51]. In vivo models, however, are subject to greater biological variability and are less amenable to rapid iteration or precise time-point manipulation. Nonetheless, they remain indispensable for capturing systemic factors that cannot be replicated in isolated systems [68].

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Each model supports a distinct set of analytical techniques. In vitro systems enable a wide range of assays for gene and protein expression (e.g., qRT-PCR, ELISA, Western blot, and dot blot), cellular activity (e.g., ALP, CAII, TRAP activity, mitochondrial activity, formation and degradation of mineralized matrix), reporter assays (e.g., luciferase deposition), (immune-)histological and other staining techniques [23,26,63,64]. Ex vivo models are particularly suited for temporal profiling of circadian gene oscillations in intact tissues using bioluminescence (e.g., *Per1:Luc*), histological evaluation, and short-term culture-based assays [45,47–50]. In vivo models facilitate longitudinal assessments of bone structure and function (e.g., micro-CT, bone densitometry, dynamic histomorphometry), as well as systemic biomarker analyses (e.g., serum CTX, PICP, PINP, OPG, RANKL), providing a comprehensive physiological context for studying circadian disruption [17,31].

Reliability is a multifaceted consideration. In vitro studies yield reproducible, mechanistically focused data but may lack the complexity of in vivo circadian regulation. Ex vivo models offer intermediate reliability, preserving native tissue interactions while minimizing systemic confounders. In vivo models, although biologically relevant, are inherently more variable due to genetic, environmental, and behavioral factors. Nevertheless, they remain the gold standard for validating findings derived from cell-based approaches.

A critical challenge in translational circadian biology is the divergence between human and murine circadian patterns. As nocturnal animals, mice exhibit rhythms that differ fundamentally from those of diurnal humans [50,69]. While murine models provide essential mechanistic insights and genetic tools, extrapolation to human physiology must be approached with caution. Emerging research using human cell-based models—such as PDLFs, induced pluripotent stem cell-derived osteoblasts, and in vitro osteoblast–osteoclast co-culture systems [61], alongside clinical chronobiology studies [70,71], is vital for bridging this translational gap.

5. Conclusion

Circadian rhythms are fundamental to bone homeostasis, regulating the activity of osteoblasts and osteoclasts [39,50,72]. Environmental or genetic disruptions can impair bone metabolism and elevate fracture risk [19,73]. A variety of models, from in vivo to in vitro, have been used to explore this relationship, each with unique strengths and limitations as illustrated in Figures 4–6. In this review, we compared circadian dynamics across different models of osteoblast and osteoclast function and emphasized that model selection should be aligned with specific research objectives: in vitro systems for mechanistic and high-throughput studies, ex vivo systems for intermediate complexity, and in vivo models for physiological and systemic relevance, as summarized in Table 2. Ultimately, a multi-model, integrative strategy, supported by incorporating human-relevant systems, real-time monitoring, and time-based therapies, holds the greatest potential for investigating circadian bone biology.

Table 2. Overview of in vivo, ex vivo, and in vitro models for circadian rhythm research in bone cells.

Model Type	What Exists	Advantages	Disadvantages
In vivo	- mouse models with controlled light/dark cycles. - Core clock gene knockout mice (Bmal1, Clock, Per1/2, Cry1/2). - human studies (e.g., astronauts or controlled light environments).	- Reflects systemic rhythms (hormonal, neural, metabolic). - Simultaneous assessment of central and peripheral clocks. - Captures the true physiological environment.	- Species-specific differences in activity (diurnal/nocturnal). - Cost-, time- & labor-intensive. - Limited flexibility for detailed mechanistic studies (detection methods / available tissue). - Ethical restrictions.
Ex vivo	- Culture of isolated bone tissues.	- Maintains native cell architecture and interactions.	- Lacks systemic hormonal and neural cues.

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	- Organotypic bone explants.	- Control over environmental factors (light, temperature). - May reduce/refine animal experiments (several bones from one animal).	- Dampened oscillations with time. - Limited flexibility for detailed mechanistic studies (detection methods / available tissue). - Cannot fully mimic whole-body responses.
In vitro	- 2D and 3D mono-cultures of osteoblasts, osteoclasts, or MSCs. - 2D and 3D co-culture models (osteoblast–osteoclast). - Cell synchronization by serum-shock, PTH, and human serum.	- Cost-effective. - Can be planned, up-scaled & replicated (high-throughput). - Control over environmental factors (light, temperature). - Ideal for mechanistic and pharmacological studies due to a wide selection of detection methods. - May reduce/refine animal experiments.	- Lacks systemic hormonal and neural cues. - Oscillations may reduce with time in primary cells / immortalized cell lines may show incomplete rhythms. - Cannot fully mimic whole-body responses.

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Abbreviations

ALP	alkaline phosphatase
CAII	carbonic anhydrase II
CTX	collagen type I C-telopeptide
ERK	extracellular signal-regulated protein kinases
MSC	mesenchymal stromal cells
NTX	n-terminal cross-linked telopeptide of type I collagen
MAPK	mitogen-activated protein kinase
OPG	osteoprotegerin
OCN	osteocalcin
PICP	carboxy-terminal propeptide of type I procollagen
PINP	procollagen type I N-terminal propeptide
PTH	parathyroid hormone
PDLF	periodontal ligament fibroblast
qRT-PCR	quantitative reverse-transcription polymerase-chain reaction
RANKL	receptor activator of nuclear factor kappa-B ligand
TRAP	tartrate-resistant acidic phosphatase
ZT	zeitgeber Time

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