Implications of the circadian clock in implant dentistry

Hiroko OKAWA¹,², Hiroshi EGUSA² and Ichiro NISHIMURA¹

¹ Weintraub Center for Reconstructive Biotechnology, UCLA School of Dentistry, Los Angeles, CA 90095-1668, USA
² Division of Molecular and Regenerative Prosthodontics, Tohoku University Graduate School of Dentistry, 4-1 Seiryo-cho, Aoba-ku, Sendai, Miyagi 980-8575, Japan
Corresponding authors, Ichiro NISHIMURA; E-mail: inishimura@dentistry.ucla.edu, Hiroshi EGUSA; E-mail: egu@tohoku.ac.jp

Circadian rhythms are approximately 24-h cell-autonomous cycles driven by transcription and translation feedback loops of a set of core circadian clock genes, such as circadian locomotor output cycles kaput (CLOCK), brain and muscle arnt-like protein-1 (Bmal1), period (Per), and cryptochrome (Cry). The genetic clockwork of these genes produces circadian rhythms in cells throughout the body, including the craniofacial region. During development, dento-alveolar bone tissue formation could be regulated by site-specific circadian patterns. Studies using knockout mice and mesenchymal stem cells (MSCs) to evaluate clock genes revealed regulatory effects of clock function on bone remodeling, suggesting involvement of the circadian clockwork in osseointegration of titanium implants. Indeed, rough surface titanium modulates specific clock genes, Neuronal PAS domain protein-2 (Npas2) and Per, in MSCs to facilitate osseointegration. Further understanding of the bone clock machinery associated with biomaterial surface properties might improve preoperative diagnosis for dental implant treatments.

Keywords: Circadian clock, Dental implant, Osseointegration, Stem cells, Titanium

INTRODUCTION

Titanium dental implants are among of the most well-established treatments for missing teeth, with a 10-year survival rate of 90–97%⁸. However, a recent report raised the possibility of overestimation for the previously determined survival rates because a more rigorous meta-analysis demonstrated a potentially high risk of implant loss in older age groups⁹. These findings should particularly be taken into consideration in super-aged societies such as Japan. To improve the success rate of implant treatments in older and medically compromised patients, increased osseointegration may thus still be an important future direction for dental implants. However, the detailed molecular mechanisms of osseointegration are still not well understood.

To date, various osseointegration studies have been performed to evaluate the osteogenic behavior of peri-implant cells and tissues associated with surface properties of implant materials⁵. Comprehensive genome analyses have revealed that specific genetic networks associated with implant placement include not only osteogenic marker genes, but also bone collagen/cross-link genes and cartilage-related extracellular matrix genes⁵. In addition, a whole-genome microarray analysis identified several circadian rhythm-related genes as the genes most significantly affected by titanium implant placement⁶. The circadian rhythm is generally recognized as an internal clock that operates on an approximately 24-h cycle in the body. The discovery of the molecular mechanisms underlying the circadian rhythm resulted in the 2017 Nobel Prize in Physiology or Medicine, awarded to Drs. Jeffrey Hall, Michael Rosbash and Michael Young⁶. The circadian rhythm, appearing at first glance to be hardly related to dental implants, also exists in peripheral bone tissue⁴,⁵,⁷. Indeed, recent studies have suggested a unique role of circadian rhythm-related genes in osseointegration that is regulated by surface properties of titanium materials⁴,⁵,⁷,⁹.

In this review, we first outline the molecular mechanisms of the circadian clock and chronobiology in bone tissues and stem cells. We then describe the peripherally controlled circadian rhythm in bone tissue during osseointegration in association with surface properties of titanium implant materials.

MOLECULAR MACHINERY OF THE CIRCADIAN CLOCK

Mammals synchronize their activities to approximately 24-h cycles set by day and night through a light-sensing neuronal mechanism. The molecular clock controls the circadian rhythms of organs, peripheral tissues, and cells. The master clock located in the suprachiasmatic nucleus (SCN) in the hypothalamus synchronizes peripheral tissue clocks through the neuro-endocrine signals⁵,⁷. The central clock in the SCN is primarily regulated by light/dark cycles, whereas peripheral clocks are also affected by physical stimuli such as feeding⁷. However, the SCN central clock and peripheral clocks essentially share a core molecular mechanism to maintain coordinated time keeping.

The molecular mechanism regulating this 24-h cycle consists of a transcriptional/translational feedback loop orchestrated by clock genes⁷ (Fig. 1). In this feedback
CHRONOBIOLOGY OF CRANIOFACIAL BONE TISSUES

Most craniofacial bones are formed through intramembranous ossification without a cartilage precursor tissue despite having a dual embryonic origin from neural crest cells and paraxial mesoderm. Neonatal calvarial bones are connected by a fibrous suture tissue that undergoes postnatal mineralization. Ex vivo mouse calvarial organ culture has provided a unique model to investigate biological and pathological processes of suture fusion and craniosynostosis. McElderry et al. measured suture mineral deposition in mouse calvarial organ culture every hour for a period of 6 days using automated Raman microscopy. Suture mineralization did not occur constantly but rather exhibited bursts of mineralization, followed by a lag period with no mineral deposition. The average periodicity of suture mineralization was 26.8±9.6 h, and suture mineralization occurred 7 h after the peak expression time of Per1.

Suture mineralization is regulated by protein factors. Gene mutations in fibroblast growth factor receptors (Fgfr1, 2 and 3) and the gene transcription factors Msx2 and Twist, as well as other genetic contributions, have been linked to craniosynostosis. Thus, calvarial gene expression may demonstrate a circadian rhythm. Zvonic et al. examined this possibility. Calvarial bone was harvested from 8-week-old male mice every 4 h for 48 h, and gene expression was determined by whole-genome microarray. Core circadian clock genes such as Bmal1, Neuronal PAS domain protein 2 (Npas2), Per1, Per2, Per3, Cry1, and Cry2 as well as the downstream targets D-box binding protein (Dhp) and Rev-erba showed oscillating expression profiles. This finding confirmed the presence of a functional molecular clock mechanism in adult calvarial bone. Furthermore, the microarray data revealed a circadian expression pattern for more than 26% of the genes tested. KEGG pathway analyses suggested that genes related to cell signaling and proliferation as well as energy and metabolism exhibited a circadian rhythm. In addition, the expression of key genes associated with bone remodeling such as Bone morphogenetic protein (Bmp), Fibroblast Growth Factor (Fgf), Fgf Receptor (Fgfr), and Runt-related transcription factor (Runx) was also found to oscillate. Gafni et al. examined the temporal and spatial expression of osteocalcin in the maxillomandibular complex using a transgenic mouse model carrying a human osteocalcin promoter/luciferase reporter system (hOC-Luc). The bioluminescence derived from hOC-Luc displayed a circadian rhythm, and this circadian pattern of osteocalcin promoter activation was also found in all bone tested. Osteocalcin was previously implicated in the regulation of bone mineralization; however, new insights suggest that it has hormonal effects in regulating glucose metabolism. Osteocalcin is predominantly synthesized and secreted by mature osteoblasts, and thus it has been routinely used as an osteoblastic molecular marker. The oscillatory activation of the osteocalcin promoter suggests that osteoblasts in the skeletal system function under circadian regulation.

Tonna et al. suggested that cells in the alveolar bone periosteum and cementum have a circadian

---

**Fig. 1** Simplified schematic representation of the clockwork mechanism for the core transcriptional/translational feedback loop.

The heterodimeric transcription factors CLOCK and BMAL1 bind to E-box and activate transcription of the Per and Cry genes. The PER and CRY proteins translocate into the nucleus and suppress transcriptional activation by the CLOCK-BMAL1 complex. The CLOCK-BMAL1 complex also activates the transcription of the Rev-Erb nuclear receptor and ROR, which rhythmically regulate the transcription of Bmal1 by binding to RORE.

loop, circadian locomotor output cycles kaput (CLOCK) and brain and muscle arnt-like protein-1 (Bmal1) serve as positive feedback signals. The heterodimeric transcriptional factors CLOCK and BMAL1 activate transcription of period (Per) and cryptochrome (Cry) genes by binding to a cis-regulatory enhancer sequence called the E-box element on target gene promoters. The translated Per and Cry proteins form heterodimers of PER-CRY and translocate into the nucleus to inhibit the transcriptional activation by CLOCK and BMAL1. Therefore, their own transcriptional products suppress Per and Cry gene expression. This negative feedback loop acts as a core circadian regulator to maintain the 24-h rhythm.

Moreover, the CLOCK and BMAL1 complex controls the transcription of reverse orientation c-erb (Rev-erb) and RAR-related orphan receptor (Ror). REV-ERB and ROR bind the orphan receptor response element (RORE) in the Bmal1 promoter, and REV-ERB suppresses Bmal1 gene transcription, whereas ROR enhances this transcription. These subloops contribute to the stabilization of molecular oscillation and regulate approximately 24-h cycles (Fig. 1).
proliferation pattern. In that study, mice were injected with 3H-thymidine one hour prior to euthanasia and maxillary samples were collected every 3 h for 24 h. The proliferation activity was determined by 3H-thymidine incorporation during DNA synthesis at each time point using autoradiography. The external surface of the maxillary alveolar bone periosteum showed a single peak of DNA synthesis activity at 6 pm. However, surprisingly, the inner periosteum and cementum showed double peaks of DNA synthesis. This study indicated potential heterogeneity in site-specific cell proliferation and growth circadian patterns in dentoalveolar bone tissues. Thus, dental and craniofacial chronobiology may be regulated not only by SCN-derived central regulation but also by complex local demand and the site-specific environment.

CIRCADIAN CLOCK IN STEM CELL DIFFERENTIATION

Pluripotent stem cells

Most, if not all, adult cells in humans and mice have a clock function regulated by circadian expression of core clock genes. Embryonic stem cells (ESCs) express core circadian genes as demonstrated by RT-PCR; however, ESCs transduced with Bmal1-dLuc or Per2-dLuc lentiviral luciferase constructs demonstrated bioluminescent signals that did not follow a circadian pattern. This study confirmed the findings of previous studies that demonstrated a lack of functional circadian rhythm in ESCs. Recent studies further identified a significant role of circadian clock molecules in complex cell division cycles and checkpoint33,34). Thus, it has been postulated that the clock molecules have been shown to modulate the cell division cycle, which consists of G1, S, G2, and M stages. Entry into the DNA synthesis (S) and mitosis (M) stages can be terminated by the checkpoint system in the G1 and G2 stages, respectively. The p21 gene is a G1/S checkpoint regulator that is regulated by BMAL1 through Rev-erba response elements (RREs) in hepatocytes. Another checkpoint regulator, p16, acts in the G2/M stages and is regulated by non-POU domain-containing octamer-binding protein (NONO), which was found to be a partner molecule for PER2. Recent studies further identified a significant role of clock molecules in complex cell division cycles and checkpoint. Thus, it has been postulated that the lack of circadian rhythmicity in ESCs may facilitate their proliferation without a “resting” period.

Once ESCs are exposed to a differentiation-guiding environment, a clear circadian expression pattern appears. Yagita et al. exposed ESCs stably expressing the Bmal1:Luc reporter gene to a neuronal differentiation medium containing all-trans retinoic acid and monitored the bioluminescence. After 8 days of differentiation, Bmal1:Luc expression demonstrated a circadian cycle, and persistent circadian cycles developed after 15 days of differentiation. Therefore, clock molecules have been postulated to play a role in cell differentiation. Lu et al. generated Clock-knockout ESCs using CRISPR/Cas9-mediated genetic editing and found that Clock knockout mutation did not affect ESC morphology or the pluripotency of undifferentiated cells, as demonstrated by teratoma formation including all germ layers. However, the Clock-knockout ESCs exhibited spontaneous differentiation, indicating an exit from the pluripotent state into differentiation. These findings suggest that clock genes are critically involved in not only the maintenance of circadian rhythm but also cell differentiation.

Fully differentiated adult fibroblasts can be dedifferentiated via introduction of critical factors such as Oct3/4, Sox2, Klf4, and c-Myc (the so-called “Yamanaka factors”), generating induced pluripotent stem cells (iPSCs). iPSCs exhibit robust proliferation and ESC-like morphology. The source dermal and gingival fibroblasts have a well-established circadian pattern of core clock gene expression, and iPSC induction (reprogramming) diminishes this pattern (Fig. 2A). Umemura et al. reported that ESCs and iPSCs shared a post-translation regulatory mechanism, which contributed to the lack of coordinated circadian rhythm, even though both ESC and iPSC expressed circadian clock genes. Therefore, the coordinated circadian clock function may play an intrinsic role in regulating cellular differentiation.

Adult stem cells

Adult tissues are under constant stress from injury,
inflammation, and infection, as well as from exposure to biomaterials. Adult stem cells are involved in the proper healing and regeneration in response to these stimuli. Mesenchymal stem cells (MSCs) are adult stem cells with multipotent differentiation capability isolated from bone marrow, adipose tissue, amniotic fluid, dental tissues, and other sources. MSCs isolated from the bone marrow of Per1: luc rats demonstrated circadian expression of PER1, as demonstrated by luciferase bioluminescence. The effect of the core circadian genes on phenotypic differentiation of MSCs has been extensively investigated.

Per1/Per2 double-knockout and Cry1/Cry2 double-knockout mice show increased trabecular bone mass and bone mineral apposition rate (BMR), suggesting that the clock mechanism inhibits anabolic bone remodeling. The BMR of Bmal1 knockout mice was similarly increased, however, the cortical and trabecular bone mass was significantly reduced. The low-bone-mass phenotype was recapitulated in MSCs with conditional Bmal1 knockout (Bmal1-Prx1-cre). Abnormal mandibular condyle morphology and mandibular hypoplasia were also demonstrated in Bmal1 knockout mice.

MSCs isolated from global Bmal1 knockout and MSC-specific Bmal1 knockout mice demonstrated marked impairment of in vitro osteogenic differentiation. Chen et al. reported age-related reduction of Bmal1 expression in MSCs isolated from old mice, which also showed decreased proliferation and in vitro osteogenic differentiation as compared to MSCs from young mice. Early-aging phenotypes have also been reported in Bmal1 knockout mice. The circadian expression profile of genes related to the osteogenic differentiation of MSCs suggests that the molecular clock regulates the differentiation of MSCs.

Lentivirus-derived Bmal1 overexpression in mouse fibroblasts (NIH3T3-E1) resulted in significantly increased expression of osteogenic genes such as Runx and osteocalcin. Mechanistic dissection of this model revealed up-regulation of Bmp-2 and β-catenin as well as down-regulation of the Wnt antagonist Dkk1 in MSCs exposed to osteogenic differentiation medium. Down-regulation of Wnt signaling by Bmal1 knockout was also demonstrated in 3T3-L1 pre-adipocytes and C3H10T1/2 MSCs, resulting in inhibition of adipogenic differentiation.

Role of the circadian clock in osteogenic differentiation

The roles of endocrine, paracrine and autocrine effects of bone-related growth factors on bone homeostasis and regeneration have been intensely investigated. The signal transduction of growth factor receptors is partly mediated through cAMP and its response elements. Adenylate cyclase and the cAMP pathway in osteoblasts also mediate the effects of catecholamine receptors such as β-adrenergic receptors (β-ARs). Increased orthotopic bone formation after treatment with “β blockers” was observed in rats and humans, and initially postulated to be due to modulation of cAMP pathway. However, the hypothalamic-autonomous nervous system (ANS) was found to directly regulate osteoblasts by activating β-ARs. Leptin, a hormone derived from adipocytes, activates leptin receptor-bearing neurons such as proopiomelanocortin (POMC)/cocaine- and amphetamine-regulated transcript (CART)-containing neurons in the hypothalamus. Leptin-influenced hypothalamic activity modulates vertebral and appendicular bone remodeling through the sympathetic arm of the ANS, causing reduction of bone turnover in leptin-deficient obese mice.

The ANS-derived regulation of bone homeostasis has been termed “neuroskeletal” regulation. The hypothalamic ANS signal to β2-ARs activates cAMP response element-binding protein (CREB), which induces transcription of downstream circadian clock genes including Per1 and Per2. Per1/Per2 double-knockout mutation reversed the effects of a β2-AR agonist and increased the vertebral and appendicular bone volume. The effects of environmental stimuli on osteogenic differentiation thus appear to be at least partly mediated by the cellular circadian clock (Fig. 2).

The core clock transcriptional factors regulate the expression of not only core clock genes but also non-core clock genes called clock-controlled genes (CCGs). The tissue-specific behavior of MSCs may be influenced by core clock gene expression through the CCG repertoire. The understanding of this mechanism is still incomplete; however, as compared to bone marrow- and adipose-derived MSCs, dental pulp-derived MSCs were found to exhibit robust upregulation of Per1, Bmal1, and Rev-erba. Therefore, it is tempting to speculate that the trait-specific differentiation of MSCs may be regulated by the interaction between the niche environment and endogenous molecular clock mechanism.

![Fig. 3 Simplified schematic drawing of the role of circadian-controlled genes (CCGs) in bone tissue.](image-url)
CIRCADIAN RHYTHM AND OSSEOINTEGRATION

Environmental stimulation after dental implant placement

The placement of titanium implants generates a unique cellular environment that has been postulated to increase osteogenic differentiation of bone marrow MSCs. Increased osteogenic differentiation has been demonstrated for MSCs exposed to a variety of titanium substrates in vitro\(^{11,70}\). However, when the effect of titanium substrates on the whole-genome transcriptome of MSCs was evaluated, the expression of genes associated with osteogenic differentiation was surprisingly less affected than that of other genes (reviewed in\(^{90}\)). A whole-genome microarray analysis of peri-implant tissue in rats found that differential expression of circadian rhythm genes was most strongly associated with titanium implant placement\(^6\). In particular, Npas2 and Bmal1 were significantly upregulated, whereas Per2 was significantly downregulated.

Ti4V6AI with dual-acid etching and the discrete crystalline deposition of hydroxyapatite nanoparticles was the titanium biomaterial used in the above study\(^6\). Titanium biomaterials with complex surfaces preferentially alter the expression of specific circadian genes compared to those with a simple machined surface\(^{5,9}\). A complex titanium surface (generated by sandblasting and double acid etching followed by discrete apposition of hydroxyapatite nanoparticles), but not a smooth surface (machined titanium surface with polishing), significantly upregulated Npas2 and downregulated Per1 in human MSCs when compared to a polypropylene surface\(^9\). Human MSCs cultured on titanium discs with another rough surface modification (SLA: sandblasting with large grits and acid etched) showed significantly increased Npas2 expression and also markedly suppressed Per2 expression compared to MSCs cultured on a control polystyrene culture plate or titanium discs with a smooth surface\(^9\). Titanium implants with complex surfaces seem to preferentially activate the expression of circadian rhythm-related genes around the implant.

Molecular mechanism of Npas2 up-regulation by titanium biomaterials

It remains unclear how titanium implants modulate the molecular clock mechanism and particularly how they up-regulate Npas2. Mutant mouse models are effective in addressing the specific function of identified genes\(^{77}\), whereas the causal molecular mechanism may be identified by chemical genomic analyses in an unbiased fashion. Such analyses utilize high-throughput screening of a large number of chemical compounds. Libraries of hit compounds are used to determine putative molecular targets\(^{78,79}\).

Mouse MSCs carrying the Npas2-LacZ reporter gene system were used for high-throughput screening of pharmacologically active compounds (LOPAC)\(^9\). A chemical genomics analysis of the identified compounds revealed involvement in signal transduction related to the α-adrenergic receptor (α-AR)\(^9\). MSCs have been shown to predominantly express β-ARs, particularly β2-ARs, during osteogenic differentiation\(^{80}\). As described above, β2-ARs mediate sympathetic signaling and induce osteogenic gene transcription through cAMP and CREB pathways\(^{81,83}\). Strikingly, when MSCs were cultured on titanium discs with a complex surface, α-AR expression was significantly increased, whereas β2-AR expression was unaffected\(^9\). β-AR antagonists and α-AR agonists are commonly used for treating hypertension. In recent clinical and animal studies, anti-hypertension drugs significantly increased dental implant survival\(^{82}\) and improved osseointegration\(^{83}\). Therefore, it has been hypothesized that environmental cues from titanium biomaterials induce selective upregulation of α-ARs in MSCs to modulate their differentiation, enabling osseointegration.

Unique role of Npas2 during osseointegration

Osseointegration of an experimental SLA implant was tested in femur bones of wild-type and Npas2 knockout (KO) mutant mice\(^9\). The biomechanical withholding load of the femur implants, as measured by implant push-out force, was reduced in Npas2 KO mice. Uniquely, bone histology and bone-to-implant contact (BIC) were unaffected in Npas2 KO mice. Therefore, loss of bonding of bone to Ti biomaterials was thought to be the primary mechanism underlying the decrease in biomechanical withholding load.

NPAS2 is a b-HLH transcription factor and considered orthologous to CLOCK due to its genetic sequence similarity\(^{84}\). As b-HLH factors, NPAS2 and CLOCK form a dimeric protein complex with BMAL1 to activate gene transcription. Whereas CLOCK-BMAL1 dimers play the dominant role in maintaining circadian rhythm, NPAS2 is a redundant molecule that serves as a backup system for CLOCK\(^{85,86}\). In the absence of CLOCK, NPAS2 is thought to serve as a substitute to form the heterodimer NPAS2-BMAL1\(^{87}\).

MSCs cultured on titanium biomaterials with complex surfaces consistently showed increased expression of Npas2 but not Bmal1 and CLOCK\(^{8,9}\). Coordinated gene expression plays a pivotal role in cell proliferation and differentiation. Gene expression profiling may thus provide insights into cellular behavior. Weighted gene co-expression network analysis (WGCNA)\(^{88}\) of microarray data for peri-implant tissue revealed co-expression of Bmal1 and CLOCK in naïve bone. Titanium implantation significantly rearranged the co-expression landscape, and Npas2 became a partner gene of the Bmal1-containing gene cluster\(^8\). Therefore, the gene co-expression data strongly suggest that NPAS2 should become a preferential molecular partner of BMAL1 in the presence of titanium implants.

Chromatin immunoprecipitation and DNA sequencing (ChIP-SEQ) of mouse hepatocytes revealed 5,900 BMAL1 sites, 4,600 CLOCK sites and 2,300 Npas2 sites\(^{89}\). It has been postulated that the CLOCK-BMAL1 dimer and NPAS2-BMAL1 dimer activate different sets of genes. Many CCGs are under clock
that NPAS2 is a gas-sensing transcription factor91,92). Only observed for BMAL1-NPAS2-HEME, suggesting carbon monoxide-induced hypoxia, DNA binding activity was diatomic gases under hypoxic conditions. Under carbon bind to HEME, which coordinates the transportation of also suggests this possibility. NPAS2 has been shown to increased DNA binding activity of NPAS2. It therefore seems likely that the micro-environment created by Ti increased under oxidation-reduction conditions93,94).

The DNA binding activity of NPAS2 was also shown to increase under oxidation-reduction conditions93,94). The upregulation of hypoxia inducible factor (HIF)-1α is a co-founder and consultant of ABR LLC and CLIN Inc. The surface of titanium biomaterials undergoes oxidation, resulting in the formation of a TiO2 layer. Thus, the microenvironment around titanium biomaterials may be characterized as O2-deficient, or hypoxic95. The upregulation of hypoxia inducible factor (HIF)-1α signaling in MSCs exposed to titanium biomaterials90) also suggests this possibility. NPAS2 has been shown to bind to HEME, which coordinates the transportation of diatomic gases under hypoxic conditions. Under carbon monoxide-induced hypoxia, DNA binding activity was only observed for BMAL1-NPAS2-HEME, suggesting that NPAS2 is a gas-sensing transcription factor90,92). The DNA binding activity of NPAS2 was also shown to increase under oxidation-reduction conditions93,94). Thus, it has been postulated that implantation of titanium biomaterials may induce multiple effects on Npas2-expressing MSCs, leading to upregulation and increased DNA binding activity of NPAS2. It therefore seems likely that the micro-environment created by Ti biomaterials favors the function of NPAS2, which may activate the coordinated expression of CCGs mediating bone and implant bonding.

CONCLUSION

Growing evidence has demonstrated the importance of the peripheral circadian rhythm system in osseointegration. Titanium biomaterials substantially affect networks of circadian clock genes, which are proposed to regulate the establishment of osseointegration. We believe that a complete understanding of molecular clock machinery from both host and material sides during osseointegration is crucial to design new and more effective clinical strategies for dental implant treatments.

CONFLICTS OF INTEREST

Nishimura received in-kind and research funding support from NeoBiotech, USA and Zimmer Biomet. Nishimura is a consultant to FUJIFILM Corp and BioVinc LLC and a co-founder and consultant of ABR LLC and CLIN Inc.

REFERENCES

1) Howe MS, Keys W, Richards D. Long-term (10-year) dental implant survival: A systematic review and sensitivity meta-analysis. J Dent 2019; 84: 9-21.
2) Shibata Y, Tanimoto Y. A review of improved fixation methods for dental implants. Part I: Surface optimization for rapid osseointegration. J Prosthodont Res 2015; 59: 20-33.
3) Nishimura I. Genetic networks in osseointegration. J Dent Res 2013; 92: 1098-1108.
4) Mengatto CM, Mussano F, Honda Y, Colwell CS, Nishimura I. Circadian rhythm and cartilage extracellular matrix genes in osseointegration: a genome-wide screening of implant failure by vitamin D deficiency. PLoS One 2011; 6: e15848.
5) Huang RC. The discoveries of molecular mechanisms for the circadian rhythm: The 2017 Nobel Prize in Physiology or Medicine. Biomed J 2018; 41: 5-8.
6) Zvonie S, Ptitsyn AA, Kilroy G, Wu X, Conrad SA, Scott LK, et al. Circadian oscillation of gene expression in murine calvarial bone. J Bone Miner Res 2007; 22: 357-365.
7) McElderry JD, Zhao G, Khmaladze A, Wilson CG, Franceschi RT, Morris MD. Tracking circadian rhythms of bone mineral deposition in murine calvarial organ cultures. J Bone Miner Res 2013; 28: 1846-1854.
8) Hassan N, McCarville K, Morinaga K, Mengatto CM, Langfelder P, Hokugo A, et al. Titanium biomaterials with complex surfaces induced aberrant peripheral circadian rhythms in bone marrow mesenchymal stromal cells. PLoS One 2017; 12: e0183359.
9) Morinaga K, Sasaki H, Park S, Hokugo A, Okawa H, Tahara Y, et al. Neuronal PAS domain 2 (Npas2) facilitated osseointegration of titanium implant with rough surface through a neuroskeletal mechanism. Biomaterials 2019; 192: 62-74.
10) Weaver DR. The suprachiasmatic nucleus: a 25-year retrospective. J Biol Rhythms 1998; 13: 100-112.
11) Damolla F, Le Minh N, Preitner N, Kornmann B, Fleury-Olela F, Schibler U. Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. Genes Dev 2000; 14: 2950-2961.
12) Dunlap JC. Molecular bases for circadian clocks. Cell 1999; 96: 271-290.
13) Gekakis N, Staknis D, Nguyen HB, Davis FC, Wilsbacher LD, King DP, et al. Role of the CLOCK protein in the mammalian circadian mechanism. Science 1998; 280: 1564-1569.
14) Shearman LP, Siriram S, Weaver DR, Maywood ES, Chaves I, Zheng B, et al. Interacting molecular loops in the mammalian circadian clock. Science 2000; 288: 1013-1019.
15) Brown SA, Kowalska E, Dallmann R. (Re)inventing the circadian feedback loop. Dev Cell 2012; 22: 477-487.
16) Guillaumond F, Dardente H, Giguere V, Cermakian N. Differential control of Bmal1 circadian transcription by REV-ERB and ROR nuclear receptors. J Biol Rhythms 2005; 20: 391-403.

17) Ferguson JW, Atit RP. A tale of two cities: The genetic mechanisms governing calvarial bone development. Genesis 2019; 57: e23248.

18) Melville H, Wang Y, Taub PJ, Jabs EW. Genetic basis of potential therapeutic strategies for craniosynostosis. Am J Med Genet A 2010; 152A: 3007-3015.

19) Rice DP, Rice R, Thesleff I. Molecular mechanisms in calvarial bone and suture development, and their relation to craniosynostosis. Eur J Orthod 2003; 25: 139-148.

20) Goos JAC, Mathijssen IMJ. Genetic causes of craniosynostosis: An update. Mol Syndromol 2019; 10: 6-23.

21) Wilkie AOM, Johnson D, Wall SA. Clinical genetics of craniosynostosis. Curr Opin Pediatr 2017; 29: 622-628.

22) Gafni Y, Pitsyn AA, Zilberman Y, Pelleg G, Gimble JM, Gazit D. Circadian rhythm of osteocalcin in the maxillomandibular complex. J Dent Res 2009; 88: 45-50.

23) Zoch ML, Clemens TL, Riddle RC. New insights into the biology of osteocalcin. Bone 2016; 82: 42-49.

24) Wei J, Karsenty G. An overview of the metabolic functions of osteocalcin. Rev Endocr Metab Disord 2015; 16: 93-98.

25) Tonna EA, Singh IJ, Sandhu HS. Autoradiographic study of bone marrow stromal cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006; 126: 663-676.

26) Dierickx P, Vermunt MW, Muraro MJ, Creyghton MP, Samsa WE, Vasanjai A, Midura RD, Kondratov RV. Deficiency of circadian clock protein BMAL1 in mice results in a low bone mass phenotype. Bone 2016; 84: 194-203.

27) Yagita K, Horie K, Koinuma S, Nakamura W, Yamanaka S, et al. Role of circadian clock protein BMAL1 in mice results in a low bone mass phenotype. Bone 2016; 84: 194-203.

28) Takarada T, Xu C, Ochi H, Nakazato R, Yamada D, Nakamura S, et al. Bone resorption is regulated by circadian clock in osteoblasts. J Bone Miner Res 2017; 32: 872-881.

29) Tsang K, Liu H, Yang Y, Charles JF, Ermann J. Defective circadian control in mesenchymal cells reduces adult bone mass in mice by promoting osteoclast function. Bone 2019; 121: 172-180.

30) Hirai S, Hayashi Y, Ito M, Amemiya T, Dezawa K, Arai Y, et al. Micro-CT observation of in vivo temporal change in mandibular condyle morphology in BMAL1 knockout mice. J Oral Sci 2018; 60: 473-478.

31) Zhao J, Zhou X, Tang Q, Yu R, Yu S, Long Y, et al. BMAL1 deficiency contributes to mandibular dysplasia by upregulating MMP3. Stem Cell Reports 2018; 10: 180-195.

32) Chen Y, Xu X, Tan Z, Ye C, Zhao Q, Chen Y. Age-related BMAL1 change affects mouse bone marrow stromal cell proliferation and osteo-differentiation potential. Arch Med Sci 2012; 8: 30-38.

33) Kondratov RV, Kondratova AA, Gorbacheva VV, Vykhovanets OV, Antoch MP. Early aging and age-related pathologies in mice deficient in BMAL1, the core component of the circadian clock. Genes Dev 2006; 20: 1868-1873.

34) Bruner MK, Walisser JA, Sullivan R, Manley PA, Moran SM, Kalscheur VL, et al. Progressive arthropathy in mice with a targeted disruption of the Mops3/Bmal1 locus. Genesis 2005; 41: 122-132.

35) Dudek M, Meng QJ. Running on time: the role of circadian clocks in the musculoskeletal system. Biochem J 2014; 463: 1-8.

36) Guntur AR, Kawai M, Le P, Bouxsein ML, Bornstein S, Green CB, et al. An essential role for the circadian-regulated gene nocturnin in osteogenesis: the importance of local timekeeping in skeletal homeostasis. Ann N Y Acad Sci 2011; 1237: 58-63.

37) Takahashi K, Yamazaki S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006; 126: 663-676.

38) Egusa H, Okita K, Kayashima H, Yu G, Fukuyasu S, Saeki M, et al. Gingival fibroblasts as a promising source of induced pluripotent stem cells. PLoS One 2010; 5: e12743.

39) Sasaki H, Hokugo A, Wang L, Morinaga K, Ngo JT, Okawa H, et al. Neuronal PAS domain 2 (Npas2)-deficient fibroblasts accelerate skin wound healing and dermal collagen reconstruction. Anat Rec (Hoboken) 2019; doi: 10.1002/ar.24109.

40) Sandu C, Liu T, Malan A, Challet E, Pevet P, Felder-Schmittbuhl MP. Circadian clocks in rat skin and dermal fibroblasts: differential effects of aging, temperature and melatonin. Cell Mol Life Sci 2015; 72: 2237-2248.

41) Hoyle NP, Seinikmane E, Putker M, Feeney KA, Krogger TP, Chesham JE, et al. Circadian actin dynamics drive rhythmic fibroblast mobilization during wound healing. Sci Transl Med 2017; 9: eaai2774.

42) Fu L, Patel MS, Bradley A, Wagner EF, Karsenty G. The molecular clock mediates leptin-regulated bone formation. Cell 2005; 122: 803-815.

43) Sasaki H, Hokugo A, Wang L, Morinaga K, Ngo JT, Okawa H, et al. Molecular clock mediates leptin-regulated bone formation. Cell 2005; 122: 803-815.

44) Hirai S, Hayashi Y, Ito M, Amemiya T, Dezawa K, Arai Y, et al. Micro-CT observation of in vivo temporal change in mandibular condyle morphology in BMAL1 knockout mice. J Oral Sci 2018; 60: 473-478.

45) Zhao J, Zhou X, Tang Q, Yu R, Yu S, Long Y, et al. BMAL1 deficiency contributes to mandibular dysplasia by upregulating MMP3. Stem Cell Reports 2018; 10: 180-195.

46) Chen Y, Xu X, Tan Z, Ye C, Zhao Q, Chen Y. Age-related BMAL1 change affects mouse bone marrow stromal cell proliferation and osteo-differentiation potential. Arch Med Sci 2012; 8: 30-38.

47) Kondratov RV, Kondratova AA, Gorbacheva VV, Vykhovanets OV, Antoch MP. Early aging and age-related pathologies in mice deficient in BMAL1, the core component of the circadian clock. Genes Dev 2006; 20: 1868-1873.

48) Bruner MK, Walisser JA, Sullivan R, Manley PA, Moran SM, Kalscheur VL, et al. Progressive arthropathy in mice with a targeted disruption of the Mops3/Bmal1 locus. Genesis 2005; 41: 122-132.

49) Dudek M, Meng QJ. Running on time: the role of circadian clocks in the musculoskeletal system. Biochem J 2014; 463: 1-8.

50) Guntur AR, Kawai M, Le P, Bouxsein ML, Bornstein S, Green CB, et al. An essential role for the circadian-regulated gene nocturnin in osteogenesis: the importance of local timekeeping in skeletal homeostasis. Ann N Y Acad Sci 2011; 1237: 58-63.

51) Min HY, Kim KM, Wee G, Kim EJ, Jang WG. Bmal1 induces osteoblast differentiation via regulation of BMP2 expression in MC3T3-E1 cells. Life Sci 2016; 162: 41-46.

52) Lin F, Chen Y, Li X, Zhao Q, Tan Z. Over-expression of circadian clock protein Bmal1 affects mouse bone marrow stromal cell proliferation and osteo-differentiation potential. Arch Med Sci 2012; 8: 30-38.

53) Min HY, Kim KM, Wee G, Kim EJ, Jang WG. Bmal1 induces osteoblast differentiation via regulation of BMP2 expression in MC3T3-E1 cells. Life Sci 2016; 162: 41-46.

54) Lin F, Chen Y, Li X, Zhao Q, Tan Z. Roles of brain and muscle ARNT-like 1 and Wnt antagonist Dkk1 during osteogenesis of bone marrow stromal cells. Cell Proli 2013; 46: 644-653.
56) Guo B, Chatterjee S, Li L, Kim JM, Lee J, Yeohor VK, et al. The clock gene, brain and muscle Arnt-like 1, regulates adipogenesis via Wnt signaling pathway. FASEB J 2012; 26: 3453-3463.

57) Mezawa M, Araki S, Takai H, Sasaki Y, Wang S, Li X, et al. Regulation of human bone sialoprotein gene transcription by platelet-derived growth factor-BB. Gene 2009; 435: 80-87.

58) Liou SF, Hsu JH, Chu HC, Lin HH, Chen IJ, Yeh JL. KMUP-1 promotes osteoblast differentiation through cAMP and cGMP pathways and signaling of BMP-2/Smad1/5/8 and Wnt/beta-Catenin. J Cell Physiol 2015; 230: 2038-2048.

59) Zhou W, Yu L, Fan J, Wan B, Jiang T, Yin J, et al. Endogenous parathyroid hormone promotes fracture healing by increasing expression of BMPR2 through cAMP/PKA/CREB pathway in mice. Cell Physiol Biochem 2017; 42: 551-563.

60) Minkowitz B, Boskey AL, Lane JM, Pearlman HS, Vigorita VJ. Effects of propranolol on bone metabolism in the rat. J Orthop Res 1991; 9: 869-875.

61) Turker S, Karatosun V, Gunal I. Beta-blockers increase bone mineral density. Clin Orthop Relat Res 2006; 443: 73-74.

62) Pasco JA, Henry MJ, Sanders KM, Kotowicz MA, Seeman E, Minkowitz B, Boskey AL, Lane JM, Pearlman HS, Vigorita VJ. Effects of propranolol on bone metabolism in the rat. J Orthop Res 1991; 9: 869-875.

63) Patel MS, Elefteriou F. The new field of neuroskeletal biology. Calcif Tissue Int 2007; 80: 337-347.

64) Rosen CJ. Bone remodeling, energy metabolism, and the molecular clock. Cell Metab 2008; 7: 7-10.

65) Fleet JC. Leptin and bone: does the brain control bone biology? Biochim Biophys Acta 2014; 1840: 354-355.

66) Meister B. Control of food intake via leptin receptors in the hypothalamus. Vitam Horm 2000; 59: 265-304.

67) Karsenty G. Convergence between bone and energy homeostasis: leptin regulation of bone mass. Cell Metab 2006; 4: 341-348.

68) Meister B. Control of food intake via leptin receptors in the hypothalamus. Vitam Horm 2000; 59: 265-304.

69) Ueda HR, Matsumoto A, Kawamura M, Iino M, Tanimura T, Hashimoto S. Genome-wide transcriptional orchestration of circadian rhythms in Drosophila. J Biol Chem 2002; 277: 14048-14052.

70) Rogers EH, Fawcett SA, Pekovic-Vaughan V, Hunt JA. Comparing circadian dynamics in primary derived stem cells from different sources of human adult tissue. Stem Cells Int 2017; 2017: 2057168.

71) Olivares-Navarrete R, Hyzy SL, Hailthcock DA, Cundiff CA, Schwartz Z, Boyan BD. Coordinated regulation of mesenchymal stem cell differentiation on microstructured titanium surfaces by endogenous bone morphogenetic proteins. Bone 2015; 73: 208-216.

72) McCafferty MM, Burke GA, Meenan BJ. Mesenchymal stem cell response to conformal sputter deposited calcium phosphate thin films on nanostructured titanium surfaces. J Biomed Mater Res A 2014; 102: 3585-3597.

73) Cowden K, Dias-Netipanji MF, Popat KC. Effects of titanium nanotube surfaces on osteogenic differentiation of human adipose-derived stem cells. Nanomedicine 2019; 17: 380-390.

74) Petecchia L, Usai C, Vassalli M, Gavazzo P. Biophysical characterization of nanostructured TiO2 as a good substrate for hBM-MSC adhesion, growth and differentiation. Exp Cell Res 2017; 358: 111-119.

75) Lotz EM, Olivares-Navarrete R, Berner S, Boyan BD, Schwartz Z. Osteogenic response of human MSCs and osteoblasts to hydrophilic and hydrophobic nanostructured titanium implant surfaces. J Biomed Mater Res A 2016; 104: 3137-3148.

76) Hayashi R, Ueno T, Migita S, Tsutsumi Y, Doi H, Ogawa T, et al. Hydrocarbon deposition attenuates osteoblast activity on titanium. J Dent Res 2014; 93: 698-703.

77) Fontaine DA, Davis DB. Attention to background strain is essential for metabolic research: C57BL/6 and the international knockout mouse consortium. Diabetes 2016; 65: 25-33.

78) Chan TF, Carvalho J, Riles L, Zheng XF. A chemical genomics approach toward understanding the global functions of the target of rapamycin protein (TOR). Proc Natl Acad Sci U S A 2006; 97: 13227-13232.

79) MacBeath G. Chemical genomics: what will it take and who gets to play? Genome Biol 2001; 2: COMMENT2005.

80) Li H, Fong C, Chen Y, Cai G, Yang M. beta2- and beta3-, but not beta1-adrenergic receptors are involved in osteogenesis of mouse mesenchymal stem cells via cAMP/PKA signaling. Arch Biochem Biophys 2010; 496: 77-83.

81) Spencer GJ, Hitchcock IS, Genever PG. Emerging neuroskeletal signalling pathways: a review. FEBS Lett 2004; 559: 6-12.

82) Wu X, Al-Abedalla K, Eimar H, Arekumath Madathil S, Abi-Nader S, Daniel NG, et al. Antihypertensive medications and the survival rate of osseointegrated dental implants: A cohort study. Clin Implant Dent Relat Res 2016; 18: 1171-1182.

83) Al-Subaie AE, Laurenti M, Abdallah MN, Tamimi I, Yaghoubi F, Eimar H, et al. Propranolol enhances bone healing and implant osseointegration in rats tibiae. J Clin Periodontol 2016; 43: 1180-1170.

84) Reick M, Garcia JA, Dudley C, McKnight SL. NPAS2: an analog of clock operative in the mammalian forebrain. Science 2001; 293: 506-509.

85) DeBruyne JP, Weaver DR, Reppert SM. CLOCK and NPAS2 have overlapping roles in the suprachiasmatic circadian clock. Nat Neurosci 2007; 10: 543-545.

86) Bertolucci C, Cavallari N, Colognesi I, Aguzzi J, Chen Z, Caruso P, et al. Evidence for an overlapping role of CLOCK and NPAS2 transcription factors in liver circadian oscillators. Mol Cell Biol 2008; 28: 3070-3075.

87) Asher G, Schibler U. A CLOCK-less clock. Trends Cell Biol 2006; 16: 547-549.

88) Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics 2008; 9: 559.

89) Koike N, Yoo SH, Huang HC, Kumar V, Lee C, Kim TK, et al. Transcriptional architecture and chromatin landscape of the core circadian clock in mammals. Science 2012; 338: 349-354.

90) Ran Q, Yu Y, Chen W, Shen X, Mu C, Yuan Z, et al. Deferoxamine loaded titania nanotubes substrates regulate osteogenic and angiogenic differentiation of MSCs via activation of HIF-1alpha signaling. Mater Sci Eng C Mater Biol Appl 2018; 91: 44-54.

91) Dioum EM, Rutter J, Tuckerman JR, Gonzalez G, Gilles-Gonzalez MA, McKnight SL. NPAS2: a gas-responsive transcription factor. Science 2002; 293: 506-509.

92) Ascenzi P, Bocchi A, Leonl L, Visca P, Zennaro E, Milani M, et al. CO sensing through hemi-based sensor proteins. JIBMB Life 2004; 56: 309-315.

93) Rutter J, Reick M, Wu LC, McKnight SL. Regulation of clock and NPAS2 DNA binding by the redox state of NAD cofactors. J Biol Chem 2006; 281: 3137-3148.