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REVIEW

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Circadian rhythms in stem cells and their therapeutic potential

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Abstract

Circadian rhythms are present in almost all cells, but their existence in stem cells has remains not well established. Circadian clock appears to be closely associated with differentiated mature cells and rarely detected in immature embryonic stem cells. Recent evidence reveals the presence of circadian genes and rhythmic physiologic activities in stem cells as well as stem cell-derived extracellular vesicle (EV) characteristics. The circadian clock entails diverse physiologic and pathological mechanisms underlying cell fate. Integration of circadian rhythm to clinical applications, such as chronotherapy, chrono-biomarker, and environment modification, may facilitate therapeutic outcomes of stem cell-based regenerative medicine. Understanding circadian rhythms in stem cells can optimize stem cell-based therapies by determining the best times for harvesting and administering stem cells, thereby enhancing therapeutic efficacy. Further research into the circadian properties of stem cells will refine stem cell-based therapies, contributing to advancements in regenerative medicine.

Keywords Biological clock, Synchronization, Stem cell therapy, Neurological disorders

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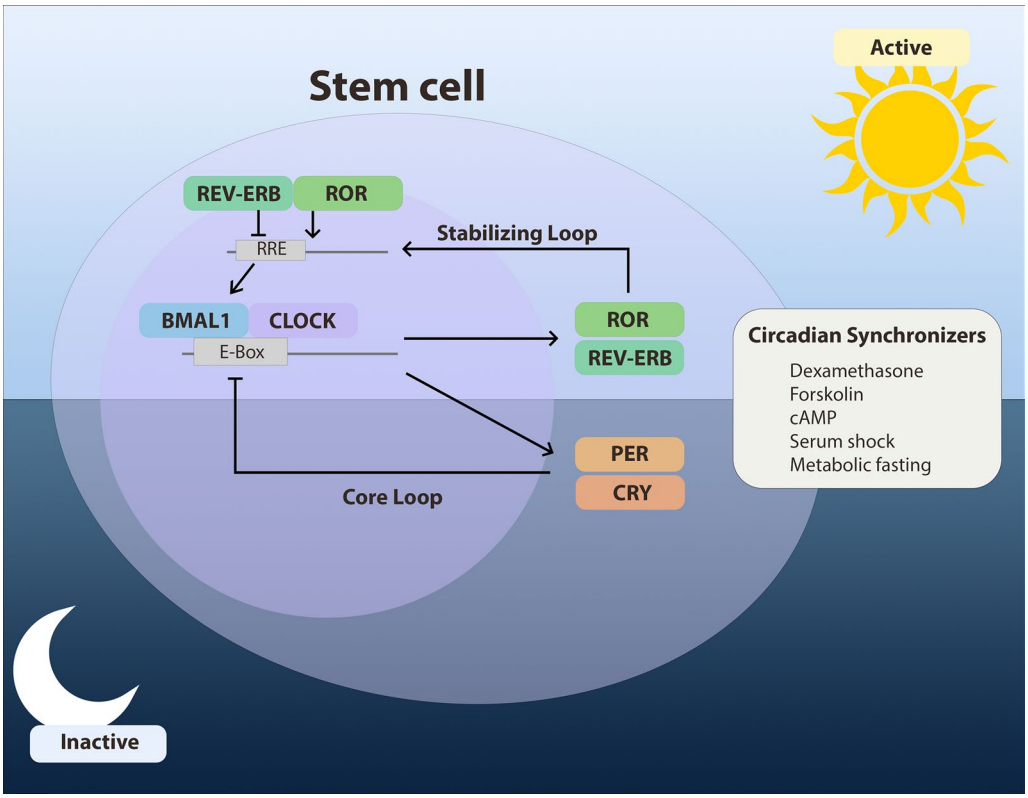
Graphical abstract



Introduction

The concept of the circadian clock dates back to 1729 when it was observed that the plant *Mimosa pudica* displayed self-sustaining movement cycles even in the absence of light [1]. This observation set the stage for subsequent pivotal research on circadian rhythms. In 2017, the Nobel Prize in Physiology or Medicine was conferred upon Jeffrey C. Hall, Michael Rosbash, and Michael W. Young for their pioneering research on the molecular mechanisms that govern circadian rhythms [2]. These self-sustaining oscillatory activities are inherent in nearly every cell in the body, with a substantial number of genes in peripheral tissues exhibiting circadian expression patterns [3–7]. The distinct circadian rhythms in different tissues become particularly pronounced during development [8]. In mammals, the suprachiasmatic nucleus (SCN) serves as the central regulator, maintaining a cycle of approximately 24 h and coordinating the synchronization of biological clocks across various tissues [9]. Notably, SCN destruction and transplantation can respectively disrupt and restore circadian rhythms [10–12].

Despite discoveries of circadian rhythm in almost all types of cells, circadian rhythm in stem cells remains controversial. Bioluminescence studies of well-known clock genes in multiple embryonic cell lines demonstrate no oscillation [13]. Reprogramming differentiated cells into induced pluripotent stem cells (iPSCs) also disrupts circadian oscillations, leading to a hypothesis that circadian clock regulators mature during differentiation. Furthermore, the expression of genes in the circadian core loop varies significantly between embryonic and differentiated states [14, 15]. Conversely, stem cells exhibit oscillating metabolic activity and proliferative rate, suggesting the presence of a circadian clock even in the embryonic stage [16, 17]. Furthermore, several studies suggest that circadian clock genes are not essential for cellular circadian oscillation, implying regulation via non-canonical pathways [18, 19]. These findings underscore the intricate nature of circadian regulation in stem cells, positing that circadian rhythms may exist in stem cells.

Circadian rhythm modulates both physiological and pathological processes, encompassing the regulation of the sleep–wake cycle, hormone secretion, metabolism,

cell division, and cell movement [6, 20–22]. The circadian clock is involved in the pathogenesis of various conditions, including ischemic stroke, Alzheimer's disease, Parkinson's disease, Huntington's disease, and post-traumatic stress disorder [23, 24]. Circadian rhythms can be harnessed to optimize stem cell therapy. Selective timing of stem cell and EV harvest, as well as administration, can improve treatment outcomes. The use of circadian synchronizers, such as dexamethasone, forskolin, and serum shock, holds the potential for optimizing regimens. Additionally, circadian alignment through timed light exposure and melatonin can further improve neurodegenerative disorders and possibly affect the progression of neurodegeneration by restoring circadian homeostasis [23].

This review aims to capture recent advances in circadian rhythm research in stem cells. We also highlight the implications of the circadian clock in clinical applications for neurological disorders. Understanding the pattern of varying stem cell activities can lead to treatment optimization. By aligning stem cell treatments with the body's natural circadian rhythms or selecting the most active phase of stem cells, we can improve therapeutic outcomes and reduce their side effects. Integrating circadian biology into regenerative medicine for brain diseases paves the way for innovative approaches that leverage the natural timing systems of the body to maximize therapeutic benefits (Fig. 1).

Molecular clockwork of circadian rhythm

The circadian clock's transcriptional–translational feedback loops (TTFL) comprise a core loop and a stabilizing loop [6] (Fig. 1). In the core loop, the circadian locomotor output cycles kaput (CLOCK) and Basic Helix-Loop-Helix ARNT Like 1 (BMAL1) proteins bind to E-box enhancer elements of Period (PER1-3) and Cryptochrome Circadian Regulator (CRY1/2) genes. The transcribed PER and CRY proteins translocate into the nucleus to suppress CLOCK/BMAL1 activity, forming a negative feedback loop. This mechanism is regulated by posttranslational modifications that introduce delays, resulting in a cycle duration of approximately 24 h. In the stabilizing loop, CLOCK/BMAL1 activity induces the expression of nuclear receptor REV-ERBα/β (also known as nuclear receptor subfamily 1 group D member 1 gene, Nr1d1) and Retinoid-related orphan receptor (RORα/β). REV-ERBs inhibit BMAL1 expression, while RORs compete with REV-ERBs for

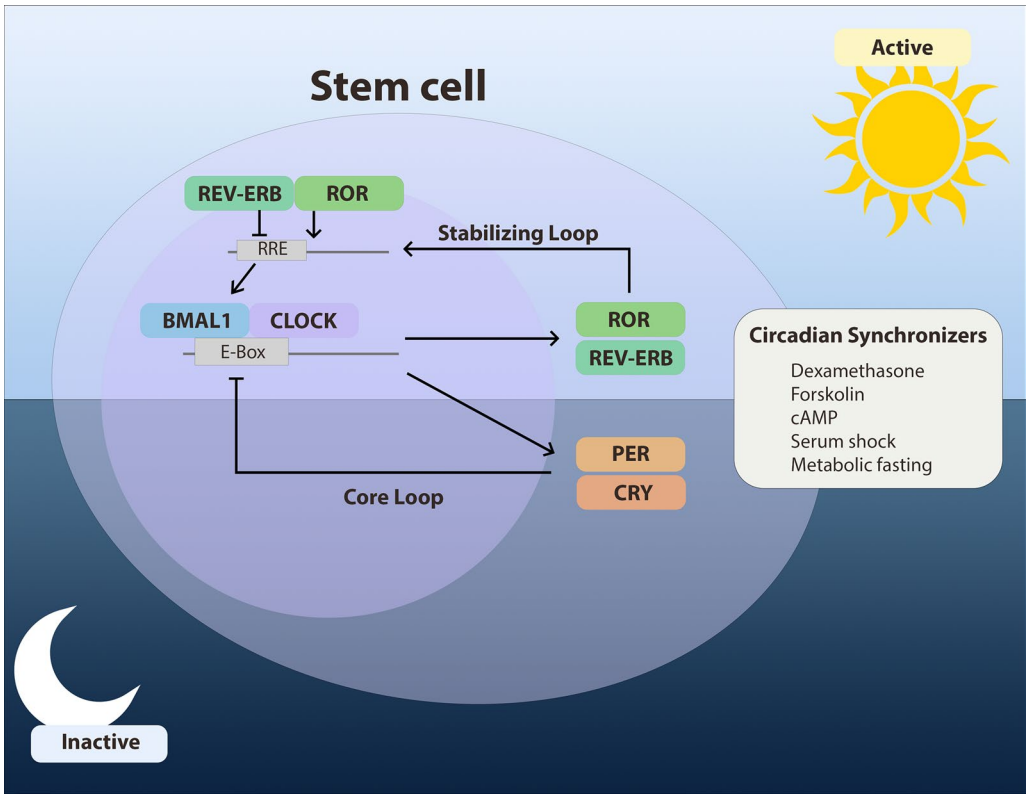


Fig. 1 Circadian clock in stem cells. Recent evidence increasingly supports the presence of circadian rhythms in stem cells. The application of circadian synchronizers, such as dexamethasone and forskolin, has been shown to stimulate and enhance these oscillations

shared DNA binding sites, REV response element (RRE), and promote BMAL1 expression.

Post-transcriptional and epigenetic modifications significantly influence circadian programs. Phosphorylation of PER2 enhances its complex formation with CRY, promoting nuclear translocation and preventing degradation [25]. Similarly, casein kinase I ε (CKIε) phosphorylates PER, regulating its nuclear entry and degradation [26]. As a result, mutations in CKIε or PER2 phosphorylation sites decrease PER2 degradation, leading to familial advanced sleep phase syndrome (FASPS) [27, 28]. SUMOylation of BMAL1, induced by the CLOCK gene, localizes BMAL1 to the promyelocytic leukemia nuclear body (NB), enhancing its transactivation and ubiquitin-dependent degradation [29, 30]. Epigenetically, rhythmic histone methylation is essential for transcriptional oscillator function [31, 32]. In Arabidopsis, circadian oscillation is marked by sequential H3 acetylation and methylation [33]. Inhibiting these modifications abolishes oscillator gene expression and increases clock-repressor binding, indicating their role in transitioning from activation to repression.

Redox processes are posited as a non-canonical circadian pathway [34]. Non-transcriptional mechanisms in circadian regulation have been challenging to study in mammals. Nevertheless, research utilizing human red blood cells, which lack DNA, has demonstrated that transcription is not essential and non-transcriptional pathways alone can sustain cellular circadian rhythms [35]. Similarly, circadian rhythms have been observed in cyanobacteria through the exclusive action of KaiA, KaiB, and KaiC proteins, independent of transcription and translation [36]. mammals, oscillations in flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide phosphate (NADP) redox states have been detected in SCN tissue slices, and these oscillations modulate the excitability of SCN neurons through non-transcriptional regulation of membrane potassium channels [37]. Comprehensive studies of both classical and novel mechanisms controlling circadian rhythm will pave the way for a complete picture of its functions.

SCN functions as the central pacemaker, synchronizing circadian rhythms across the body. SCN activates clock gene transcription and is reset daily by light-stimulating melanopsin-containing intrinsically photosensitive retinal ganglion cells (ipRGCs) via the retinohypothalamic tract [34, 38]. Light, the primary external cue for time, activates the N-methyl-D-aspartate (NMDA) receptor on SCN neurons, which triggers calcium-mediated activation of calmodulin-dependent protein kinase II (CaM kinase II or CaMKII) and gene transcription [39]. SCN efferent pathways extend to the hypothalamus, thalamus, and other brain regions [23], and communicate with peripheral organs via humoral signals like transforming growth factor-α (TGF-α) [40]. Despite SCN impairment, peripheral clocks remain synchronized to light–dark cycles but eventually desynchronize under constant darkness (DD). Mice with silenced BMAL1 in the SCN exhibit impaired circadian rhythms in DD but maintain 24-h activity rhythms under light–dark conditions. SCN clock-deficient mice show accelerated resetting of PER2 rhythms after a light–dark cycle shift, indicating the SCN's role in stabilizing peripheral clocks against external perturbations [41–43].

Notwithstanding these significant advancements in understanding the mechanisms of the circadian clock over recent decades, much remains to be explored. The discovery of non-transcriptional pathways, such as redox, suggests the potential existence of additional pathways yet to be uncovered. Furthermore, a comprehensive understanding of circadian-regulated physiological and pathological processes remains an important field of inquiry. Although numerous circadian output genes involved in critical pathways have been identified, the extent to which circadian rhythm optimization can alleviate diseases is a question that warrants further investigation.

Circadian clock in stem cells

Several studies have demonstrated that circadian clocks do not function in stem cells (Table 1). For instance, a bioluminescence study in mouse embryonic stem cells (ESCs) revealed no circadian rhythm; however, the

Table 1 Negative evidence for circadian rhythm in stem cells

Species	Cell line	Passage	Indicators	References
Human	ESCs	Not specify	No circadian bioluminescence oscillations of clock genes	[14, 15]
	iPSCs	Not specify	Loss of circadian oscillations after dedifferentiation	[14]
	BM-MSCs	3–5	No circadian bioluminescence oscillations of clock genes	[44]
Mouse	ESCs	< 10, primary	No circadian bioluminescence oscillations of clock genes	[13, 45]
	iPSCs	Not specify	Loss of circadian oscillations after dedifferentiation	[13]
	NSCs	Primary	Non-rhythmic/ultradian bioluminescence oscillations of clock genes, turn into circadian rhythm after differentiation	[46]

oscillation of circadian clock genes was induced upon differentiation [13]. Reprogramming differentiated cells into induced pluripotent stem cells (iPSCs) disrupted circadian oscillation, indicating that circadian clock regulators mature during differentiation. Furthermore, gene expression in the circadian core loop significantly differs between embryonic and differentiated states, with PER2 and CLOCK expression increasing post-differentiation, while CRY expression drastically reduces. Similarly, experiments with neural progenitor cells, cardiomyocytes, and fibroblasts show that only differentiated cells exhibit oscillated activities of circadian genes [14, 15, 46]. On a molecular level, disrupted c-MYC and DNA methyltransferase 1 (DNMT1) in mouse ESCs hinder clock development. Additionally, misregulation of karyopherin Subunit Alpha 2 (KPNA2), which facilitates the cytoplasmic localization of PER1/2, results in cytoplasmic PER protein accumulation and impaired clock development [47]. These findings collectively underscore that functional circadian clocks are closely tied to the differentiation state of the stem cells rather than their immature state.

Equally compelling evidence supports the presence of circadian rhythms in various types of stem cells (Table 2). Most stem cells, including mesenchymal stem cells (MSCs), erythroid hematopoietic stem cells (HSCs), ESCs, intestinal stem cells, and anagen hair follicle, have a 24-h cycle. On the other hand, myeloid HSCs and adipose progenitor cells (APCs) have 12- and 18-h cycles respectively. Some types of stem cells, such as epidermal stem cells, maintain a connection with the brain's central clock to regulate tissue homeostasis, similar to the finding in mature peripheral tissues [58]. Proliferation activity and the rate of stem cell release also fluctuate with circadian rhythms [51, 52, 56], potentially due to intrinsic clocks within the stem cells or central regulatory mechanisms in vivo [53, 55]. Embryonic stem cells exhibit oscillating metabolism such as glucose uptake and glucose transporter (GLUT) expression [17]. As these cells differentiate, clock genes begin to show circadian patterns, and the amplitude of glucose utilization rhythms increases, suggesting the early presence of a functional circadian clock. Additionally, several studies indicate that transcription of circadian clock genes is not essential for the circadian oscillation of cellular activity, implying the existence of non-canonical regulatory pathways such as redox [18, 19].

Understanding the circadian cycle of stem cells is complex and fraught with multiple challenges, the foremost being heterogenicity. Oscillation patterns not only differed between cell types, but stem cells within the same tissue also display heterogeneous responsive states, with only subsets activated during each round of morphogenesis. This results in coexisting populations of stem cells at opposite clock phases, each responding differently to homeostatic cues [59]. Moreover, contributory factors such as cell–cell interactions and the microenvironment play significant roles in circadian regulation. Cancer stem cells, which typically exhibit robust circadian oscillations, often lack rhythm when cultured in a monolayer [60]. The pattern and frequency of clock gene oscillations in stem cells vary across different stages of differentiation. For instance, neural stem cells (NSCs) initially display a high-frequency rhythm of PER1, which transitions to a 24-h circadian rhythm upon complete differentiation [46]. This ultradian rhythm may be crucial for the high proliferation activity observed during the embryonic state. These complexities underscore the intricate nature

Table 2 Positive evidence for circadian rhythm in stem cells

Species	Cell Line	Passage	Cycle Length (hours)	Indicators	References
Human	MSCs	Not specified	20–24	Oscillation of clock genes	[48]
	UC-MSCs	5–7	24	Oscillation of clock genes	[49]
	APCs	Primary	18	Oscillation of clock genes	[50]
	Cord blood-derived CD34 + progenitor cells	Primary	24	Proliferation rate	[51]
	HSCs	Primary	Erythroid: 24 Myeloid: 12	Cell division (S phase activity)	[52]
	Endothelial progenitor cells (EPCs)	Primary	At least 14	EPC release	[53]
Mouse	ESCs	Primary	24	Glucose uptake, glucose transporter 1 and 8 (Glut1/8) expression	[17]
	Bone marrow progenitor cells	Primary	24	Proliferetion rate	[16]
	BM-MSCs	Primary	12	Oscillation of clock genes	[54]
	HSCs	Primary	24	HSC release	[55]
	Intestinal stem cells	Primary	24	Cell division (mitosis and DNA synthesis)	[56]
	Anagen hair follicle/skin	Primary	24	Per2 oscillation	[57]

of circadian regulation in stem cells and the need for tailored approaches to study their rhythms accurately.

Although significant progress has been made in elucidating the circadian clock in stem cells, much remains to be explored. While stem cells may possess circadian rhythms, it is still unknown whether their clocks exhibit cycle patterns and output genes similar to those of mature cells. Identifying the active and inactive stages of stem cells, along with their properties, will undoubtedly benefit regenerative medicine. Additionally, stem cell-derived EVs are being widely studied as a promising novel therapy for various diseases, particularly inflammation and degenerative conditions. If stem cells have circadian rhythms, this may influence the quantity and content of EVs released at different times of the day. This field of research holds the potential to enhance treatment efficacy by aligning therapeutic interventions with the natural circadian rhythms.

Clinical application of circadian clock

Circadian rhythms are involved in multiple pathogenesis processes in the brain. Some diseases exhibit circadian preferences; for instance, ischemic strokes with nighttime onset result in larger ischemic core volumes compared to those with daytime onset, due to the influence of circadian rhythms on the integrity of the blood–brain barrier (BBB) and hypoxic signaling [24, 61, 62]. Additionally, cluster headaches and sundowning syndrome in Alzheimer's disease demonstrate circadian variations [24]. The relationship between circadian rhythms and diseases is complex, encompassing both direct pathogeneses, as seen in sleep disorders, and more ambiguous connections, as observed in neurodegenerative diseases. Understanding these relationships can provide insights into disease mechanisms and potential therapeutic targets.

The circadian clock can be strategically utilized to optimize treatment efficacy [63]. Since many common medications directly target the products of clock genes, optimizing regimens to align with circadian timing, known as chronotherapy, can significantly enhance therapeutic outcomes [7, 64]. For instance, hypertension is more pronounced during the daytime, rationalizing the administration of antihypertensive medications in the morning [65]. Another point to consider is circadian oscillations of efflux transporters in the intestine can lead to varied enteral absorption, contributing to drug chronotoxicity [66]. Chrono-biomarkers, which fluctuate throughout the day, necessitate different reference ranges to improve the accuracy of diagnosis. The extent of circadian disruption in patients with systemic disorders can vary by up to 12 h [67]. Enhancing hospital environments to promote circadian synchronization through controlled light, noise, and external time cues has been shown to significantly improve treatment outcomes [68]. This underscores the critical role of aligning therapeutic interventions with the body's natural rhythms to maximize efficacy and minimize adverse effects.

Circadian optimization holds significant potential for enhancing stem cell-based therapy. Over the past decade, stem cell therapy has emerged as a promising therapeutic field for various neurological disorders [69]. Stem cells, especially MSCs derived from the umbilical cord (UC-MSCs), adipose tissue (ASCs), and bone marrow (BM-MSCs), exhibit multiple therapeutic mechanisms [70], including cell replacement [71], trophic factor secretion [72], and immunomodulation [73]. Since the division cycle of stem cells varies with the time of day they are harvested, their efficacy may also fluctuate accordingly [16]. Additionally, the timing of stem cell transplantation plays a critical role in treatment outcomes, as host immune activities demonstrate circadian variations [74]. Strategically selecting the time of transplantation can potentially minimize tissue damage and rejection. Moreover, the release rate, size, and content of EVs exhibit diurnal variation, emphasizing the importance of timing in EV harvest [75, 76]. Moreover, genetic modification to facilitate the circadian clock has also proved to be beneficial. BMAL1-overexpressing MSCs rescue the rhythm and promote osteoblast differentiation in aged mice [77]. These insights underscore the importance of circadian optimization in maximizing the therapeutic efficacy of stem cell-based therapies.

Due to the debate over whether stem cells possess intrinsic circadian rhythms, several studies have explored the use of circadian synchronizers to enhance circadian activities in stem cells (Table 3). Dexamethasone has been shown to activate oscillations of circadian clock genes in multiple lines of stem cells, mimicking systemic signaling in vivo [78, 80, 81]. Forskolin has proven effective in somatic cells [82], but studies in human iPSCs demonstrate oscillation only in clock output D-Box Binding PAR BZIP Transcription Factor (DBP), not in core clock genes [79]. Other interventions, such as cyclic adenosine monophosphate (cAMP), serum shock, and metabolic fasting, can also activate and increase the amplitude of circadian clock gene oscillation. Melatonin, released by the pineal gland to regulate the sleep–wake cycle, can synchronize circadian rhythms in mature cells; however, studies in stem cells remain incomplete [83]. Nevertheless, it is important to note that circadian stimulation may increase the risk of tumorigenesis in stem cell therapy due to enhanced cell proliferation [84, 85]. These findings suggest that while circadian synchronizers hold promise for optimizing stem cell therapies, their potential risks must be carefully considered.

Table 3 Circadian synchronizors in stem cells

Intervention	Cell model	Cycle length (hours)	Outcomes	References
Dexamethasone	Mouse/human BM-MSCs	24	Oscillating clock gene expression	[78]
	Human iPSCs	24	DBP rhythm but not BMAL1/PER2, more likely from temperature rhythm	[79]
	Human ASCs, BM-MSCs, dental pulp stem cells (DPSCs)	12–24	Oscillating clock gene expression	[80]
Forskolin	Human iPSCs	24	DBP rhythm but not BMAL1/PER2, more likely from temperature rhythm	[79]
cAMP	Human MSCs	20–24	Oscillating clock gene expression	[44]
Serum shock	Human MSCs	20–24	Oscillating clock gene expression	[44]
Fasting	Human MSCs	24	Increased amplitude of clock gene oscillation	[48]

Circadian rhythms are intricately involved in multiple disorders, yet the relationship between circadian rhythms and diseases remains complex with unclear connecting mechanisms. Understanding these connections can provide valuable insights into disease mechanisms and potential therapeutic targets. The circadian clock can be leveraged to optimize treatment efficacy through chronotherapy, chrono-biomarkers, and environmental adjustments. Additionally, circadian optimization can enhance stem cell-based therapy by selective timing of harvest and transplantation of stem cells and EVs. Furthermore, circadian synchronizers, such as dexamethasone, may enhance the effects of stem cell therapy. Despite these advancements, extensive research is still required to further develop and refine treatment regimens.

Key enabling studies to advance therapeutic use of circadian rhythmicity of stem cells

Despite significant progress in circadian clock studies in stem cells, much remains to be learned to advance therapeutic applications of circadian rhythmicity of stem cells. There are at least three key areas of research that will significantly impact the clinical application of stem cell therapy. First, it is crucial to capture circadian rhythmicity in essential stem cell functions, particularly cell motility and division, encompassing immaturity and differentiated state. Understanding these rhythms could reveal how stem cells function and interact with their microenvironment differently by the time of the day. Second, confirming the direct cause-and-effect mediation of specific core clock genes, such as BMAL1 and PER, in stem cells is essential. This can be achieved through targeted gene silencing and upregulation studies, which will elucidate the precise roles these genes play in regulating stem cell behaviors. Such insights could lead to novel strategies for manipulating stem cell activity to optimize their regenerative capabilities. Third, once circadian rhythmicity and its pathways are established in stem cells, we can explore the therapeutic effects of stem cell therapy at different circadian stages that promote safe and effective regenerative modalities. In particular, investigating the transplantation of "active" versus "inactive" stem cells in disease models, such as ischemic stroke, could guide the optimal timing for stem cell therapy, potentially reducing the risk of graft rejection and improving transplant integration and function. Recent clinical studies have highlighted a significant diurnal variation in stroke incidence and outcomes, with both detection and mortality rates peaking during morning hours [86–90]. Additionally, normobaric hyperoxia has been shown to effectively reduce infarct size in rodents only when administered during the daytime. These findings underscore the importance of chronopharmacology [91]. In parallel, optimizing the timing of stem cell harvest and transplantation to align with circadian rhythms may offer a promising strategy to mitigate the pathophysiological conditions associated with stroke such as neurovascular unit integrity and inflammation. The circadian clock is intricately intertwined with multiple pathologies, yet it has been overlooked for stem cell-based regenerative medicine. By integrating circadian biology into stem cell therapeutic protocols, the appropriate timing to harvest stem cells and to transplant them will likely enhance their therapeutic applications especially for human diseases which display inherent biological clock alterations.

Conclusion

The circadian clock plays a vital role in synchronizing physiological activities across the body. The intricate connection between circadian rhythms and disease pathogenesis involves a wide array of clock output genes, making it a complex area of study. Understanding these rhythms has significant clinical applications; by adjusting disrupted rhythms, we can potentially

improve disease progression. The discovery of circadian rhythms in stem cells opens new avenues for optimizing stem cell-based therapies to maximize their efficacy. Nonetheless, further research is essential. Confirming the oscillatory activity in stem cells, elucidating the underlying molecular mechanisms and their outputs, and comparing the outcomes of stem cell therapies at different time points are crucial next steps in this field.

Abbreviations

APCs	Adipose progenitor cells
ASCs	Adipose tissue-derived mesenchymal stem cells
BBB	Blood–brain barrier
BMAL1	Basic Helix-Loop-Helix ARNT Like 1
BM-MSCs	Bone marrow-derived mesenchymal stem cells
CaM kinase II or CaMKII	Calmodulin-dependent protein kinase II
cAMP	Cyclic adenosine monophosphate
CLOCK	Circadian locomotor output cycles kaput
CKIε	Casein kinase Iε
CRY	Cryptochrome Circadian Regulator
DBP	D-Box Binding PAR BZIP Transcription Factor
DD	Constant darkness
DNMT1	DNA methyltransferase 1
DPSCs	Dental pulp stem cells
EPCs	Endothelial progenitor cells
ESCs	Embryonic stem cells
EV	Extracellular vesicle
FAD	Flavin adenine dinucleotide
FASPS	Familial advanced sleep phase syndrome
GLUT	Glucose transporter
HSCs	Hematopoietic stem cells
ipRGCs	Intrinsically photosensitive retinal ganglion cells
iPSCs	Induced pluripotent stem cells
KPNA2	Karyopherin Subunit Alpha 2
MSCs	Mesenchymal stem cells
NADP	Nicotinamide adenine dinucleotide phosphate
Nr1d1 (REV-ERBα/β)	Nuclear receptor subfamily 1 group D member 1 gene
NSCs	Neural stem cells
PER	Period
RORα/β	Retinoid-related orphan receptor
RRE	REV response element
SCN	Suprachiasmatic nucleus
TGF-α	Transforming growth factor-α
UC-MSCs	Umbilical cord-derived mesenchymal stem cells

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Competing Interests
The authors declare that they have no competing interests.

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