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Review Article

Osteogenic action of the natural plant material icariin and its potential use in biomedical tissue engineering

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Abstract: Bone tissue regeneration remains a critical research focus in biomedical tissue engineering. Icariin, a flavonoid compound extracted from the medicinal herb *Epimedium*, has been traditionally used in Chinese medicine to treat bone-related disorders such as lumbago associated with kidney Yang deficiency, impotence, and musculoskeletal weakness. Recent advances in biomedical research have increasingly clarified its biological mechanisms, revealing that icariin can promote osteoblast proliferation and differentiation while simultaneously inhibiting osteoclast activity. However, current application of icariin in biomedical tissue engineering remains in the exploratory phase, with its molecular mechanisms not yet fully characterized. Moreover, the *in vivo* and *in vitro* evaluation systems for its therapeutic efficacy and safety remain to be optimized. A comprehensive investigation into icariin's osteogenic properties and its translational potential in biomedical tissue engineering are warranted. Such investigations may provide a theoretical basis and experimental evidence for developing novel bone repair materials and therapeutic strategies, offering substantial scientific significance and clinical application value.

Keywords: Natural plants, icariin, osteogenesis, biomedicine, tissue engineering

Introduction

Icariin (C33H40O15), a prenylated flavonol glycoside, is recognized as the principal active constituent and quality control marker compound derived from plants of the *Epimedium* genus (Berberidaceae family), commonly known as Horny Goat Weed or Yin Yang Huo in Traditional Chinese Medicine (TCM) [1-3]. Its chemical structure features a kaempferol backbone substituted with methoxy groups at positions 3 and 5, a hydroxyl group at position 4', and a rhamnosylglucoside moiety attached at position 7 (**Figure 1**). Notably, it possesses a characteristic prenyl group at position 8, which is believed to contribute significantly to its unique biological activities, particularly its osteogenic properties [4-7]. Icariin exhibits relatively low water solubility and limited oral bioavailability in its native form, which are challenges addressed in modern pharmaceutical research and delivery system design [8-10].

Osteogenic effects of icariin have been extensively explored in both domestic and international studies. In vitro experiments have demonstrated that icariin promotes the proliferation and differentiation of osteoblasts. For example, Wang et al. [11] employed MTT and MTB colorimetric assays to show that icariin and other five flavonoids enhance extracellular matrix mineralization and facilitate osteoblast proliferation and differentiation, as well as the osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs). Khezri et al. [12] reported that icariin can promote osteoblast proliferation and differentiation by inducing BMSC differentiation, potentially mediated through BMP-2 mRNA upregulation, when alkaline enzymes reach a certain concentration. Wu et al. [13] showed that icariin initially suppresses alkaline phosphatase (ALP) activity in the early stage but significantly enhances ALP activity in later stages. Chen et al. [14] found that while low concentrations of icariin exert limited effects on osteoblast proliferation, they significantly improve ALP activity, promote the timely expression of type I collagen and synthesis of bone Gla protein (BGP), thus completing the proliferation and differentiation of osteo-

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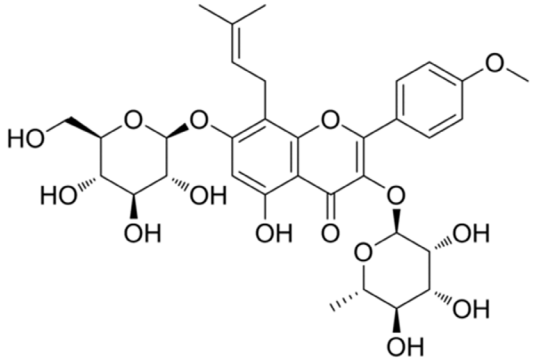

Figure 1. Icariin structure.

blasts *in vitro*. Animal studies have also achieved fruitful results. Ding et al. [15] used ovariectomized rats as a model for postmenopausal osteoporosis. The results showed that oral administration of icariin at 225 mg/d increased bone mineral density and positively influenced bone growth and development. Yao et al. [8] demonstrated that icariin modulates the age-related expression of osteoblast-related markers and alters the RANKL/OPG ratio, thereby reducing age-related osteoporosis.

Icariin also showed a positive effect in inhibiting osteoclasts. Jia et al. [16] have shown that icariin inhibits osteoclast induction and bone resorption in a dose-dependent manner, ultimately promoting osteoclast apoptosis and suppressing resorptive activity. Li et al. [17] confirmed that icariin not only hinders osteoclast formation but also inhibits their bone resorption capacity. The study of Yuan et al. [4] further supported the role of icariin in enhancing osteoblast-osteoclast coupling and effectively inhibiting osteoclastic activity. Although clinical trials remain limited, existing studies have revealed the therapeutic potential of icariin in treating bone-related diseases. In early clinical trials involving osteoporotic patients, treatment with icariin-containing preparations led to modest improvements in bone mineral density and symptoms relief. However, due to the small sample size and short study period, the efficacy and safety of icariin in clinical trials still need further large-scale and long-term in-depth studies [18].

Icariin has also made progress in the application of tissue engineering [19]. Studies have shown that its osteo-inductive effects can be improved when compounded with suitable biomaterials. For example, icariin-loaded bioceramic materials applied to bone defect model have been shown to promote new bone formation and accelerate defect repair [20]. However challenges remain, including the controlled release of icariin, optimization of its compatibility with biomaterials, and its stability in complex *in vivo* environments.

The purpose of this study is to elucidate the molecular mechanisms underlying the osteogenic effects of icariin within the context of biomedical tissue engineering. Specifically, we seek to clarify its regulatory roles in osteoblast proliferation, differentiation, and osteoclast activity using cellular and molecular biology techniques, thereby providing a theoretical foundation for developing novel bone repair strategies. At the same time, we aim to optimize the application of icariin in tissue engineering by investigating its combination with various biomaterials, determining the optimal concentration, and developing controlled release systems to enhance its osteoinductive capacity, in vivo stability, and the efficacy of bone defect repair. In addition, through integrated studies involving preclinical animal models and preliminary clinical trials, the effectiveness and safety of icariin-bases interventions will be systematically evaluated, laying the foundation for its future clinical transformation [19, 20].

The osteogenic mechanism of icariin

Icariin significantly promotes the proliferation and differentiation of osteoblasts at the cellular level [11]. Studies have shown that icariin can directly act on osteoblasts by modulating their cell cycle, accelerating cell transition from G1 to S phase, thereby enhancing cell proliferation [21]. For example, in vitro study has shown a marked increase in osteoblast number and cellular activity following treatment with appropriate amount of icariin. In addition to promoting proliferation, icariin plays an outstanding role in promoting osteoblast differentiation. It upregulates the expression of key osteogenic genes, such as runt-related transcription factor 2 (Runx2) [22]. As the core transcription factor of osteoblast differentiation, elevated Runx2 expression subsequently activates downstream osteogenic markers, including ALP and osteocalcin (OCN), thereby promoting osteoblast maturation and the synthesis and miner-

alization of the bone matrix. *In vitro* studies have confirmed that icariin significantly increases ALP activity and enhances calcium nodule formation in both number and size, indicating effective promotion of osteoblast differentiation and mineralization [23].

The osteogenic effects of icariin are mediated through activating multiple intracellular signal pathways. Among them, the ERα-Wnt/β-catenin signaling pathway plays a pivotal role. Icariin binds to estrogen receptor α (ERα) to form a complex that translocates into the nucleus and regulates the expression of genes related to Wnt signaling pathway [24]. Activation of Wnt signaling pathway inhibits the activity of glycogen synthase kinase 3β (GSK-3β), stabilizing β-catenin, which then accumulates in the cytoplasm and translocates to the nucleus. There, it interacts with T-cell factor/lymphoid enhancer-binding factor (TCF/LEF) family members to activate osteogenic gene transcription, including Runx2 and OCN, promoting the proliferation and differentiation of osteoblasts [25]. It was found that the effect of icariin on the proliferation and differentiation of osteoblasts was obviously weakened when the ERα-Wnt/β-catenin signaling pathway was blocked by specific inhibitors, confirming the pathway's essential role in the osteogenic mechanism of icariin.

In addition, icariin activates the mitogen-activated protein kinase (MAPK) signaling pathway, which includes extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 [26]. Icariin enhances the phosphorylation of ERK and JNK, leading to activation of downstream transcription factors such as c-Fos and c-Jun. These transcription factors bind to promoter regions of osteogenesis-related genes and upregulate their expression, further enhancing osteoblast proliferation and differentiation [27]. At the same time, the activation of MAPK signaling pathway enhances the expression of type I collagen, osteocalcin, and osteopontin, promoting bone matrix synthesis and deposition and providing the structural basis for bone formation.

Besides stimulating osteogenesis, icariin inhibits the proliferation of osteoclast precursor cells and reduces the number of osteoclasts [28]. In *in vitro* experiments, the addition of icariin to osteoclast precursor cultures significantly suppressed cell proliferation, as evidenced by reduced cell counts and downregulation of proliferation-related markers [29]. Besides, icariin interferes with the differentiation of osteoclasts. Specifically, it suppresses the expression of key osteoclastogenic genes, such as receptor activator of nuclear factor-κB (RANK) and calcitonin receptor (CTR), thereby preventing precursor cells from progressing to mature osteoclasts. Morphological observations revealed that osteoclasts treated with icariin showed a reduction in typical osteoclast features, such as an inhibition of the formation of multinucleated giant cells, and a marked decline in functional enzyme activities, including tartrate-resistant acid phosphatase (TRAP), indicating that icariin significantly impairs osteoclast differentiation and bone-resorbing function [30]. The anti-osteoclastogenic effect of icariin involves the regulation of multiple molecular signaling pathways, with the RANKL-RANK-OPG axis playing a particularly critical role [31]. RANKL is an important inducer of osteoclast differentiation and activation. Icariin can bind with RANK to block signal transduction of osteoclast differentiation [32]. Concurrently, icariin upregulates the expression of osteoprotegerin (OPG), a bait receptor that competitively binds RANKL and prevents it from activating RANKL. This dual modulation effectively inhibits the RANKL-RANK signaling cascade and reduces osteoclast formation [5]. Both *in vitro* and *in vivo* studies have demonstrated a significant reduction in the RANKL/OPG ratio following icariin treatment, correlating with a decrease in osteoclast formation [33]. In addition, icariin also regulates the nuclear factor κB (NF-κB) signaling pathway, which is critically involved in osteoclast formation and function [2]. Icariin inhibits NF-κB activation, preventing its nuclear translocation and subsequent transcription of osteoclast-related genes. Molecular assays have confirmed that icariin treatment significantly suppresses NF-κB activity and downregulates the expression of osteoclast proliferation and differentiation genes [29, 30], further validating its inhibitory effect on osteoclastogenesis through the NF-κB pathway. **Figure 2** summarizes the regulatory effects of icariin on osteoblast and osteoclast and the involved signaling pathways.

Application of icariin in biomedical tissue engineering

Icariin can be loaded in a variety of scaffold materials for its application in biomedical tis-

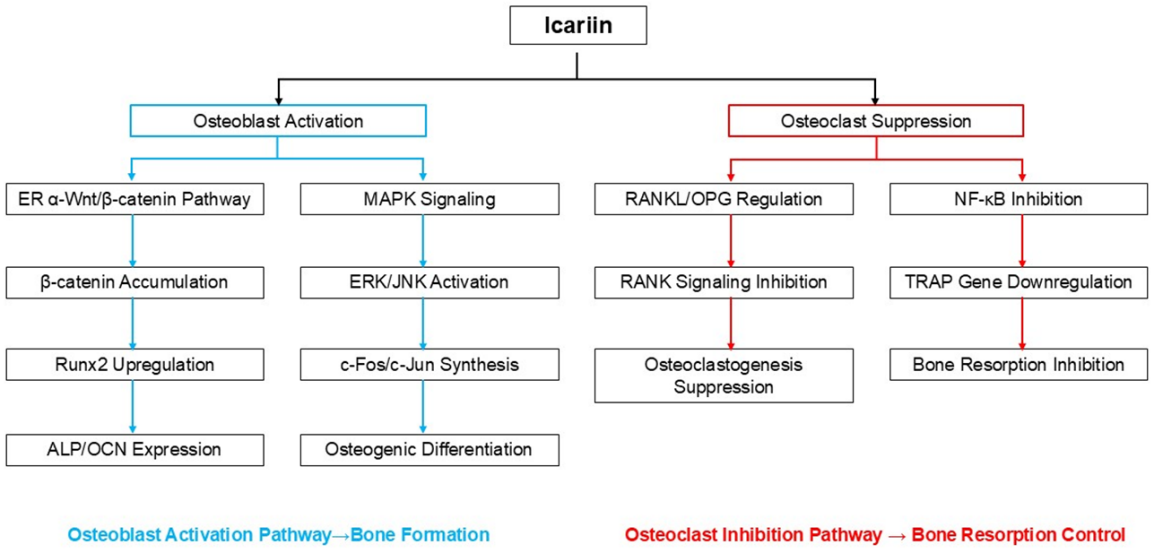

Figure 2. Mechanism of icariin's effect on osteoblast and osteoclast precursor.

sue engineering [34]. Among these, natural polymers such as collagen and chitosan are widely used due to their excellent biocompatibility and biodegradability. Collagen, a major component of the extracellular matrix, has low immunogenicity and promotes cell adhesion [35]. When loaded onto collagen scaffold, icariin acts synergistically to enhance the adhesion, proliferation, and differentiation of osteoblasts [36]. Chitosan has antibacterial activity and an adjustable degradation rate. Its amino groups can interact with icariin, enabling effective drug loading and sustained release. For example, icariin can be immobilized on chitosan scaffold through physical adsorption or chemical crosslinking to prepare composite scaffold materials with osteogenic induction [37]. Synthetic polymer materials such as polylactic acid (PLA), polyglycolic acid (PGA), and their copolymer poly (lactic-co-glycolic acid) (PLGA) are also often used to construct icariin-containing scaffolds. These materials have precisely controllable physicochemical properties, such as molecular weight, degradation rate, and porosity. PLA exhibits high mechanical strength and slow degradation, making it suitable as a structural material for long-term mechanical support for bone tissue regeneration [38]. PGA, with its fast degradation rate, enables quick release of icariin in the early stage, promoting osteogenesis. By adjusting the PLA-to-PGA ratio, PLGA copolymer scaffolds with varying degradation rates and mechanical properties can be tailored for different bone defect scenarios [39]. Icariin can be incorporated into these synthetic polymers by dispersing it in a polymer solution, followed by scaffold fabrication using techniques such as solvent casting, particulate leaching, and 3D printing. These processes enable the creation of scaffolds with specific geometries and pore structures, offering a favorable microenvironment for cell growth and tissue regeneration. Inorganic materials such as hydroxyapatite (HA) and tricalcium phosphate (TCP) can also be used in conjunction with icariin [40]. HA, with its chemical composition similar to natural bone, has good biological activity and bone conductivity, serving as a favorable matrix for new bone deposition. Icariin-loaded HA scaffolds combine structural support with enhanced osteoinduction. TCP, on the other hand, has higher solubility and degradation rate, allowing for rapid icariin release and providing calcium and phosphorus ions that contribute to new bone formation.

Various strategies are available for incorporating icariin into scaffold materials, including physical blending, chemical grafting, and electrospinning [41]. Physical blending involves directly mixing icariin with the scaffold raw materials in solution or molten state, followed by molding into scaffolds. Despite the simple operation, this method offers limited control over drug loading and release kinetics. Chemical grafting enables covalent bonding of

icariin to polymer chains, resulting in more stable drug incorporation and controlled release [42]. Electrospinning is another widely used technique to fabricate nanofibrous scaffolds with high surface area and porosity. Icariin is added to the spinning solution and evenly distributed in the scaffold with the formation of fibers. The resulting scaffolds are conducive to cell adhesion, proliferation, and differentiation, while also enabling sustained release of icariin [43].

In terms of cell affinity, many cell experiments have shown that osteoblasts can adhere, spread, and proliferate well on the icariin-loaded scaffolds. For example, BMSCs cultured on icariin-collagen composite scaffolds showed close adhesion to the scaffold surface and extended pseudopodia along the scaffold fibers under scanning electron microscopy. The cells maintained normal morphology and exhibited active proliferation, which is attributed to the combined effects of scaffold surface properties and icariin-mediated signaling [44].

The microstructure of scaffolds provides physical anchoring sites, while icariin may promote the interaction between cells and scaffolds by regulating receptor expression or activating related signal pathways. In terms of biocompatibility, animal experiments showed that icariin-containing scaffolds did not cause obvious inflammatory reaction or immune rejection upon implantation and integrate well with surrounding tissues. For example, when icariin-PLGA scaffolds were implanted into a rat skull defect model, histological and hematological analyses revealed only mild inflammatory infiltration at the implantation site, which subsided over time, and no significant increase in serum inflammatory factors was observed [45].

With the passage of time, the scaffold material gradually degrades, and new bone tissue forms and integrates with the surrounding bone tissue, demonstrating excellent *in vivo* biocompatibility and therapeutic potential in bone tissue engineering applications (**Table 1**).

Icariin activates osteogenic signaling pathways

Icariin exerts its osteogenic effects through the activation of multiple intracellular signaling pathways, reflecting a high degree of complexity and diversity [46]. Current research indicates that icariin regulates the proliferation and differentiation of osteoblasts via both non-nuclear and genomic pathways. On the non-nuclear level, icariin influences several critical signaling cascades, including the mitogen-activated protein kinase (MAPK), protein ubiquitination, mechanistic target of rapamycin (mTOR), and phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) pathways. In one study, primary osteoblasts were isolated from neonatal Sprague-Dawley rats, cultured, and subjected to different treatment conditions. Phosphoproteomic analysis using liquid chromatography-mass spectrometry (LC-MS/MS) revealed that icariin induces phosphorylation of specific intracellular proteins, thereby supporting its non-nuclear mechanism of action and implicating these pathways in its osteogenic activity [46]. Phosphorylation events within these pathways lead to functional modulation of target proteins. For example, activation of the MAPK pathway facilitates the expression of a series of downstream target genes associated with osteoblast proliferation and differentiation, promoting cells towards osteogenesis. The mTOR pathway contributes to metabolic regulation by ensuring sufficient energy and substrates for cell proliferation during osteogenesis. The PI3K/Akt pathway supports cell survival, proliferation, and cytoskeleton remodeling, all of which are essential for osteoblast function and bone tissue formation [47].

Icariin also exerts regulatory effects on signaling pathways involved in pathological bone conditions, such as hormone-induced avascular necrosis of the femoral head. Unreasonable hormone use can cause apoptosis and dysfunction of osteoblasts [48]. In this context, icariin modulates key signaling pathways, including Wnt, PI3K/Akt, mTOR, and estrogen receptor (ER) pathways, to restore cellular homeostasis and enhance osteogenic activity. The Wnt/β-catenin signaling pathway is extremely important for maintaining bone homeostasis and promoting osteoblast differentiation and trabecular bone formation. Icariin may stabilize β-catenin or alter its subcellular localization, enhancing transcriptional activation of osteogenesis-related genes and accelerating the bone formation process. Concurrent activation of the PI3K/AKT and mTOR pathways further improves the intracellular environment, supporting the normal function of osteoblast cells and promoting bone repair [49].

Table 1. Characteristics of scaffold materials for Icariin delivery in bone tissue engineering

Material Category	Specific Material	Advantages	Limitations	Loading Method	Drug Release Profile
Natural Polymers	Collagen	- Low immunogenicity - Promotes cell adhesion (ECM component)	- Low mechanical strength	Physical adsorption for osteoblast differentiation	Synergistic sustained release
	Chitosan	- Antibacterial activity - Tunable degradation - Amino group interactions	- Requires chemical modification for stability	Physical adsorption/chemical crosslinking	Effective loading & sustained release
Synthetic Polymers	PLA	- High strength - Slow degradation (>6 months)	- Hydrophobicity may hinder drug dispersion	Solvent casting/3D printing for structural support	Long-term release (>3 months)
	PGA	- Rapid degradation (<1 month) - Early-stage drug release	- Rapid mechanical strength loss	Short-term defect filling	Burst release (1-2 weeks)
	PLGA	- Tunable degradation (1-6 months) - Controllable mechanics	- Acidic degradation byproducts	Ratio adjustment of PLA/PGA	Phase-specific release
Inorganic Materials	Hydroxyapatite (HA)	- High bioactivity - Bone conductivity (similar to natural bone)	- Brittleness - Processing challenges	Surface loading for bone deposition	Sustained release + Ca/P ion supply
	Tricalcium Phosphate (TCP)	- High solubility - Fast Ca/P ion release	- Overly rapid degradation (2-4 weeks)	Short-term defect repair	Rapid release + ionic supplementation
Fabrication Techniques	Physical Blending	- Simple operation - Low cost	- Poor control over loading/release kinetics	Solution/melt blending	Initial burst + gradual release
	Chemical Grafting	- Stable loading - Controlled release	- Complex process - Potential drug activity alteration	Covalent bonding	Linear controlled release
	Electrospinning	- High surface area (>80 m^2/g) - Porosity >90%	- Limited mechanical strength	Nanofiber scaffold preparation	Sustained release (1-3 months)

Notes: PLA, polylactic acid; PGA, polyglycolic acid; PLGA, poly lactic-co-glycolic acid; ECM: extracellular matrix.

Moreover, icariin has been shown to promote the osteogenic differentiation of MC3T3-E1 pre-osteoblasts via activation of the Hedgehog signaling pathway [50]. This pathway plays an indispensable role in embryonic development, adult tissue homeostasis, and damage repair [51]. In bone tissue, Hedgehog signaling regulates osteoblast differentiation, proliferation, and mineralization. Icariin may act through Hedgehog receptors to trigger downstream signaling cascades and transcription factor activation, leading to upregulation of osteogenic genes. This promotes the transition of osteogenic progenitor cells into mature osteoblasts and promotes new bone formation [52].

The potential of icariin in bone tissue engineering has attracted increasing attention, with its regulatory role in osteogenic signaling pathways emerging as a key research focus [53]. In addition to several well-characterized signaling pathways, recent studies have revealed its potential associations with other signaling pathways, further highlighting the mechanistic complexity of its osteoinductive effects. Notably, icariin plays a key role in activating the bone morphogenetic protein (BMP) signaling pathway [54-57]. BMP signaling induces the differentiation of undifferentiated mesenchymal stem cells (MSCs) into osteoblasts and promotes their maturation and matrix mineralization. Icariin may strengthen the transduction efficacy of BMP signaling by interacting with BMP receptors and upregulating downstream effector molecules such as Smad1, Smad5, and Smad8 [58, 59]. Phosphorylated Smads further regulate the transcription of osteogenesis-related genes, promoting matrix synthesis and mineralized nodule formation. In vitro studies showed that icariin significantly enhanced the expression and activity of BMP pathway components in cultured osteoblasts, accompanied by significantly improved osteogenesis ability, providing robust evidence of BMP-mediated mechanisms. Moreover, the Notch signaling pathway has also been implicated in the osteogenic effects of icariin [60]. The Notch signaling pathway plays an important role in cell proliferation, differentiation, and fate determination. In bone tissue, it regulates the balance between osteoblasts and osteoclasts and is involved in bone development and repair. Icariin may influence osteoblast function by modulating Notch signaling pathway activity [2, 61]. Specifically, icariin alters the expression of Notch receptors and their ligands, affecting the downstream expression of transcriptional targets such as *Hes 1* and *Hey1* [62, 63]. These genetic changes may enhance the proliferation and differentiation of osteoblasts, while concurrently inhibiting osteoclastogenesis, supporting bone formation and regeneration.

At a deeper molecular level, icariin regulates these signaling pathways through several molecular mechanisms [64]. First, it may directly act on the key proteins in the signaling pathway, modifying their structure, function, and/or phosphorylation status, thereby altering downstream signaling activity. For example, by interacting with the catalytic site of a protein kinase, icariin could modulate its phosphorylation efficiency and the subsequent activation of target genes. Second, icariin may indirectly affect pathway activity by regulating intracellular environmental factors, including oxidative stress status and calcium ion concentrations [65]. Third, icariin may interact with membrane receptors or surface proteins to initiate intracellular signaling cascades that culminate in osteogenic gene activation.

In terms of clinical application prospects, it is of great significance to elucidate the mechanisms by which icariin activates osteogenic signaling pathway. Such knowledge provides a solid theoretical foundation for the development of novel bone regeneration therapies. For example, advanced drug delivery systems can be designed to precisely deliver icariin to bone defect sites, thereby enhancing local bioavailability and therapeutic efficacy [33, 66]. Furthermore, a deeper mechanistic understanding enables the design of combination therapies - pairing icariin with synergistic drugs or treatment modalities to further improve the efficiency and quality of bone repair.

Despite encouraging progress, the precise mechanism of icariin activating osteogenic signaling pathway remain incompletely understood [67]. Future research should adopt multidisciplinary approaches, integrating cell biology, molecular biology, biochemistry and biophysics, to provide more scientific basis for its wide application in clinical practice.

In conclusion, by modulating multiple osteogenic signaling pathways, icariin affects osteoblast

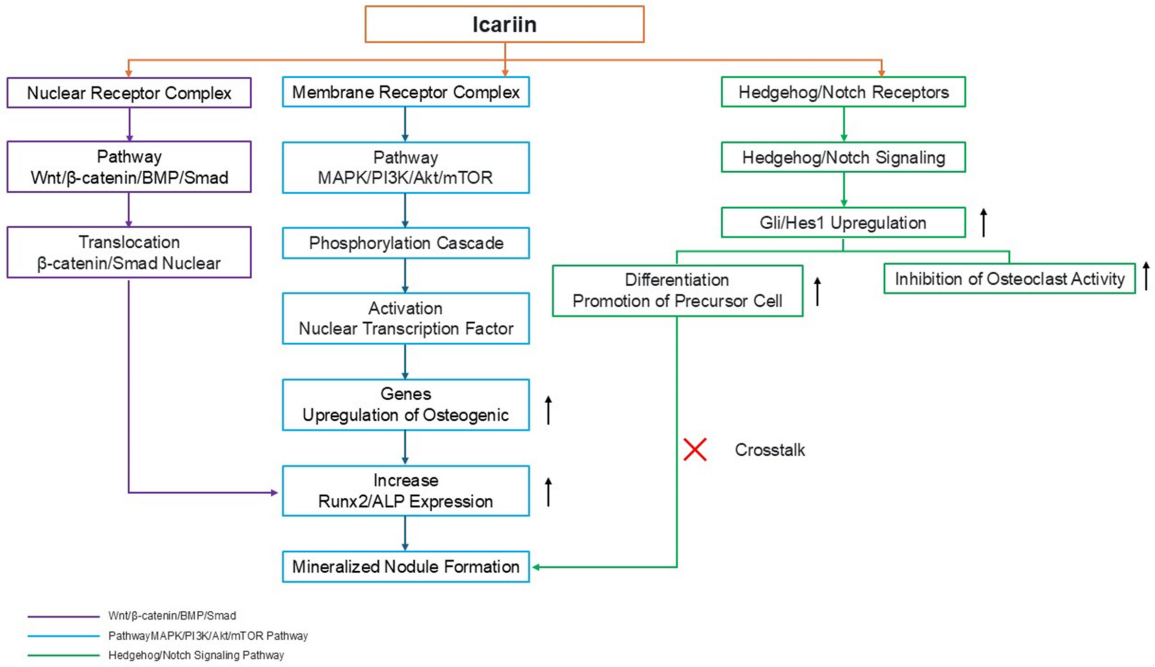

Figure 3. Icariin-mediated signaling network in osteogenesis.

function and orchestrates bone formation and repair at various molecular and cellular levels, underscoring its significant therapeutic potential in bone tissue engineering (**Figure 3**).

Effects of icariin on osteoblast differentiation

Icariin exerts multidimensional regulatory effects on osteoblast differentiation throughout the entire process of osteoblast differentiation [68]. In the early differentiation stage, ALP serves as a landmark enzyme. Changes in ALP activity are widely recognized as indicators of osteogenic differentiation. Numerous studies have shown that icariin can significantly modulate ALP activity in osteoblasts. For example, *in vitro* experiments using the mouse pre-osteoblast cell line (MC3T3-E1) demonstrated that treatment with an appropriate concentration (e.g., 10^{-6} mol/L) of icariin led to a significant increase in ALP activity, indicating that icariin promotes early osteoblast differentiation [69]. The enhancement is likely due to icariin's involvement in the regulation of signaling pathways or molecular mechanisms related to ALP synthesis and activation, resulting in increased enzyme production and activity. Consequently, this lays the foundation for the subsequent osteogenesis-related activities such as deposition and mineral matrix formation.

As differentiation progresses, the expression and secretion of OCN, a late-stage osteogenic marker, become a key observation indicator of osteoblasts maturation. Icariin has also been shown to upregulate OCN expression and secretion [70]. ELISA and other detection means have confirmed that icariin treatment increases the content of OCN in cell culture supernatants and bone tissues. In vivo experimental model have further validated that icariin administration leads to elevated OCN levels in bone tissue, reflecting the enhanced secretory function and terminal differentiation of osteoblasts under icariin stimulation. These findings suggest that icariin promotes not only early-stage osteoblast activation but also supports terminal maturation, which is essential for bone matrix mineralization and the establishment of structurally mature bone tissue [71-73]. **Figure 4** presents stage-specific mechanism of icariin action in osteogenesis.

The formation of mineralized nodules is a hallmark of osteoblast differentiation and maturation, and icariin plays a significant role in promoting this process [74]. *In vitro* studies using Alizarin Red S staining have demonstrated that treatment with appropriate concentration of icariin (such as 10^{-6} mol/L) results in a substantial increase in mineral nodule formation, with

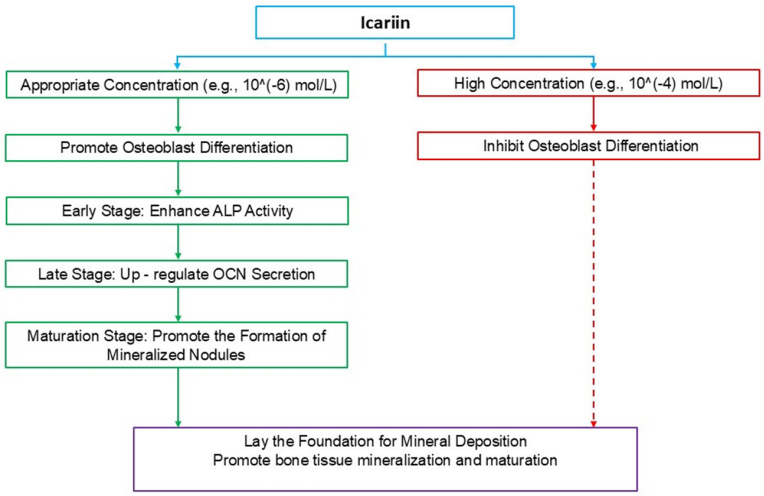

Figure 4. Schematics of stage-specific action of Icariin during osteoblast formation.

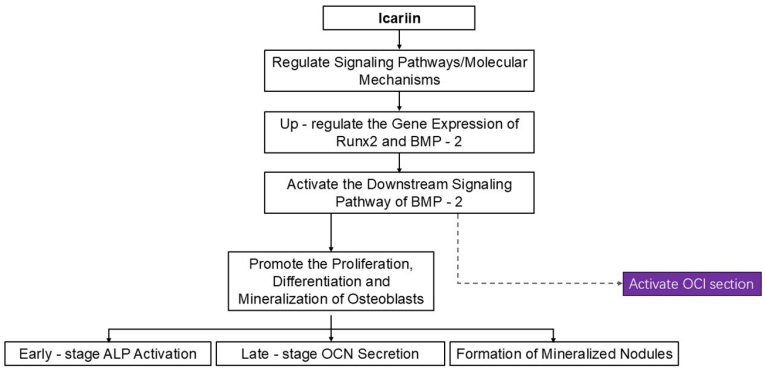

Figure 5. Molecular mechanism network of icariin's osteogenic activity.

notable increases in both nodule number and size, as well as in their clustered distribution. These findings indicate that icariin enhances calcium salt deposition and accelerates the mineralization capacity of differentiating osteoblasts [32, 75]. However, when the concentration of icariin is too high (e.g., 10^{-4} mol/L), mineral nodule formation is markedly inhibited, and few or no nodules are observed. This phenomenon reflects a bidirectional regulatory mechanism of icariin, whereby moderate doses promote osteoblast differentiation and mineralization, whereas excessive high concentrations exert inhibitory effects.

At the molecular level, qRT-PCR has revealed that icariin increases mRNA levels of osteogenic markers such as Runx 2 and BMP-2, while immunoblot analysis confirms corresponding upregulation at the protein level [14, 76, 77]. For example, enhanced expression of BMP-2 facilitates the activation of downstream signaling cascades that regulate osteoblast proliferation, differentiation, and mineralization, collectively validating the promotive effect of icariin on osteoblast differentiation at the molecular level.

To sum up, icariin plays a crucial role in promoting osteoblast differentiation across multiple stages. By regulating the stage-specific biomarkers and the expression of related genes and proteins, icariin effectively drives osteoblasts toward maturation and supports the physiological processes of bone formation and repair (**Figure 5**).

The promoting effect of icariin on bone repair

Icariin has shown significant efficacy in promoting bone repair, with mechanisms that operate at multiple biological levels. These effects have been verified in various experimental models and application scenarios [31]. At the cellular level, icariin maintains osteoblast viability and function, thereby creating a favorable cellular environment for bone repair [78]. Within an appropriate concentration range, icariin promotes the proliferation of osteoblasts, resulting in an increase in the cell numbers. For instance, when MC3T3-E1 pre-osteoblast cells were treated with icariin at a concentration of 10^{-6} mol/L, the optical density (OD) values from proliferation assays increased significantly, and direct cell counting confirmed a rapid growth trend [79-81]. At the same time, icariin's role in osteoblast differentiation is indispensable. It enhances key differentiation indicators, including ALP activity, osteocalcin secretion, mineralization nodule formation, and the expression of osteogenesis related genes and protein, thereby promoting osteoblast maturation and functional competence. For example, at the bone defect site, mature osteoblasts can secrete

extracellular matrix (ECM) components and promote calcium salt deposition, gradually filling the defect area.

In terms of ECM regulation, icariin demonstrates significant positive effects [1]. The ECM provides both structural scaffolding and a biochemical milieu essential for bone regeneration, supporting cell survival, proliferation, and intercellular communication. Icariin enhances osteoblast-mediated synthesis and secretion of essential ECM components such as type I collagen, thereby enriching the cellular microenvironment [1]. Furthermore, in cases of ECM damage, icariin accelerates matrix repair process by stimulating osteoblasts to rapidly produce matrix constituents, restoring both the structural integrity and biological function of the ECM [77]. This restoration ensures effective cell signaling transmission and maintains normal nutrient exchange, which are essential for the continuous progression of bone regeneration [32, 74, 75].

In the field of bone tissue engineering, icariin exhibits promising application potential by enhancing bone repair efficacy. When incorporated into conventional bone tissue engineering scaffolds (e.g., PLGA) to form composite scaffolds, icariin confers dual benefits: the scaffolds maintain excellent biocompatibility while significantly improving osteoinductive capacity [33]. *In vitro* experiments using rabbit bone marrow-derived MSCs has demonstrated that icariin-containing composite scaffolds significantly increase ALP activity and OCN secretion. Additionally, the expression of osteogenesis-related genes (e.g., RUNX2 and COL1A1) and corresponding protein levels also reached maximum values, indicating robust osteogenic differentiation potential [66]. These properties effectively guide MSC lineage commitment toward the osteoblast phenotype, thereby accelerating new bone tissue formation [2]. *In vivo* studies further confirmed the regenerative potential of icariin-containing scaffolds. When implanted into bone defects, these scaffolds promoted early-stage bone formation, progressively filled the defect areas and restored both the structural integrity and mechanical properties of bone tissue [82]. Compared to control scaffolds without icariin, the composite materials demonstrated significantly superior outcomes in promoting bone regeneration [83].

In addition, icariin promotes bone repair through dual mechanisms: modulating inflammatory responses and inhibiting osteoclast differentiation [84-86]. During bone injury repair, excessive or prolonged inflammatory responses can impede the healing process. Icariin has been shown to effectively suppress the release of pro-inflammatory cytokines and inhibit NF-κB pathway activation, thereby reducing local inflammation [68, 70, 78]. Concurrently, the overactivation and persistence of osteoclasts significantly delay bone regeneration. Icariin activates the STAT3 signaling pathway to enhance transcription of downstream osteogenic genes, including OCN, while simultaneously inhibiting osteoclast differentiation [53, 68]. This dual action helps maintain the dynamic balance between osteoblast and osteoclast activity, ensuring effective bone regeneration.

Research perspective

From a research and development (R&D) perspective, icariin's multifaceted bioactivity positions it as a promising candidate for innovative applications in biomedical tissue engineering. Current efforts primarily focus on optimizing its delivery systems, enhancing molecular stability within complex biological environments, and exploring synergistic interactions with biomaterials to precisely modulate the regeneration microenvironment [64].

Delivery system innovations: The hydrophobic nature of icariin presents challenges for its incorporation into aqueous-based formulations, prompting the development of advanced nanotechnology-based delivery systems. Lipid-based nanoparticles (LNPs) and polymer-based nanocarriers (e.g., PLGA) have been investigated to enhance icariin's aqueous solubility, protect it from premature degradation, and enable controlled release [87]. Surface modification of these carriers with targeting ligands (e.g., peptides specific to tissue repair niches) can further enhance site-specific accumulation, minimizing off-target effects. Additionally, integrating icariin into hydrogel-based scaffolds - either through physical entrapment or chemical conjugation - allows for localized delivery within 3D tissue constructs, supporting sustained release over extended periods, which is critical for tissue remodeling and regeneration [34].

Synergies with biomaterial scaffolds: In tissue engineering, icariin's osteogenic, angiogenic, and anti-inflammatory properties offer unique advantages when integrated with biomaterial scaffolds. For bone tissue engineering, incorporating icariin into calcium phosphate ceramics or collagen-based matrices has been shown to promote MSC differentiation into osteoblasts while inhibiting osteoclast activity, thereby enhancing new bone formation. In vascular tissue engineering, icariin-loaded electrospun nanofibrous scaffolds have demonstrated enhanced endothelial cell proliferation and tube formation, suggesting improved vascularization of engineered tissues [21, 26]. Moreover, in neural tissue applications, icariin-loaded chitosan scaffolds have exhibited neuroprotective effects and stimulated neurite outgrowth, suggesting potential utility in repairing central nervous system injuries. These interactions highlight the need for interdisciplinary research in designing smart biomaterials that dynamically respond to cellular signals, with icariin serving as both a bioactive modifier and therapeutic agent.

Translational potential and challenges: While preclinical studies strongly supports icariin's efficacy in promoting tissue regeneration, several challenges must be addressed to enable successful clinical translation. Standardization of icariin purity and bioactivity remains critical, as natural sources may yield variable compositions. The integration of herbal-derived compounds with synthetic biomaterials introduces unique regulatory complexities, necessitating comprehensive toxicological assessments and long-term safety data [76]. Future efforts should prioritize large-animal models to evaluate biodegradation kinetics of icariin-loaded scaffolds, immune responses to sustained icariin release, and functional integration of regenerated tissues into host environments. Additionally, exploring gene-delivery systems that induce endogenous icariin-like pathways may offer an alternative strategy to overcome current delivery limitations, though this approach introduces new challenges in translational regulation [24, 27].

To sum up, icariin promotes bone repair through multiple mechanisms, including regulation of osteoblast and osteoclast function, maintenance of extracellular matrix integrity, optimization of scaffold bioactivity, and modulation of inflammation. These properties make icariin a highly promising therapeutic candidate for addressing clinical challenges associated with bone defects and tissue regeneration.

Conclusion

This study presents a systematic analysis of the osteogenic potential of icariin and its translational applications in biomedical tissue engineering. Experimental evidence demonstrates that icariin significantly enhances osteoblast proliferation and differentiation, improves ECM synthesis and regeneration efficiency in both *in vitro* and *in vivo* models. Its multifaceted mechanisms of action involve activation of osteogenic signaling pathways (e.g., Wnt/β-catenin), upregulation of key osteogenic gene expression, promotion of angiogenesis, and modulation of immune cell activity. Despite these promising findings, further studies are urgently needed to clarify icariin's pharmacokinetic profiles, assess long-term safety profiles, and optimize its delivery strategies for clinical use. Looking ahead, as research progresses, icariin holds strong potential as a novel therapeutic agent for bone tissue engineering, offering promising therapeutic avenues for the treatment of bone defect and osteoporosis.

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Disclosure of conflict of interest

None.

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