



Review

Epimedium: A Versatile Player in Bone Tissue Engineering

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Abstract

Epimedium is a traditional herb that has been investigated for its potential osteogenic and bone-regenerative effects, yet systematic analyses of its multi-component, multi-target mechanisms in bone metabolism and tissue engineering applications are limited. To address this knowledge gap, this narrative review systematically summarizes more than 20 bioactive constituents extracted from various parts of major medicinal *Epimedium* species. We elaborate on their pivotal roles in modulating osteogenic differentiation, suppressing osteoclastogenesis and bone resorption, regulating inflammatory responses, exerting antioxidant effects, promoting angiogenesis, demonstrating antitumor activity, exerting chondroprotective effects, and exhibiting antibacterial activity during bone defect repair. Furthermore, we highlight recent advancements and current trends in applying these bioactive constituents within the field of bone tissue engineering. Additionally, we compared the osteogenic and osteoprotective potentials of three metabolically related flavonoids (icariin, icaritin, and icariside II). Network pharmacology was utilized to elucidate their multi-target mechanisms in modulating bone metabolism, providing insights for future mechanistic studies. Despite the long-standing clinical use and extensive scientific investigation of *Epimedium*, this review identifies several challenges associated with its application in bone tissue engineering and offers constructive recommendations to overcome these limitations.

Keywords: *Epimedium*; bioactive constituents; bone tissue engineering; bone-related biological functions; network pharmacology

Academic Editor: Umberto Garagiola

Received: 16 August 2026

Revised: 19 October 2026

Accepted: 21 October 2026

Published: 26 October 2026

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1. Introduction

Bone, a marvel of nature's engineering, is a dynamic and highly vascularized tissue that undergoes continuous remodeling throughout an individual's lifetime. Although bone possesses a robust regenerative capacity, severe bone injuries and pathological conditions often fail to heal spontaneously due to the lack of structural scaffolds for new bone formation and positive regulatory signals required for bone regeneration [1]. Traditional

autologous or allogeneic bone grafts present clinical limitations, such as donor site morbidity, restricted availability, and potential long-term complications, thereby urgently necessitating advanced bone tissue engineering solutions [2].

Successful bone regeneration is a highly complex process involving the regulation of osteogenic and osteoclastic differentiation, inflammatory responses, oxidative stress, and angiogenesis. Among these, the coordinated regulation of osteogenic differentiation and the suppression of osteoclastogenesis constitute the core mechanism for maintaining bone metabolic homeostasis in bone tissue engineering. A key challenge in current research is the development of biomaterial-based delivery systems to achieve targeted inhibition of osteoclast activity while preserving normal physiological bone remodeling processes [3,4]. Because chronic inflammation and oxidative stress can impair osteoblast function and hyperactivate osteoclasts, developing scaffold materials with integrated antioxidant and anti-inflammatory properties to modulate the local tissue microenvironment has emerged as a major challenge [3–6]. Angiogenesis supports osteogenesis by providing essential nutrients and signaling molecules, such as vascular endothelial growth factor (VEGF) secreted by endothelial cells [5–7]. However, the architectural design of vascular channels within scaffolds must balance adequate mechanical strength with optimal biological performance. Furthermore, treatments for specific clinical conditions impose additional requirements on ideal scaffolds. For instance, following bone tumor resections, implants (e.g., titanium (Ti) and its alloys, polyetheretherketone) must simultaneously release anticancer drugs to inhibit tumor recurrence and promote rapid osteogenesis [8–10]. For osteochondral interface defects, scaffold design should focus on the unique biomechanical properties of cartilage (e.g., a low friction coefficient and high compressive resilience) and its avascular microenvironment to effectively repair the bone–cartilage junction [11]. Finally, implant materials must overcome bacterial biofilm formation and local microenvironmental alterations caused by infections (such as hypoxia and acidic pH). Thus, antibacterial properties represent a critical factor in ensuring successful bone regeneration.

Epimedium, recognized as a potent promoter of bone healing [12], was first documented in the Shennong's Herbal Classic (circa 200–300 AD). In the Compendium of Materia Medica, compiled by Shizhen Li during the Ming Dynasty, *Epimedium* was described in detail for its therapeutic properties, including strengthening muscles and bones, enhancing vital energy, benefiting the liver and kidneys, and improving mental clarity. Modern pharmacological studies further support these traditional claims, demonstrating that multiple bioactive constituents of *Epimedium* exhibit significant bone-protective effects [13–17]. Due to its diverse chemical composition and broad pharmacological activities, *Epimedium* has become a focal point in the research of medicinal plants and their applications in treating various diseases. Overall, in the context of bone tissue engineering, *Epimedium* exerts its pharmacological effects through multiple biological pathways. With respect to bone metabolism, its active constituents promote the differentiation of mesenchymal stem cells into osteoblasts and modulate key signaling pathways to suppress osteoclast activity, thereby enhancing the balance between bone formation and resorption [18,19]. The antioxidant properties are characterized by the scavenging of free radicals and the upregulation of endogenous antioxidant enzyme activities, which mitigate oxidative stress-induced cellular damage [20,21]. Its anti-inflammatory effects are mediated through the inhibition of pro-inflammatory signaling cascades and the regulation of immune factor secretion [22–24]. The anti-tumor activity involves the induction of tumor cell apoptosis, suppression of angiogenesis, and enhancement of immune surveillance [25–28]. Furthermore, icariin, a major flavonoid of *Epimedium*, induces endothelium-dependent vasorelaxation and improves vascular endothelial function, effects that may promote local per-

fusion and support vascularized bone regeneration [29,30]. This multi-target, multi-pathway mechanism of action positions *Epimedium* as a bridging agent between traditional medicine and modern bone repair technologies, thereby opening new avenues for research in the clinical management of complex bone defects. Furthermore, its antibacterial properties and chondroprotective effects contribute to establishing a favorable microenvironment throughout the entire bone defect repair process [31,32].

Based on the aforementioned rationales, this narrative review aims to inspire researchers to explore further potential applications of *Epimedium* in bone tissue engineering, by artfully synthesizing the latest advancements in elucidating its bioactive compounds' regulatory mechanisms. The review focuses on analyzing over twenty bioactive compounds extracted from key medicinal *Epimedium* species (including *Epimedium brevicornum* Maxim., *Epimedium sagittatum* Maxim., *Epimedium pubescens* Maxim., *Epimedium sutchuenense* Franch., *Epimedium wushanense* T. S. Ying, *Epimedium acuminatum* Franch., and *Epimedium koreanum* Nakai.) to explore their intricate roles in bone defect repair. We elucidate their diverse biological activities across multiple targets and highlight the advanced drug delivery systems engineered to enhance therapeutic potency. Flavonoids are the most widely utilized active constituents derived from *Epimedium*. Notably, icariin, icaraside II, and icaritin exhibit a well-defined metabolic conversion relationship [14,23]. However, ongoing debates regarding their physicochemical properties and relative bone-protective activity have hindered a comprehensive elucidation of the pharmacological substance basis of *Epimedium*. In closing, we reflect on prevailing challenges and chart a visionary course for future research, encompassing the convergence of multi-omics technologies, the design of intelligent biomaterials, artificial intelligence, and the development of sophisticated preclinical models, aiming to accelerate research progress and clinical translation in bone regeneration derived from *Epimedium* (Figure 1).

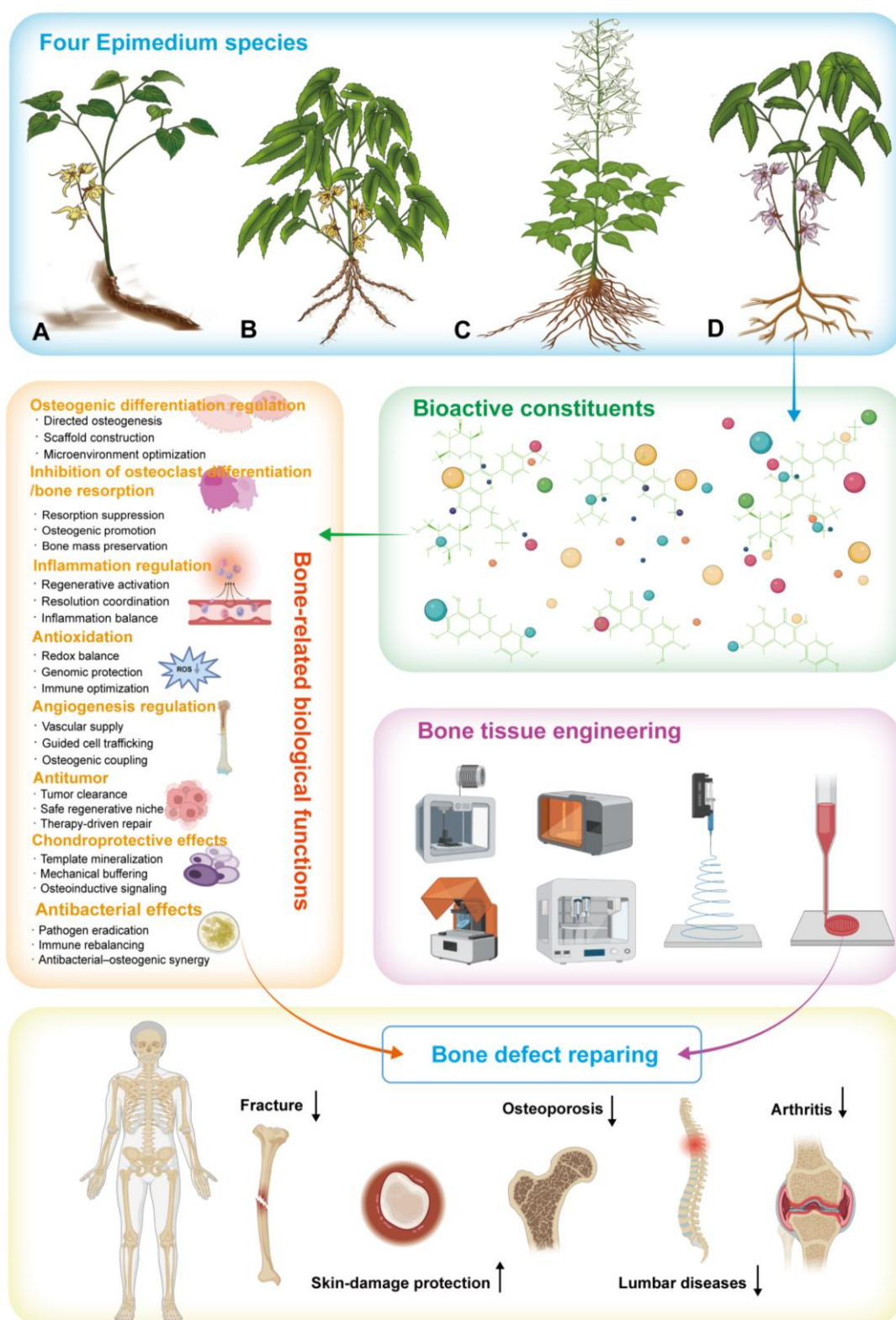


Figure 1. Schematic of “Traditional Medicinal Herb-to-Bone Tissue Engineering-to-Bone Defect Repair”: Integration of bioactive constituents from four *Epimedium* species: (A) *Epimedium koreanum* Nakai., (B) *Epimedium sagittatum* (Sieb. et Zucc.) Maxim., (C) *Epimedium brevicornum* Maxim., and (D) *Epimedium sutchuenense* Franch. and their modulation of key biological mechanisms, including osteogenic differentiation, inhibition of osteoclast differentiation and bone resorption, inflammation regulation, antioxidant effects, angiogenesis regulation, antitumor effects, chondroprotective effects, and antibacterial effects, in combination with advanced bone tissue engineering strategies. These

functionalized constructs are specifically designed to meet clinical demands, enabling effective repair of bone defects and the treatment of diverse pathologies, including fractures, osteoporosis, and arthritis. The circle colors distinguish different *Epimedium*-derived bioactive constituents, while the circle sizes represent their molecular weights. Arrows show the flow from the four *Epimedium* species to these constituents (blue), from the constituents to their biological effects (green), and from these effects (orange) or tissue-engineering approaches (magenta) to bone defect repair. Black arrows indicate whether the labeled outcomes decrease (↓) or increase (↑).

2. Active Constituents of *Epimedium*

The chemical investigation of *Epimedium* commenced in 1935, initially pioneered by the Japanese scholar Akai [33]. To date, more than 270 constituents have been isolated and identified from *Epimedium*, including flavonoids, polysaccharides, alkaloids, organic acids, phytosterols, and terpenoids [34]. These constituents collectively constitute the complex and diverse pharmacological basis of *Epimedium*, thereby providing a scientific rationale for its application in traditional Chinese medicine (Figure 2 and Table 1).

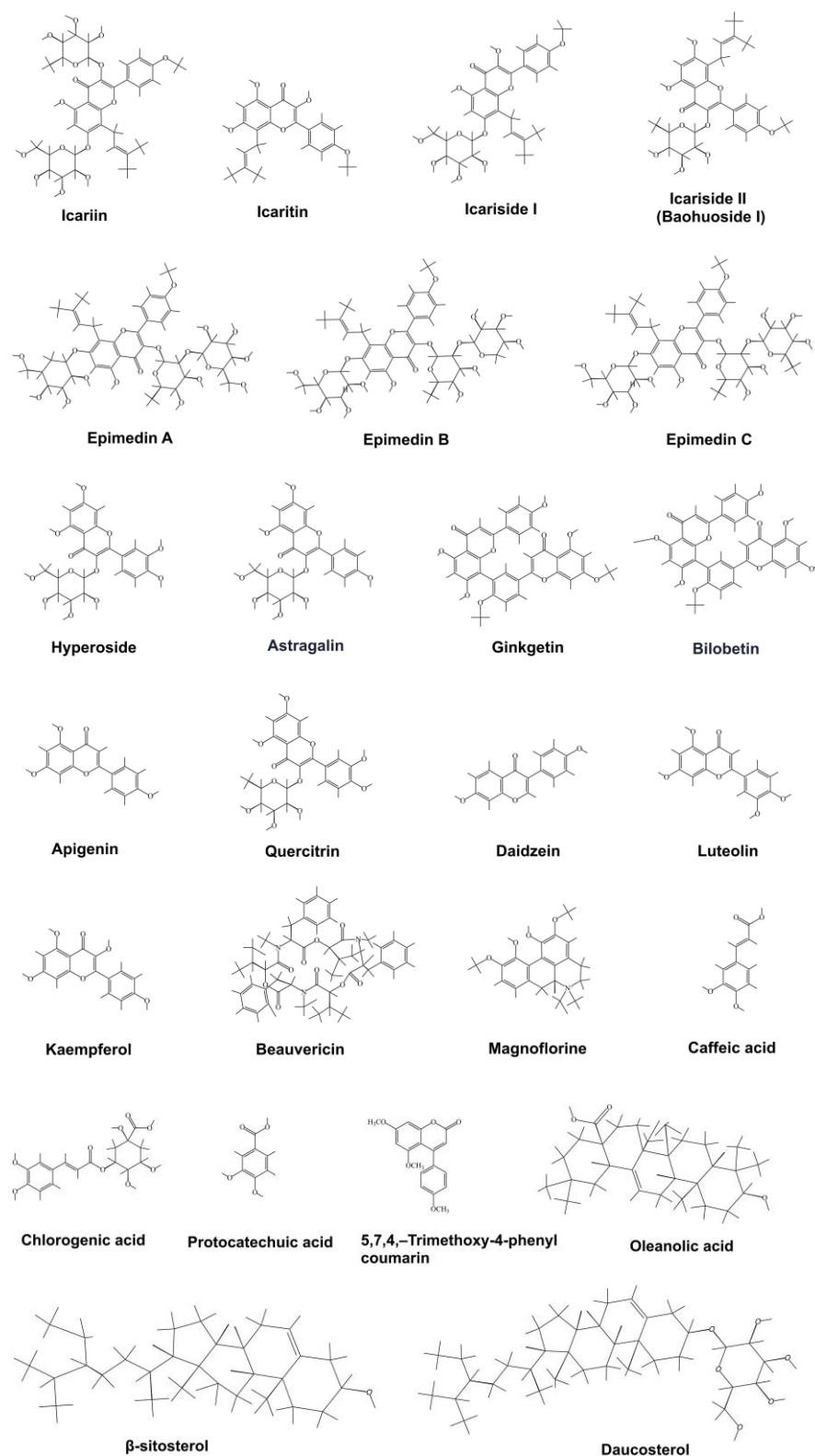


Figure 2. Structural formulae of active constituents of *Epimedium*.

2.1. Flavonoids

Flavonoids are a common class of plant phenolic compounds, which can be categorized into bioflavonoids (e.g., vitamin P), isoflavones (e.g., phytoestrogens), and neoflavonoids (e.g., leucoanthocyanidins) [35]. Flavonoids represent the primary bioactive constituents and function as characteristic chemotaxonomic markers for the genus *Epimedium*.

[36]. The compounds constitute approximately 1% to 8% of the dry weight of *Epimedium* [37]. *Epimedium* contains a variety of 8-prenylflavonoids, such as icariin, icaritin, icariside I, icariside II (also known as baohuoside I), sagittatoside A, sagittatoside B, icaritin dirhamnoside, epimedin A, epimedin B, and epimedin C [38,39]. Among these, icariin, icariside II, epimedin A, epimedin B, and epimedin C are recognized as the most abundant bioactive flavonoids, with icariin, epimedin A, epimedin B, and epimedin C collectively accounting for over 52% of total flavonoids in dried *Epimedium* [40]. Notably, icariin and icariside II are regarded as the most promising bioactive components in *Epimedium* for their potential in bone metabolism regulation [41]. However, the concentrations of these bioactive components in *Epimedium* can vary significantly depending on the species, harvesting time, storage duration, and geographical origin. For instance, icariin predominates in *Epimedium brevicornum* Maxim. and *Epimedium koreanum* Nakai. varieties, followed by epimedin B, epimedin C, and epimedin A [40,42]. Conversely, in *Epimedium sagittatum* Maxim., *Epimedium pubescens* Maxim., and *Epimedium wushanense* T. S. Ying, epimedin C emerges as the most abundant constituent, succeeded by icariin, epimedin B, and epimedin A [40]. Quantitative analysis reveals the following mean proportions of icariin, epimedin A, epimedin B, and epimedin C among 15 investigated flavonoids: *Epimedium brevicornum* Maxim. (85.6%), *Epimedium koreanum* Nakai (82.3%), *Epimedium sagittatum* Maxim. (68.8%), *Epimedium pubescens* Maxim. (74.9%), and *Epimedium wushanense* T. S. Ying (69.8%) [40]. *Epimedium sagittatum* Maxim. demonstrates notably higher concentrations of epimedin C (10.88 ± 0.83 mg/g dry-weight basis (DWB)), icariin (11.21 ± 1.12 mg/g DWB), and icariside II (3.23 ± 0.24 mg/g DWB) compared with other *Epimedium* species, with these levels being influenced by geographical provenance [42]. *Epimedium pubescens* Maxim. is generally considered to possess the highest overall bioactive content [43]. Comparative studies under identical field conditions demonstrate that *Epimedium sagittatum* Maxim. exhibits significantly greater concentrations of key bioactive components (icariin, icariside II, and epimedin C) than *Epimedium pubescens* Maxim. when grown in equivalent abiotic and biotic environments [43].

2.1.1. Principal Flavonoids

Icariin is the most abundant bioactive compound found in *Epimedium*, as supported by pharmacological research [44,45]. Structurally, icariin contains a glucose moiety attached at the C-3 position, a rhamnose group at the C-7 position, a methoxy group at C-4 position, and an isopentenyl group at C-8 position. The isopentenyl moiety at the C-8 position is believed to be the active component responsible for osteoblast differentiation [46]. Icaritin, a flavonoid aglycone derivative of icariin, is formed through the hydrolytic cleavage of glycosidic moieties from icariin, icariside I, and icariside II [47,48]. It serves as a major metabolite of icariin in both the intestine and serum [49–53]. Icariside is one of the bioactive constituents of *Epimedium*. It is demonstrated that following oral administration of icariin, various metabolites such as icariside I, icariside II, and icaritin are generated through degradation by intestinal flora [54].

Epimedin A, epimedin B, and epimedin C are flavonol glycosides and principal bioactive components of *Epimedium* [16,17,55]. Epimedin C is the most abundant flavonol glycoside among certain *Epimedium* species, such as *Epimedium brevicornum* Maxim. [55]. It serves as a key chemical marker for the quality control of *Epimedium* medicinal materials, with reported content ranging from 1.22 to 40.63 mg/g DWB [56,57]. Studies have indicated that other herbal components present in *Epimedium* extracts may reduce the oral bioavailability of epimedin C [58]. Moreover, Islam et al. investigated the relationship between the chemical fingerprint of *Epimedium* extracts and their estrogenic activity, revealing a strong correlation between the total content of epimedin A, epimedin B, epimedin C and the estrogenic activity of the extracts [59].

Hyperoside is also one of the common flavonoid components in *Epimedium*. Its content varies significantly depending on the plant species and processing methods [60,61]. It is also known as quercetin-3-O- β -D-galactoside pyran glycoside and belongs to the flavonol glycoside category [60,61]. The aglycone of hyperoside, quercetin, is the active component in plants. Astragalin is a major flavonoid compound in *Epimedium*. It has a great oral bioavailability (45%) and drug similarity (0.24), and is one of the important active components that enable it to exert anti-osteoporosis effects [62].

2.1.2. Minor Flavonoids

Studies have quantified trace flavonoid aglycones present in *Epimedium* species, including apigenin, quercitrin, daidzein, luteolin, and kaempferol. These flavonoid aglycones exhibit an average concentration of 0.038 ± 0.0061 g per 100 g of ethanol extract. Apigenin, i.e., 4',5,7-trihydroxyflavone, constitutes one of the minor flavonoids in *Epimedium*, with a concentration of 0.0062 ± 0.0019 g/100 g of ethanol extract [63]. The extraction of quercitrin (also known as quercetin-3-O-rhamnoside) from *Epimedium* yields only trace quantities, presenting significant challenges for its large-scale application. Daidzein is a flavonoid compound isolated from *Epimedium pubescens* Maxim, with relatively low concentrations [34]. Kaempferol is a trace flavonoid compound derived from *Epimedium brevicornum* Maxim, quantified at 0.0035 ± 0.0009 g per 100 g of ethanol extract [34]. Luteolin is a naturally occurring flavonoid extracted from *Epimedium sagittatum* Maxim. and *Epimedium koreanum* Nakai [34]. Compared with the flavonols such as quercetin or kaempferol, luteolin occurs at lower levels [64].

2.2. Polysaccharides

To date, over 20 types of polysaccharides have been isolated from *Epimedium*, which are primarily categorized into neutral polysaccharides (containing mannose and rhamnose) and acidic polysaccharides (containing uronic acids such as glucuronic acid) [65–68]. Polysaccharides purified from *Epimedium acuminatum* Franch. contain more than 50% xylose [68]. Notably, *Epimedium* polysaccharides enhance the solubility of flavonoids, such as icariin and icariside II, in a concentration-dependent manner, with acidic polysaccharides exhibiting a more pronounced solubilizing effect [69].

2.3. Alkaloids

To date, seven alkaloids have been isolated from plants of the genus *Epimedium* [34]. Among these compounds, epimedipine represents a novel alkaloid, magnoflorine is classified as a non-lipophilic alkaloid, and mortieridinone, chloramphenicol, beauvericin, (–)-4,6'-anhydrooxysporidinone, as well as the 4'-hydroxy derivative of (–)-oxysporidinone, are identified as amide-type alkaloids [34]. These compounds are characterized by complex ring structures and exhibit significant biological activities [70]. These alkaloids often act synergistically with flavonoids, such as epimedin, to modulate immune responses, inflammation, and oxidative stress [71]. The alkaloids present in *Epimedium* are predominantly composed of magnoflorine [34]. Magnoflorine is an isoquinoline alkaloid that has been proven to have significant anti-inflammatory effects.

2.4. Phenylpropanoids

Phenylpropanoids are a class of natural organic substances characterized by the phenylpropane skeleton (C6-C3), and their derivatives include simple phenylpropanoids, coumarins, lignans, and lignins [34]. Simple phenylpropanoids isolated from *Epimedium* species include chlorogenic acid, cryptochlorogenic acid, neochlorogenic acid, protocatechuic acid, and caffeic acid. Chlorogenic acid, an ester formed between caffeic acid and

quinic acid, is present in the whole plants of *Epimedium* and occurs at relatively high concentrations in *Epimedium koreanum* Nakai [34]. Its caffeic acid moiety contains a benzene ring, a vinyl group, and two phenolic hydroxyl groups, structural features that confer potent biological activities.

Protocatechuic acid, chemically known as 3,4-dihydroxybenzoic acid, is a metabolite of anthocyanins. Structurally similar to gallic acid, caffeic acid, and vanillic acid, protocatechuic acid is a naturally occurring phenolic acid that is widely distributed in nature [72]. It has been isolated from *Epimedium koreanum* Nakai and is considered one of the key phenolic constituents in this plant species [73]. Caffeic acid, also referred to as 3,4-dihydroxycinnamic acid, is a phenolic compound commonly found in medicinal plants, coffee, and olive oil [74]. It has been identified as a significant aromatic organic acid in *Epimedium koreanum* Nakai and demonstrates notable anti-inflammatory and antioxidant effects [73–75]. Coumarin compounds feature a benzopyranone ring as their core structure, which is formed through the lactonization of o-hydroxycinnamic acid. Their defining structural characteristic is the fusion of a benzene ring with a pyranone ring. These compounds are distinguished by their low molecular weight, exceptional synthetic accessibility, and remarkable bioavailability [76]. However, the coumarin compounds in the *Epimedium* genus consist of only one type of compound, i.e., 5,7,4-trimethoxy-4-phenylcoumarin [34]. This compound is isolated from the leaves of *Epimedium brevicornum* Maxim. and its content is extremely low [34]. Although the quantity and diversity of coumarins in *Epimedium* are relatively limited, numerous other derivatives found in this genus have attracted significant research interest in the field of bone biology, highlighting their potential as promising therapeutic agents.

The lignan components identified in *Epimedium* comprise twelve structural types, including monoepoxy lignans, propyl neolignans, rhamnosides, podophyllotoxin A, podophyllotoxin C, rhododendrol, isopodophyllotoxin, and cycloeuphoral [34]. These compounds are predominantly distributed in the stems, leaves, and rhizomes of *Epimedium* and contribute to its pharmacological effects through synergistic interactions with flavonoids, alkaloids, and other constituents. Lignans are primarily lipophilic and possess complex molecular architectures, often conjugating with sugar moieties to form glycosides (e.g., rhamnosides), thereby influencing their bioavailability.

2.5. Phytosterols and Terpenoids

The phytosterols found in *Epimedium* are primarily composed of β -sitosterol, with additional compounds including stigmasterol, campesterol, and daucosterol [34]. These phytosterols act synergistically with other bioactive constituents, such as icariin, to produce various pharmacological effects. Terpenoids, natural organic compounds composed of isoprene units, occur at relatively low concentrations in *Epimedium*. Oleanolic acid, a pentacyclic triterpenoid compound extracted from *Epimedium wushanense* T. S. Ying, commonly exists in both free and glycoside forms with concentrations generally ranging from 0.2% to 2% [34].

Table 1. The bioactive components that have been identified in *Epimedium* (Note: ^a Rep. Comp., representative compounds; ^b Mol. For., molecular formula; ^c Mol. Wt., molecular weight; ^d Ref., references).

Species	^a Rep. Comp.	^b Mol. For.	^c Mol. Wt.	Plant	Part	Abundance	Pharmacokinetic Exposure	^d Ref.
Flavonoids	Icariin	C ₃₃ H ₄₀ O ₁₅	676.7	<i>Epimedium brevicornum</i> Maxim., <i>Epimedium sagittatum</i> (Siebold & Zucc.) Maxim., <i>Epimedium pubescens</i> Maxim., <i>Epimedium koreanum</i> Nakai, and <i>Epimedium wushanense</i> T. S. Ying	Whole	1155.98–4252.89 µg/g across five-species means; 1.28–11.21 mg/g across East-Asian species/origins; 8.50–39.9 mg/g in 419 <i>Epimedium brevicornu</i> Maxim. samples.	<i>Epimedium</i> total flavonoid capsule (ETFC) (derived from the extraction of <i>Epimedium brevicornu</i> Maxim.) rat study: among highest plasma exposure: T _{max} 0.22 ± 0.02 h, C _{max} 18.22 ± 7.16 ng/mL, AUC _{0–∞} 62.32 ± 21.59 ng·h/mL; brain AUC _{0–t} 35.36 ± 5.56 ng·h/g.	[38,42,44,77–79]
	Icaritin	C ₂₁ H ₂₀ O ₆	368.4	<i>Epimedium brevicornum</i> Maxim., <i>Epimedium sagittatum</i> (Siebold & Zucc.) Maxim., <i>Epimedium pubescens</i> Maxim., <i>Epimedium koreanum</i> Nakai, and <i>Epimedium wushanense</i> T. S. Ying	Leaves	17.45–73.76 µg/g across five-species means; a 34-ac- cession survey detected only 0.01–0.08 mg/g in three accessions.	ETFC rat study: T _{max} 8.67 ± 1.12 h, C _{max} 2.24 ± 0.43 ng/mL, AUC _{0–∞} 24.67 ± 5.34 ng·h/mL.	[47,77,78,80]
	Icariside I	C ₂₇ H ₃₀ O ₁₁	530.5	<i>Epimedium brevicornum</i> Maxim., <i>Epimedium sagittatum</i> (Siebold & Zucc.) Maxim., <i>Epimedium pubescens</i> Maxim., <i>Epimedium koreanum</i> Nakai, and <i>Epimedium wushanense</i> T. S. Ying	Aerial	4.76–36.24 µg/g across five-species means	ETFC rat study: plasma T _{max} 0.29 ± 0.04 h, C _{max} 0.25 ± 0.05 ng/mL, AUC _{0–∞} 0.27 ± 0.02 ng·h/mL; brain AUC _{0–t} 0.41 ± 0.15 ng·h/g.	[54,60,77,78,80]
	Icariside II	C ₂₇ H ₃₀ O ₁₀	514.5	<i>Epimedium brevicornum</i> Maxim., <i>Epimedium sagittatum</i> (Siebold & Zucc.) Maxim.,	Leaves	64.84–215.37 µg/g across	ETFC rat study: high plasma exposure and brain AUC _{0–t} 33.39 ±	[42,54,77–81]

			<i>Epimedium pubescens</i> Maxim., <i>Epimedium koreanum</i> Nakai, and <i>Epimedium wushanense</i> T. S. Ying		five-species means; 0.19–3.23 mg/g across selected East-Asian materials; up to 4.48 mg/g in a 34-accession survey.	6.32 ng·h/g, comparable with icariin despite lower extract content. Rat absolute oral bioavailability reported as 4.1% versus 0.1% for icariin. Human serum: principal circulating metabolite after standardized extract.
Epimedin A	C ₃₉ H ₅₀ O ₁₉	822.8	<i>Epimedium brevicornum</i> Maxim., <i>Epimedium sagittatum</i> (Siebold & Zucc.) Maxim., <i>Epimedium pubescens</i> Maxim., <i>Epimedium koreanum</i> Nakai, and <i>Epimedium wushanense</i> T. S. Ying	Whole	1254.84–2138.93 µg/g across five-species means; 2.31–8.42 mg/g in 419 <i>Epimedium brevicornu</i> Maxim.	ETFC rat study: rapid systemic absorption; brain AUC _{0–t} 5.02 ± 0.98 ng·h/g. Oral <i>Epimedium</i> extract studies also report measurable plasma exposure. [38,77,78,82,83]
Epimedin B	C ₃₈ H ₄₈ O ₁₉	808.8	<i>Epimedium brevicornum</i> Maxim., <i>Epimedium sagittatum</i> (Siebold & Zucc.) Maxim., <i>Epimedium pubescens</i> Maxim., <i>Epimedium koreanum</i> Nakai, and <i>Epimedium wushanense</i> T. S. Ying	Whole	1111.82–2370.28 µg/g across five-species means; 6.67–55.7 mg/g in 419 <i>Epimedium brevicornu</i> Maxim. samples.	ETFC rat study: measurable plasma exposure; brain C _{max} 10.68 ± 3.62 ng/g and AUC _{0–t} 18.35 ± 2.92 ng·h/g. [38,77,78,83,84]
Epimedin C	C ₃₈ H ₅₀ O ₁₉	822.8	<i>Epimedium brevicornum</i> Maxim., <i>Epimedium sagittatum</i> (Siebold & Zucc.) Maxim., <i>Epimedium pubescens</i> Maxim., <i>Epimedium koreanum</i> Nakai, and <i>Epimedium wushanense</i> T. S. Ying	Whole	13.02–71.62 µg/g across five-species means; trace/low in a	ETFC rat study: among plasma exposure: T _{max} 1.80 ± 1.04 h, C _{max} ng/mL, AUC _{0–∞} 24.68 ± 5.50 ng·h/mL; brain C _{max} 17.49 ± 6.04 ng/g and AUC _{0–t} 28.53 ± 4.51 ng·h/g. [38,42,55,77,78,83]

					34-accession survey.		
Hyperoside	C ₂₁ H ₂₀ O ₁₂	464.4	<i>Epimedium brevicornum</i> Maxim., <i>Epimedium sagittatum</i> (Siebold & Zucc.) Maxim., <i>Epimedium pubescens</i> Maxim., <i>Epimedium koreanum</i> Nakai, and <i>Epimedium wushanense</i> T. S. Ying	Aerial	98.85–201.18 µg/g across five-species means; no significant difference among the five species in one survey ($p = 0.234$).	/	[60,77,80]
Astragalin	C ₂₁ H ₂₀ O ₁₁	448.4	<i>Epimedium brevicornum</i> Maxim., <i>Epimedium sagittatum</i> (Siebold & Zucc.) Maxim., <i>Epimedium pubescens</i> Maxim., <i>Epimedium koreanum</i> Nakai, and <i>Epimedium wushanense</i> T. S. Ying	leaves	32.88–180.32 µg/g across five-species means; trace/low in a 34-accession survey.	/	[62,77]
Quercitrin	C ₁₅ H ₁₀ O ₇	302	<i>Epimedium brevicornum</i> Maxim., <i>Epimedium sagittatum</i> (Siebold & Zucc.) Maxim., <i>Epimedium pubescens</i> Maxim., <i>Epimedium koreanum</i> Nakai, and <i>Epimedium wushanense</i> T. S. Ying	Whole	About 0.0035 ± 0.0009 g/100 g ethanol extract (about 35 ± 9 µg/g extract) in the manuscript-cited 37-specimen survey; dry-herb equivalent not reported.	/	[34,77,80]
Kaempferol	C ₂₇ H ₃₀ O ₁₄	578.6	<i>Epimedium sagittatum</i> Maxim.	Whole	/	/	[34,63]
Ginkgetin	C ₃₂ H ₂₂ O ₁₀	566.5	<i>Epimedium koreanum</i> Nakai.	Aerial	/	/	[85,86]
Bilobetin	C ₃₁ H ₂₀ O ₁₀	552.5	<i>Epimedium koreanum</i> Nakai.	Aerial	About 0.0062 ± 0.0019 g/100 g ethanol extract	/	[31,86]

						(about 62 ± 19 µg/g extract) in the manuscript-cited 37-specimen survey; dry-herb equivalent not reported.		
	Apigenin	C ₁₅ H ₁₀ O ₅	270.2	<i>Epimedium brevicornum</i> Maxim.	Whole	5.54–30.99 µg/g across five-species means.	/	[63,87]
	Luteolin	C ₁₅ H ₁₀ O ₆	286.2	<i>Epimedium brevicornum</i> Maxim., <i>Epimedium sagittatum</i> (Siebold & Zucc.) Maxim., <i>Epimedium pubescens</i> Maxim., <i>Epimedium koreanum</i> Nakai, and <i>Epimedium wushanense</i> T. S. Ying.	Whole	/	/	[34,77]
	Daidzein	C ₁₅ H ₁₀ O ₄	254.2	<i>Epimedium sutchuenense</i> Franch.	Leaves	/	/	[34]
Coumarin	5,7,4-Tri-methoxy-4-phenylcoumarin	C ₁₈ H ₁₆ O ₅	312.3	<i>Epimedium brevicornum</i> Maxim.	Leaves	706.85–1369.82 µg/g across five-species means (sample range 470.45–2165.07 µg/g); a broader 80-sample study reported 0.003–2.603% in leaves across species/parts/time s.	/	[34,88]
Alkaloid	Magnoflorine	C ₂₀ H ₂₄ NO ₄	342.4	<i>Epimedium koreanum</i> Maxim.	Whole	/	Oral <i>Epimedium</i> extract in rats: dual plasma peaks, C _{max} about	[34,77,83,89]

						411 and 276 ng/mL, $t_{1/2}$ 17.77 h, AUC _{0–∞} 4003 ng·h/mL.		
Phytosterol	β-sitosterol	C ₂₉ H ₅₀ O	414.7	<i>Epimedium koreanum</i> Maxim.	Whole	/	/	[34,90]
	Daucosterol	C ₃₅ H ₆₀ O ₆	576.9	<i>Epimedium koreanum</i> Maxim.	Whole	/	/	[34]
						249.06–664.78		
Terpenoids	Oleanolic acid	C ₃₀ H ₄₈ O ₃	456.7	<i>Epimedium wushanense</i> T. S. Ying	Root	μg/g across five-species means.	/	[34]
Phenylpro- panoid	Chlorogenic acid	C ₁₆ H ₁₈ O ₉	354.3	<i>Epimedium sagittatum</i> Maxim. and <i>Epimedium koreanum</i> Maxim.	Whole	/	Oral <i>Epimedium</i> extract in rats: C _{max} about 540 ng/mL, $t_{1/2}$ 0.45 h, [34,77,83] AUC _{0–∞} about 886 ng·h/mL.	
	Protocatechuic acid	C ₇ H ₆ O ₄	154.1	<i>Epimedium koreanum</i> Maxim.	Aerial	2.98–18.73 μg/g across five-spe- cies means.	/	[34]
	Caffeic acid	C ₉ H ₈ O ₄	180.2	<i>Epimedium koreanum</i> Maxim.	Aerial	/	/	[34,77]
	Stachydrine	C ₇ H ₁₄ NO ₂	144.2	<i>Epimedium acuminatum</i> Franch.	Aerial	/	/	[26,34]

3. Bone-Related Biological Functions of the Active Constituents

3.1. Osteogenic Differentiation Regulation

Osteogenic differentiation regulation serves as a central driving force in the process of bone regeneration, facilitating functional bone remodeling through multi-dimensional coordination of cellular behaviors and interactions with the surrounding microenvironment. This regulatory mechanism is primarily reflected in three key aspects: Firstly, by activating specific transcriptional programs, it directs stem cells to undergo lineage-specific differentiation into osteogenic cells, thereby ensuring a continuous supply of bone-forming cells [91,92]. Secondly, it governs the dynamic synthesis and remodeling of the extracellular matrix, leading to the formation of a mineralized scaffold that supports vascularization and cell migration, while the mechanical properties of the matrix provide feedback to reinforce the differentiation process [91,92]. Finally, it integrates signals from the microenvironment and coordinates the temporal interplay between inflammation resolution and tissue reconstruction, establishing conditions favorable for regeneration [91,92]. This multi-level regulatory network not only sustains homeostasis during bone regeneration but also offers critical therapeutic targets for clinical intervention. Enhancing the efficiency of this regulatory process through physical, chemical, or biological strategies can significantly improve the outcomes of repairing complex bone defects [91,92].

3.1.1. Flavonoids

Stem cells, particularly bone marrow-derived MSCs (BMSCs), have the capability to transform into osteoblasts and chondrocytes under proper inductive conditions, making them a significant biological resource for the repair of bone defects. Furthermore, stem cells can enhance cellular proliferation and extracellular matrix production by secreting bioactive chemicals, such as bone morphogenetic proteins (BMPs), OCN, osteopontin (OPN), and Col I [93,94]. Flavonoids derived from *Epimedium* have demonstrated the ability to augment cell proliferation, motility, and differentiation in the osteogenic differentiation of stem cells. Jiao et al. established that icariin promotes the migration of BMSCs both in vivo and in vitro through the activation of the MAPK signaling pathway [95]. Tang et al. reported that in a rat model of osteoporosis, the combination of icariin and stem cells significantly increases bone mass and improves bone microarchitecture [96]. Furthermore, icariin can activate the Ras homolog family member A (RhoA)-transcriptional coactivator with PDZ-binding motif (TAZ) signaling pathway to promote the proliferation and osteogenic differentiation of rat adipose-derived stem cells (ASCs), with 0.1 μM identified as the optimal concentration [18]. Under conditions of *Porphyromonas gingivalis* infection, icariin can downregulate the erythropoietin-producing hepatocellular A2 (EphA2)-RhoA signaling pathway, thereby enhancing osteogenic differentiation and bone regeneration, with 10 μM icariin showing the most pronounced effect [97]. Research has demonstrated that icariside II can enhance the osteogenic differentiation of BMSCs, with this effect being at least partially mediated through the modulation of MAPK/extracellular signal-regulated kinase (ERK) signaling pathway activation [98]. Additionally, apigenin has been shown to promote the osteogenic differentiation of BMSCs via the c-Jun N-terminal kinase (JNK) and p38 MAPK signaling pathways [99]. Furthermore, icaritin can significantly increase the expression levels of osteogenesis-related mRNAs in human BMSCs, with notable effects observed at concentrations ranging from 10^{-7} to 10^{-5} M [100]. It has been reported that hyperoside promotes the proliferation and osteogenic differentiation of BMSCs by activating the NF- κB signaling pathway, leading to improvements in experimental periodontitis in rats [101]. In osteoporotic models, kaempferol has been shown to

enhance the osteogenic differentiation of MSCs by down-regulating miR-10a-3p, up-regulating C-X-C motif chemokine ligand 12 (CXCL12), or inhibiting caveolin-1 expression [102,103]. Quercitrin can increase alkaline phosphatase (ALP) activity and mineralization in human BMSCs, thereby promoting their differentiation toward the osteoblastic lineage [104].

It is widely recognized that the differentiation of BMSCs into either osteoblasts or adipocytes is determined by the coordinated regulation of multiple signaling pathways [105,106]. However, an increase in bone marrow adiposity has been associated with a higher risk of fractures [107]. Although icariin lacks direct estrogenic activity, it has been reported to exhibit properties similar to those of selective estrogen receptor (ESR) modulators [108]. Consequently, icariin can indirectly promote osteogenic differentiation while suppressing adipogenic differentiation in rat BMSCs through modulation of the ESR α (ESR α) signaling pathway. Furthermore, icariin has been shown to restore osteogenic differentiation and enhance bone formation in bone marrow stromal cells within an estrogen-deficiency-induced osteoporotic rat model [109]. Also, icaritin not only enhances the proliferation and osteogenic differentiation of BMSCs, but also suppresses their adipogenic differentiation, thereby promoting periosteal bone formation [14,52,100,110–117].

Osteoblasts are specialized bone-forming cells within bone tissue, typically derived from BMSCs. Although osteoblasts are not terminally differentiated cells, they undergo further differentiation into osteocytes upon appropriate induction. As key effector cells in bone formation, osteoblasts play a central role in bone remodeling and repair by synthesizing osteoid, initiating mineralization, and participating in cellular signal transduction. Dysregulation of osteoblast function can contribute to pathological conditions such as osteoporosis and osteosclerosis. It has been reported that icariin can rapidly induce phosphorylation of ESR α at the serine 118 and serine 167 residues in osteoblasts via the MAPK/ERK and phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) signaling pathways, respectively [108]. Moreover, icariin can regulate the anabolic metabolism of osteoblasts by up-regulating the expression of BMP-2, BMP-4, and drosophila mothers against decapentaplegic homolog 4 (Smad4) [118–120]. Studies have demonstrated that icariin may enhance fracture healing in both young and aged rats through activation of the BMP-2/Smad5/Runx2 pathway [121]. Additionally, icariin has been shown to prevent glucocorticoid-induced osteoporosis by increasing the expression of differentiated embryonic chondrocyte expressed gene 1 [122]. Wu et al. reported that icariin can promote bone formation in rats with chronic high-dose alcohol-induced osteoporosis, inhibit bone loss, and effectively restore bone architecture and mechanical strength [123]. Primary cilia are evolutionarily conserved structures present in bone cells, including osteoblasts and chondrocytes, and play essential roles in osteogenesis and cartilage development [124–126]. Accumulating evidence suggests that primary cilia are closely associated with the onset and progression of diabetes mellitus [127,128]. Liu et al. investigated diabetic-induced bone loss and found that icariin can reverse this condition by scavenging reactive oxygen species (ROS) and activating the Cilia/Gli2/OCN signaling pathway [129]. Moreover, icariin may play a potential role in the prevention and treatment of osteoporosis by upregulating the mRNA expression levels of OPN, BMPs, and Col I in OVX rats [130]. Notably, clinical studies have indicated that icariin can increase bone density and improve bone strength in postmenopausal women [131]. In addition to icariin, icaritin and icariside II has also been demonstrated to enhance the osteogenic differentiation of BMSCs, potentially via regulation of the MAPK/ERK signaling pathway [132]. Also, icaritin can induce the differentiation and maturation of MC3T3-E1 subclone 14 cells by activating MAPK signaling components, including ERK1/2 and p38 [133]. Lead exposure has been found to inhibit the differentiation of MC3T3-E1 subclone 14 cells [134]. This may be attributed to the inhibition of the canonical Wnt signaling pathway by lead through the regulation of key

components, including Wnt3a, Dickkopf-1, p-glycogen synthase kinase 3 β (pGSK-3 β), and β -catenin [134]. As expected, icaritin treatment can partially restore Wnt/ β -catenin signaling and counteract the inhibitory effects of lead on osteogenesis [134]. The focal adhesion kinase (FAK) signaling pathway is constitutively activated during the osteogenic differentiation of human MSCs, and its activity is further enhanced upon induction of osteoblastic differentiation [135,136]. However, FAK knockout has been shown to significantly attenuate the activation of the Wnt/ β -catenin and MAPK signaling pathways and effectively inhibit BMP-9-induced bone formation in vivo [137]. Recent studies have shown that epimedin A and epimedin B can interact with FAK to modulate the signaling activity, offering therapeutic potential for osteoporosis [138]. Furthermore, RNA sequencing and KEGG enrichment analyses have revealed that epimedin B exerts its anti-osteoporotic effects by regulating the PI3K/AKT, MAPK, and peroxisome proliferator-activated receptor (PPAR) signaling pathways in mouse models [139]. Due to its structural similarity to icariin, epimedin C is hypothesized to possess comparable pharmacological properties [140]. It has been demonstrated that there are no significant differences in osteoblast function or osteogenic efficacy between epimedin C and icariin [140]. Both compounds exhibit high efficacy in lipopolysaccharide (LPS)-induced osteoblast models, where they can reverse LPS-mediated inhibition of osteoblast proliferation, mineralization, and BMP-2/Runx2 signaling [140]. Moreover, epimedin C has been shown to alleviate dexamethasone-induced suppression of bone formation in zebrafish larvae and MC3T3-E1 cells by activating the PI3K/AKT/Runx2 signaling pathway [141]. Epimedin C also protects osteoblasts from dexamethasone-induced damage by stabilizing the nuclear factor erythroid 2-related factor 2 (Nrf2)-RhoA promoter complex [142].

Hyperoside has been found to significantly activate the expression of acetylcholinesterase associated with proline-rich membrane anchor in osteoblasts [143]. Additionally, hyperoside can promote osteogenic differentiation under inflammatory conditions by simultaneously inhibiting NF- κ B activation, β -catenin expression, and ROS production, exerting beneficial effects on human osteoblast-like cells (Saos-2 cells) [144,145]. Researchers have demonstrated that the extract of St. John's wort, which primarily contains hyperoside, hypericin, and flavonoids, can significantly ameliorate forced swimming-induced stress injury in experimental animals and restore bone density to normal levels [146]. Astragalin has been reported to promote osteogenic differentiation of MC3T3-E1 cells via the BMP and MAPK signaling pathways and enhance bone formation in OVX-induced osteoporotic mice [147]. Studies have indicated that daidzein therapy improves osteoblast viability and speeds up the development of bone progenitor cells into fully developed osteoblasts. Daidzein enhances ALP activity, OCN production, and BMP-2 mRNA expression in primary osteoblast cells [148]. These effects are mediated through activation of the BMP signaling pathway, which upregulates the phosphorylation levels of Smad1/5/8 [149]. Daidzein also promotes the secretion of osteoprotegerin (OPG) in cultured normal porcine osteoblasts while reducing the membrane expression of RANKL [150]. In an OVX rat model of post-menopausal osteoporosis, Picherit et al. demonstrated that oral administration of daidzein can prevent the loss of trabecular and cortical bone or cortical bone alone [151]. Additional studies confirm that daidzein enhances osteoblast proliferation and differentiation via the BMP pathway, further supporting its role in promoting bone formation [149]. Daily intake of daidzein and tempeh has also been shown to improve calcium content, calcium transport, and bone metabolism in ovariectomized rats [152]. Dietary soy isoflavones, including genistein and daidzein, may influence calcium homeostasis by modulating bone calcium release and protecting common fracture-risk sites such as the hip and spine [153]. Apigenin has been shown to activate hydrogen peroxide-induced PI3K and AKT2 gene expression while reducing ERK2 expression, thereby enhanc-

ing cell survival and mitigating oxidative damage in osteoblasts [154]. Additionally, apigenin stimulates the generation of bone marrow-derived progenitor cells and increases the mRNA levels of osteogenic markers such as Runx2, Col I, and BMP-2 [155]. Luteolin significantly promotes osteoblast differentiation and mineralization through activation of multiple signaling pathways, including Wnt/ β -catenin, ERK/low-density lipoprotein receptor-related protein 5/GSK-3 β , and PI3K/AKT [156,157]. Network pharmacology study indicates that luteolin demonstrates multi-target effects, engaging pathways including PI3K-AKT, TNF, and estrogen signaling, thereby elucidating its molecular foundation for therapeutic efficacy in osteoporosis and glucocorticoid-driven avascular necrosis of the femoral head [158,159]. Moreover, luteolin enhances osteoblast activity by increasing the mRNA and protein expression levels of bone formation markers, including ALP, BMP-2, OPG, Osterix, and OCN, as well as the expression of β -catenin and cyclin D1 [160,161]. Nash et al. demonstrated that luteolin interacts with ESRs in mouse osteoblasts, resulting in increased ALP activity and collagen synthesis [157]. Yang et al. reported that luteolin inhibits IL-1 β -induced expression of matrix metalloproteinases (MMP-9 and MMP-13) in mouse calvarial osteoblasts, potentially through suppression of the ERK pathway, thereby preventing excessive bone matrix degradation [162]. Notably, various concentrations of luteolin (0.01, 0.1, 1, 10, and 100 μ mol/L) were found to enhance calcified nodule formation in human periodontal ligament cells. Among these, 1 μ mol/L luteolin exhibited the most pronounced effect, promoting osteogenic differentiation via activation of the Wnt/ β -catenin signaling pathway [161]. A polysaccharide purified from *Epimedium brevicornum* Maxim. has been identified with the following glycosidic linkages: $\rightarrow$ 4)- α -D-Galp-(1 $\rightarrow$ 4)- α -D-Galp-(1 $\rightarrow$ 6)- β -D-Galp-(1 $\rightarrow$ 6)- β -D-Galp-(1 $\rightarrow$ 4)- α -D-Glcp-(1 $\rightarrow$ 4)- α -D-Glcp-(1 $\rightarrow$. In vitro assays demonstrate that this polysaccharide can enhance osteogenic differentiation in osteoblasts under high-glucose conditions. Moreover, the study suggests that polysaccharide treatment promotes osteogenesis in the context of diabetic osteoporosis, potentially through the regulation of the B-cell lymphoma/leukemia-2 (Bcl-2)/Bcl-2-associated X protein (Bax) pathway and the subsequent enhancement of osteoblastic activity [163]. Another polysaccharide isolated from *Epimedium brevicornum* Maxim., primarily composed of glucose, was used to pre-treat osteoblasts during glucocorticoid-induced osteoporosis. An in vitro study indicate that it exerts protective effects on osteoblasts by activating the PI3K/AKT/mammalian target of rapamycin (mTOR) and Wnt/ β -catenin signaling pathways and suppressing mitochondrial apoptosis [164]. Quercitrin has been reported to alleviate osteoporosis by increasing ALP expression and inhibiting the JNK, ERK, and p38 MAPK signaling pathways [165]. Additionally, quercitrin protects osteoblasts from hydrogen peroxide (H₂O₂)-induced cytotoxicity and reverses oxidative stress-induced dysfunction [166]. Bilobetin has also been shown to enhance ALP activity, collagen synthesis, and calcium deposition, thereby promoting osteoblast mineralization [31]. Kaempferol activates the osteogenic signaling pathway via the Sox2/miR-124-3p/PI3K/AKT/mTOR axis, significantly increasing ALP activity and calcium deposition [167]. Network pharmacology studies further reveal that kaempferol participates in regulating the TNF signaling pathway, atherosclerosis-related signaling pathways, and the advanced glycation end products (AGEs)/receptor for AGEs (RAGE) signaling pathway through multiple targets such as AKT1 and MMP9, supporting its therapeutic potential in osteoporosis [168].

3.1.2. Other Active Constituents

Kabashima et al. demonstrated that magnoflorine at a concentration of 25 μ g/mL significantly enhances the viability, progression through the S phase, and mineralization capacity of MC3T3-E1 cells [169]. Oleanolic acid exerts anabolic effects on bone by activating

25-hydroxyvitamin D1- α -hydroxylase in osteoblasts. Furthermore, its bone-protective effects are associated with the induction of cytochrome P450 family 27 subfamily B member 1 activity and expression in bone tissue and within the osteoblast lineage [170]. Additionally, oleanolic acid promotes the osteogenic differentiation of MSCs by suppressing the Notch signaling pathway, thereby enhancing the expression of osteogenic markers such as ALP, Runx2, and Col I, which ultimately facilitates bone formation [171]. It also enhances osteoblast activity by upregulating OPG mRNA expression and modulating the phosphorylation of ERK, JNK, p38, and AKT [172]. Caffeic acid phenethyl ester demonstrates a positive regulatory effect on bone metabolism in C57BL/6J bone marrow-derived cells by promoting osteoblast differentiation and bone maturation. Moreover, low-concentration caffeic acid can enhance MSCs mineralization and upregulates the expression of osteogenic markers [173]. It also stimulates the production of BMPs and cathepsin B in osteogenic precursor cells [173]. Chlorogenic acid promotes the osteogenic differentiation of human adipose tissue-derived mesenchymal stem cells and human dental pulp stem cells (hDPSCs) via activation of the Wnt/ Ca^{2+} signaling pathway and induction of calcium deposition [174]. Han et al. confirmed that chlorogenic acid protects osteoblasts from oxidative stress by modulating the PI3K/AKT signaling pathway and enhancing the expression of Nrf2/heme oxygenase-1 (HO-1) [175]. In addition, chlorogenic acid can mitigate dexamethasone-induced osteoblast apoptosis through activation of the Nrf2/HO-1 antioxidant signaling pathway [176]. Recent studies have also revealed that oral administration of chlorogenic acid promotes the proliferation of osteoprogenitor cells and the osteogenic differentiation of BMSCs via the SH2 domain-containing protein-tyrosine phosphatase-2/PI3K/AKT/cyclin D1 signaling cascade, thereby preventing osteoporosis in ovariectomized female rats [177]. At a dosage of 20 mg/kg, chlorogenic acid significantly improved femoral head and neck bone mineral density following ischemic injury [178]. This indicates its therapeutic potential for high-concentration treatment of hormone-induced femoral head necrosis. Notably, OVX was found to markedly reduce bone volume and mechanical integrity in rats, whereas chlorogenic acid administration effectively counteracted these adverse effects [179]. In vivo studies have further demonstrated that chlorogenic acid enhances osteoblast proliferation and differentiation, thereby promoting bone mineralization and improving bone mechanical strength [180,181]. These beneficial effects may be partially attributed to chlorogenic acid's ability to amplify TNF- α -stimulated IL-6 synthesis in osteoblasts [182,183].

3.2. Inhibition of Osteoclast Differentiation/Bone Resorption

Osteoclast-mediated bone resorption is a crucial element of the bone remodeling cycle. Although maintaining osteoclast function is necessary for sustaining bone homeostasis, a certain degree of inhibition of osteoclast differentiation during bone repair may yield synergistic positive effects [184,185]. Firstly, it mitigates premature bone resorption, prolongs the collagen mineralization period, and protects the newly formed extracellular matrix [184,185]. Secondly, it disrupts the negative feedback regulation of bone remodeling, promotes mesenchymal stem cell differentiation toward the osteogenic pathway, and consequently enhances osteogenic dominance [184,185]. Thirdly, it prevents bone mass loss caused by the imbalance between resorption and formation, maintains the structural integrity of the regenerating bone tissue, and thereby improves the overall quality of bone repair [184,185].

3.2.1. Flavonoids

To date, the most extensive and in-depth research has centered on the effects of three flavonoids derived from *Epimedium* (i.e., icariin, icaritin, and icariside) on osteoclastic bone resorption. In a study utilizing a rat OVX model, icariin was demonstrated to prevent

bone loss and maintain bone strength post-OVX [186]. It dramatically enhances the expression of OPG while diminishing RANKL levels, hence increasing the OPG/RANKL ratio, suppressing osteoclast activity, and mitigating bone loss in OVX rats [187]. Recent studies have also indicated that icariin ameliorates osteoporosis in ovariectomized rats by modulating the Cullin3/Nrf2/HO-1 signaling pathway to suppress osteoclastogenesis [19]. Furthermore, icariin inhibits osteoclast differentiation and bone resorption by suppressing the synthesis of hypoxia-inducible factor-1 α (HIF-1 α) and prostaglandin E, which are regulated through the MAPK/NF- κ B signaling pathway [187]. Additional research has demonstrated that icariin exerts inhibitory effects on titanium particle-induced inflammatory osteoclastogenesis, with an optimal concentration of 0.0148 M [188,189].

Tumor necrosis factor receptor-associated factor 6 (TRAF6) serves as a key adaptor protein in transducing signals from the RANKL/receptor activator of nuclear factor κ B (RANK) axis, while nuclear factor of activated T cells c1 (NFATc1) functions as the master transcriptional regulator of osteoclast differentiation [15,190]. Icaritin suppresses osteoclast differentiation by downregulating TRAF6, which in turn inhibits the NF- κ B, MAPK/activator protein 1 (AP-1), and ROS signaling pathways, leading to reduced expression and activity of NFATc1 [15]. Notably, studies have revealed that icaritin decreases mitochondrial mass and reduces the density of mitochondrial cristae. Additionally, icaritin attenuates mitochondrial membrane potential elevation and modulates mitochondrial fusion and fission dynamics, thereby inhibiting osteoclast differentiation and maturation [53]. Zhao et al. demonstrated that icaritin can inhibit lung cancer-induced osteoclastogenesis by downregulating the expression of IL-6 and TNF- α , as well as through modulation of the AMP-activated protein kinase (AMPK)/mTOR signaling pathway [191]. The parent ring structure of icaritin is structurally similar to that of phytoestrogens, featuring hydroxylation at the C-3 and C-7 positions, which confers estrogen-like activity [192–194]. Furthermore, preliminary experiments indicate that dietary treatment of icaritin could avoid osteoporosis linked to estrogen insufficiency [195]. Lin et al. reported that icaritin can alleviate motor dysfunction in ovariectomized Parkinson's disease model mice through activating the ER/PI3K/AKT signaling pathway [196]. Also, Zhang et al. found that icaritin effectively mitigates OVX-induced bone loss and improves bone metabolism through the modulation of the toll-like receptor 4 (TLR4) and PI3K/AKT signaling pathways, thereby providing a potential therapeutic strategy for osteoporosis [195].

Regarding icariside I, it can directly inhibit osteoclast differentiation and bone resorption by suppressing the RANKL-induced TRAF6/MAPK/p38/NFATc1 signaling cascade [197]. Furthermore, icariside I can promote osteoblast differentiation and maturation in a dose-dependent manner by inhibiting adipogenesis and enhancing osteoblast energy metabolism [197]. Icariside I also significantly improves OVX-induced osteoporosis in mice by targeting the gut microbiota dysbiosis-Th1 cell-IFN- γ -osteoclast signaling axis [197]. Additionally, icariside I may inhibit osteoclast differentiation by promoting the production of short-chain fatty acids [198]. In comparison, icariside II exhibits higher inhibitory activity against osteoclast-mediated bone resorption [14]. Studies have also shown that icariside II exerts a stronger inhibitory effect on RANKL-induced osteoclast formation than icariin. This effect is likely due to the presence of a free hydroxyl group at the C-7 position, which enhances its inhibitory potential against osteoclast differentiation and bone resorption. The urokinase-type plasminogen activator receptor (uPAR) plays a crucial role in regulating osteoclast metabolism. Icariside II can prevent OVX-induced bone loss and attenuate RANKL-stimulated expression of NFATc1 and uPAR, thereby offering therapeutic benefits for osteoporosis [199]. Intriguingly, icariside II and icariin in *Epimedium* exhibit a synergistic inhibitory impact on the expansion of osteoclast precursor RAW264.7 cells [200]. A combination of 100 μ M icariin and 100 μ M icariside II achieves

nearly a 90% inhibition rate on osteoclast formation [201]. And the bone resorption inhibition rates for these compounds were 50% and 80%, respectively [201].

Among other bioactive flavonoids in *Epimedium*, epimedin A has also been shown to inhibit osteoclast differentiation by suppressing the PI3K/AKT/NF- κ B signaling axis through negative regulation of TRAF6 expression [202]. Hyperoside exerts anti-osteoporotic effects in OVX mice by inhibiting the TRAF6-mediated RANKL/RANK/NF- κ B signaling pathway, increasing the OPG/RANKL ratio, and modulating the ER α /ITG β 3 signaling pathway [203]. Hyperoside also improves bone architecture in OVX mice by enhancing miR-19a-5p-mediated downregulation of IL-17A, thereby exerting a protective effect against post-menopausal osteoporosis [204]. By inhibiting the MAPK and ROS pathways, astragalin disrupts osteoclast differentiation [205]. Consequently, it decreases inflammatory osteolysis by downregulating critical osteoclast markers such as proto-oncogene c-Fos (c-Fos), NFATc1, cathepsin K (CTSK), and tartrate-resistant acid phosphatase (TRAP) [205]. Studies have confirmed that ginkgetin inhibits bone loss in OVX mice, potentially through suppression of the NF- κ B/inhibitory subunit of NF kappa B alpha (I κ B α) signaling pathway, which inhibits osteoclast formation and maturation [85]. Ikariside A inhibits osteoclast differentiation of RAW264.7 cells via the JNK and NF- κ B signaling pathways [206]. Daidzein plays a significant role in bone metabolism by effectively increasing bone density in postmenopausal women and preventing bone loss, potentially through enhancing the expression of ESR α , ESR β , and steroid receptor coactivator-1 in the estrogen response element pathway, thereby promoting its activation [149,207,208]. Administration of daidzein to OVX mice suppress osteoclastogenesis by significantly reducing ROS and TNF- α levels produced by activated T cells [209]. Furthermore, neonatal exposure to daidzein, genistein, or their combination has been shown to positively influence skeletal development in female mice, without exerting significant effects in male mice [210]. In mouse bone marrow-derived macrophages, luteolin inhibits RANKL-induced osteoclast differentiation and downregulates the expression of osteoclast-related genes, including NFATc1, c-Src tyrosine kinase, dendritic cell-specific transmembrane protein, MMP-9, CTSK, and TRAP [211]. In mature osteoclasts stimulated with RANKL and macrophage colony-stimulating factor (M-CSF), luteolin inhibits bone resorption in a dose-dependent manner [211]. In RAW264.7 cells, luteolin suppresses the formation of RANKL-induced mature TRAP-positive osteoclasts by inhibiting the p38 MAPK and activating transcription factor 2 signaling downstream of NFATc1, thereby reducing bone resorption [212]. Coumarin inhibits osteoclastogenesis-related gene expression by modulating signaling pathways such as NF- κ B/MAPK/AKT/Ca²⁺ [213]. Coumarin improves diabetic bone metabolism by regulating the RANK-RANKL signaling axis in RAW264.7 cells [214]. Additionally, the activation of Nrf2 by apigenin can mitigate oxidative stress in BMSCs and inhibit osteoclast-mediated bone resorption, contributing to its anti-osteoporotic effects [87]. Kaempferol suppresses IL-1 β -stimulated, RANKL-mediated osteoclastogenesis by downregulating MAPK, c-Fos, and NFATc1 signaling [215].

3.2.2. Other Active Constituents

In vitro, magnoflorine can inhibit RANKL-stimulated inflammatory responses and osteoclastogenesis by suppressing the phosphorylation of transforming growth factor- β (TGF- β)-activated kinase and the activation of downstream MAPK and NF- κ B signaling pathways [216]. As expected, it alleviates titanium particle-induced inflammatory osteolysis in vivo [216]. Boiogito is a herbal formulation rich in magnoflorine used to treat knee osteoarthritis through the inhibition of osteoclast differentiation and inflammation [217]. Zhang et al. and Jin et al. reported that a specific concentration of β -sitosterol may reduce osteoclast numbers [218,219]. Studies have also indicated that β -sitosterol inhibits osteoclast migration, formation, and function by downregulating components of the cyclic

adenosine monophosphate (cAMP) and NF- κ B signaling pathways, thereby reducing osteoclast-mediated bone resorption and preventing OVX-induced bone loss [220]. Additionally, β -sitosterol protects Wistar rats against glucocorticoid-induced osteoporosis by modulating the RANKL/OPG and Runx2 signaling pathways [221]. Moreover, β -sitosterol suppresses IL-6 secretion and RANKL-induced TRAP activity [222]. Oleanolic acid inhibits RANKL-induced osteoclast formation via the ER α /miR-503/RANK signaling pathway and remains effective even at the late stages of osteoclastogenesis, preventing bone loss in bone-protective protein knockout mice [223,224]. Protocatechuic acid inhibits osteoclast differentiation by regulating the NF- κ B and Nrf2 signaling pathways [225]. It has further been demonstrated to suppress osteoclast differentiation by modulating oxidative stress and inflammation and induces apoptosis in mature osteoclasts [225]. Jang et al. found that protocatechuic acid blocks the RANK signaling pathway by downregulating TRAF6 and NFATc1 expression, reduces serum RANKL levels, increases OPG levels, and attenuates the expression of CTSK and calcitonin receptor, thereby inhibiting bone loss in OVX mice [226]. Additionally, protocatechuic acid can inhibit osteoclast formation by suppressing the JNK/c-Fos/NFATc1 signaling pathway and thus prevent inflammatory bone loss in mice [227]. Caffeic acid inhibits RANKL-induced osteoclast formation and differentiation by suppressing the expression of osteoclast markers such as TRAP, CTSK, and MMP-9 [173]. Moreover, caffeic acid can regulate apoptosis-related bone loss in osteoclasts [173]. It has also been reported that caffeic acid inhibits the expression of NFATc1 and CTSK genes to suppress osteoclastogenesis [228]. Chlorogenic acid has been shown to inhibit RANKL-mediated osteoclast formation [229]. Furthermore, chlorogenic acid can also effectively prevent the formation and destruction of osteoclasts in myeloma cells induced by RANKL [179,230,231]. Osteoclasts require the capacity to produce protons to mediate bone resorption. Notably, the ruffled borders of osteoclasts contain vacuolar H-adenosine triphosphatase (V-ATPase), which hydrolyzes ATP to generate protons and transports them into the extracellular space. V-ATPase interacts with TRAF6, which is recruited by RANK, thereby facilitating its activation [232]. Lee et al. demonstrated that chlorogenic acid partially prevents the interaction between V-ATPase and TRAF6, thereby attenuating osteoclast differentiation and reducing bone resorption [233]. Endocytosis, secretion, and transport processes occurring at the ruffled borders of osteoclasts may be associated with cellular autophagy [234]. The formation of ruffled borders requires the coordinated action of several autophagy-related proteins and microtubule-associated protein light chain 3 to form actin rings, release CTSK, and promote osteoclastic bone resorption [235,236]. Chlorogenic acid may participate in this process by modulating autophagy to inhibit osteoclast activity [229].

3.3. Inflammation Regulation

In the course of biological evolution, the inflammatory response and tissue regeneration have co-evolved into a dialectical unity that is essential for effective tissue repair. As a tightly regulated biological process, successful bone regeneration fundamentally relies on the precise transition of the inflammatory phase from a destructive to a constructive state [237]. During the early stages, inflammation functions in a manner analogous to a scalpel, removing necrotic tissue and establishing a clean microenvironment conducive to regeneration [237]. Subsequently, as inflammation resolves in a timely fashion, it operates akin to an architect, orchestrating the migration and differentiation of reparative cells in a spatially and temporally coordinated manner [237]. This spatiotemporal regulation reflects the intrinsic self-organizing capacity of biological systems.

Achieving optimal bone regeneration necessitates the maintenance of inflammation within a so-called “golden range”: excessive inflammation may result in chronic inflammation, tissue damage, and fibrosis, whereas insufficient inflammation is inadequate to

initiate the essential repair mechanisms [238]. Studies have demonstrated that successful bone healing is typically characterized by an inflammatory kinetic profile that follows a “rise first and then fall” pattern, reflecting the non-linear, self-regulating nature of biological systems through negative feedback mechanisms [238]. Clinically, the majority of refractory nonunion cases are associated with dysregulated inflammatory responses, further highlighting the critical importance of preserving this delicate balance.

3.3.1. Flavonoids

A close interaction occurs between immune regulation and bone metabolism, creating an “anti-inflammatory-osteogenic” collaborative network that is crucial for the maintenance of bone equilibrium. Macrophages display immunomodulatory roles that facilitate proper bone repair [239,240]. A relevant study indicated that a suitable dose of icariin not only reinstates the reduced osteogenic potential of aged BMSCs but also mitigates the inflammatory senescence of aged macrophages through the activation of autophagy [241]. Shao et al. demonstrated that icariin prevents inflammatory responses and bone resorption induced by titanium particles in a murine calvarial model [242]. And Zuo et al. found that icariin diminishes excessive ROS production in chondrocytes by activating the Nrf2/antioxidant response element (ARE) signaling pathway and suppresses IL-1 β -induced matrix breakdown in an osteoarthritis model [243]. Additional research confirmed that icariin can mitigate osteoarthritis by inhibiting the NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome-mediated caspase-1 signaling cascade [244]. Also, icariin exerts immunomodulatory effects by inhibiting the NF- κ B/NLRP3 pathway and reducing the levels of pro-inflammatory cytokines such as IL-1 β , IL-6, and TNF- α in a model of degenerative intervertebral disc disease [23,24]. Icariin inhibits cartilage and bone degradation in a collagen-induced arthritis murine model via suppressing CTSK activity [245]. Moreover, icariin activates miR-186 and miR-206 to improve dexamethasone-induced bone metabolic abnormalities [246,247]. One study revealed that icariin restores LPS-induced bone loss by downregulating miR-34c expression and inhibiting the JNK, p38, and NF- κ B signaling pathways [22]. Furthermore, icariin significantly promotes the differentiation of MSCs into chondrocytes, enhances chondrocyte proliferation and the expression of related genes, reduces MMPs levels, and increases extracellular matrix (ECM) secretion, thereby facilitating cartilage repair [248,249]. Zhou et al. found that epimedin C ameliorates prednisolone-induced bone tissue damage [250]. The mechanism likely involves the activation of the PPAR and AMPK signaling pathways [250]. Apigenin can modulate inflammatory cytokines and the TGF- β and BMP signaling pathways to promote dentin formation and inflammation control [251]. PI3K regulates key cancer-related processes, including cell survival, proliferation, migration, protein synthesis, glucose homeostasis, and genomic instability [252,253]. Activation of PI3K is often driven by mutations in the p110 α subunit [254]. Research has identified p110 α as a novel therapeutic target for osteoarthritis, with its mechanism involving the inhibition of MMP13 overexpression via the PI3K/AKT/mTOR signaling pathway [255]. Specifically, p110 α reduces cartilage ECM degradation and eases osteoarthritis via activating the PI3K/AKT/mTOR pathway [256]. Quercitrin activates the p110 α /AKT/mTOR signaling pathway by targeting p110 α , demonstrating promise in halting the progression of osteoarthritis by hindering the degradation of cartilage extracellular matrix and encouraging the proliferation of chondrocytes [256]. Furthermore, quercitrin demonstrates therapeutic potential against osteoarthritis through in vivo suppression of MMP13 and enhancement of type II collagen (Col II) deposition [256]. A study of Jin et al. also found that hyperoside reduces the production of pro-inflammatory cytokines and inhibits the NF- κ B signaling pathway, thereby exerting anti-inflammatory effects and preventing joint destruction [257]. Moreover, this

compound can mitigate TNF- α -induced inflammation, ECM degradation, and endoplasmic reticulum stress-mediated apoptosis in vitro through the sirtuin 1 (SIRT1)/NF- κ B and Nrf2/ARE signaling pathways [258]. Local administration of hyperoside can partially restore the expression of forkhead box protein O1 and Runx2, thereby reducing alveolar bone resorption and periodontal ligament damage [144]. Astragalin suppresses MMPs expression by inhibiting the JNK/p38/AP-1 signaling pathway, significantly alleviating synovial inflammation and joint destruction in collagen-induced arthritis (CIA) mice [259]. Luteolin mitigates gingival fibroblast inflammation by downregulating the MAPK pathway in periodontitis and enhances the osteogenic differentiation of periodontal ligament stem cells through the Wnt/ β -catenin signaling pathway, consequently decreasing the levels of inflammatory cytokines (TNF- α and IL-6) in a periodontitis model [20]. Kaempferol, when combined with flavonoids such as quercetin and apigenin, can synergistically enhance anti-inflammatory effects. For example, in an osteoarthritis model combined with stem cell therapy, kaempferol significantly reduces cartilage degradation markers (MMP-3 and MMP-13) and promotes collagen synthesis [260]. Additionally, kaempferol suppresses IL-1 β -induced inflammation in rat osteoarthritis chondrocytes by inhibiting the NF- κ B signaling pathway [261]. It also modulates the X chromosome inactivation-specific transcription factor/miR-130a/signal transducer and activator of transcription 3 (STAT3) axis in human chondrocyte C28/12 cells, effectively attenuating inflammation and ECM degradation [262].

3.3.2. Other Compounds

As a potential inhibitor of the NF- κ B signaling pathway, magnoflorine can exert significant anti-inflammatory effects through the MAPK signaling pathway [263]. Additionally, by partially blocking the PI3K/AKT/NF- κ B axis signaling route and activating the kelch-like ECH-associated protein 1/Nrf2/HO-1 signaling pathway in vitro and in vivo, the compound can lower the inflammatory response in rheumatoid arthritis [264]. By triggering the signal transducer and activator of transcription 1 and NF- κ B signaling pathways, β -sitosterol reduces pro-inflammatory cytokines and increases IL-10 in LPS-activated J774A.1 macrophages, thereby exerting anti-inflammatory effects [265–267]. Zhang et al. confirmed that protocatechuic acid suppresses the MAPK, AKT, and NF- κ B signaling pathways, which decreases osteoclastogenesis and reduces osteoarthritis caused by anterior cruciate ligament transection [268]. Huang et al. demonstrated that chlorogenic acid not only significantly inhibits the expression of cyclooxygenase-2 and inducible nitric oxide synthase (iNOS) genes in macrophages but also reduces the secretion of inflammatory cytokines, including IL-1 β , TNF- α , and IL-6 [269].

3.4. Antioxidation Effects

From an evolutionary perspective, the antioxidant mechanism represents the primordial strategy employed by living organisms to counteract the increase in entropy [270]. Re-establishing redox homeostasis during bone repair essentially involves modulating the “metabolic breathing rhythm” to reawaken the spatiotemporal memory embedded in the developmental program [270]. This therapeutic approach extends beyond symptomatic relief and directly addresses the core driving force underlying tissue regeneration—restoring to the injured site the self-organizing capacity observed during embryonic development [270]. In the oxidative stress environment induced by trauma, the antioxidant system functions as a biological firewall, preserving genomic stability and mitochondrial functional integrity in osteoblasts [271]. Such protection not only sustains the proliferative potential of osteoprogenitor cells but also ensures fidelity in epigenetic regulation during cellular differentiation, thereby supplying high-quality cellular components for bone regeneration [271]. The polarization dynamics of macrophages are critically influenced by

redox equilibrium [272,273]. Appropriate antioxidant intervention can establish a conducive immune microenvironment that not only mitigates secondary tissue damage caused by excessive inflammatory responses but also facilitates coordinated vascular regeneration during the repair phase, thereby enabling precise transition between the “inflammation-repair” biphasic process [272,273]. ROS can act as sources of cellular damage as well as physiological signals [274–281]. When produced transiently at controlled levels within defined subcellular compartments, ROS function as redox messengers [274,275,282]. Low levels of Nox4-derived ROS mediate BMP-2-induced osteoblast differentiation and matrix mineralization in mouse pre-osteoblasts [283]. In addition, Endogenous ROS generated during osteogenic induction contribute to osteoblast differentiation and matrix mineralization in MC3T3-E1 cells [284]. Moderate levels of ROS also contribute to antimicrobial defense, angiogenesis, and adaptive stress responses [285–287]. Persistent or excessive ROS, however, can inhibit osteoblast proliferation and differentiation and increase osteoclast formation and bone resorption [288,289]. Complete or indiscriminate ROS depletion may also interfere with normal redox signaling and ROS-dependent host defense [290]. Antioxidant approaches to bone repair should therefore restore redox homeostasis at the appropriate site and time rather than eliminate ROS completely [291]. Their effects depend on the dose and timing of treatment, the cellular source of ROS, and conditions within the local microenvironment [291].

3.4.1. Flavonoids

ROS can participate in the process of ferroptosis, an iron-dependent form of programmed cell death [292]. Ferroptosis, however, has been shown to induce bone loss in mice and contribute to osteoporosis development [293]. The role of Nrf2 in osteoblast differentiation may be related to intracellular ROS levels. Nrf2 deficiency in stromal cells leads to increased ROS accumulation, which induces oxidative stress and ultimately inhibits osteoblast differentiation [176,294]. It is reported that icariin may regulate osteoblast ferroptosis by activating the Nrf2/HO-1 signaling pathway, thereby promoting the healing of osteoporotic fractures [295]. Recent evidence indicates that icariin can reduce iron deposition in the bone marrow of osteoporotic mice and inhibit iron overload-induced osteoblast apoptosis, thereby increasing bone mass [296]. Lipid peroxidation has been identified as a key mechanism in iron overload-induced osteoporosis. Interestingly, the anti-osteoporotic effects of icaritin and its glycosides are closely related to their ability to inhibit lipid peroxidation. Yan et al. found in their study on the inhibition of lipid peroxidation by icaritin and its glycosides in zebrafish that the inhibition of lipid peroxidation by icaritin and its glycosides is achieved by suppressing oxidative stress and enhancing the activity of glutathione peroxidase 4 [297]. Intracellular ROS is involved in the differentiation process of osteoclasts, and nicotinamide adenine dinucleotide phosphate oxidase (NOX) is a key source of ROS [298]. Blocking NOX can improve ROS generation and the activation of downstream MAPK (JNK, p38, and ERK) and NF- κ B, thereby inhibiting osteoclast formation [276,298]. A study has confirmed that icariin inhibits the generation of ROS by reducing the expression of NOX1 and NOX4, thereby inhibiting osteoclastogenesis in RANKL-induced RAW264.7 cells [299]. Oxidative stress can inhibit osteoblast differentiation, and reducing ROS levels can reverse the osteoclast differentiation induced by RANKL stimulation [276,300]. Hyperoside inhibits apoptosis and enhances osteoblast viability by suppressing the MAPK signaling pathway (p-JNK and p-p38) and regulating the Bcl-2/Bax ratio to lower ROS levels [301]. Quercitrin has been confirmed to be able to directly or indirectly weaken the differentiation of osteoclasts through its antioxidant properties [302]. Apigenin has been reported to have a certain therapeutic effect in bone diseases related to oxidative stress [154]. And the compound can induce apoptosis of rheumatoid arthritis fibroblast-like synoviocytes (RA-FLS) by mediating ROS and activating

the oxidative stress factor ERK1/2 [303,304]. It further mediates apoptotic cell death through activating the apoptosis effector protein caspase-3/7, thereby exerting a pro-apoptotic effect on human RA-FLS [303,304]. Luteolin has also been proven to inhibit the production of ROS [305]. In an oxidative stress model, luteolin can protect osteoblasts from H₂O₂ damage by scavenging ROS and enhancing the activity of superoxide dismutase, and inhibit adipogenic differentiation by activating the AMPK signal [20]. Its protective effect against oxidative stress is further manifested in the arthritis model by inhibiting the decomposition factors of chondrocytes (MMP1 and A disintegrin and metalloproteinase with thrombospondin motifs 5) and promoting the synthesis factors (aggrecan and Col II), and maintaining cartilage homeostasis through autophagy regulation [306]. It has been reported that kaempferol can enhance the activity of antioxidant enzymes, and its oxidation metabolites (such as 2-(4-hydroxybenzoyl)-2,4,6-trihydroxy-3(2H)-benzofuranone) have significantly better antioxidant capacity than the precursors, and can achieve cell protection at extremely low concentrations [21].

3.4.2. Other Active Constituents

Rensburg et al. reported that plant sterols such as β -sitosterol and its glucoside can reduce membrane lipid peroxidation [307]. β -sitosterol can treat rheumatoid arthritis by inhibiting inflammatory mediators and lipid peroxidation [308]. The use of protocatechuic acid in rat models can improve rotator cuff repair and reduce fatty degeneration [309]. Chlorogenic acid can inhibit apoptosis by reversing the generation of intracellular ROS, H₂O₂ concentration, and excessive mitochondrial superoxide production [229]. Chlorogenic acid can also protect osteoblasts by regulating the activation of the PI3K/AKT/Nrf2/HO-1 and p21/Nrf2/HO-1 antioxidant stress signaling pathways [175,176,310].

3.5. Angiogenesis Regulation

Angiogenesis constitutes an indispensable biological basis for bone defect repair, representing a highly coordinated regulatory mechanism through which living systems orchestrate tissue regeneration and functional reconstruction. This process extends beyond the provision of nutrients, serving as a central regulatory hub that maintains the dynamic equilibrium of the osteogenic microenvironment and determines the efficacy threshold of tissue engineering strategies. Within the complex cascade of bone defect healing, angiogenesis functions as a symphonic conductor, integrating multidimensional interactions among cellular behaviors, extracellular matrix deposition, and mechanical adaptation. The early establishment of vascular networks not only delivers oxygen and essential nutrients to the regenerating regions but also forms a “biological highway” for stem cell migration, with its branching architecture directly guiding the spatiotemporal distribution of osteoprogenitor cells [5,311]. Clinical evidence indicates that bone grafts lacking effective vascularization exhibit failure rates as high as 40%, whereas vascularized tissue-engineered bone constructs demonstrate more than a threefold increase in the rate of osseointegration [312]. The molecular “crosstalk” between vascular endothelial cells and osteoblasts reflects an evolutionarily conserved cooperative mechanism. Signaling molecules secreted by the neovasculature act as biological signals to activate the osteogenic differentiation potential of mesenchymal stem cells, while the three-dimensional (3D) topological structure of vascular networks provides a biomimetic scaffold for mineral deposition. This dynamic bidirectional interaction elucidates the necessity of prevascularization strategies in the repair of segmental bone defects exceeding 5 cm, as simple scaffold implantation frequently results in central necrosis due to ischemic conditions.

3.5.1. Flavonoids

Bone, being a highly vascularized organ, critically depends on angiogenesis during osteogenesis, the level of which represents a potential therapeutic strategy for compromised bone repair and regeneration. Chen et al. demonstrated that systemic administration of icariin enhances angiogenesis and tendon-bone healing post-rotator cuff reconstruction, yielding superior mechanical strength [313]. Notably, while both icariin and icariside II effectively stimulate angiogenesis, icariside II exhibits stronger upregulation of VEGF [314]. NOS, produced by osteocytes, has been shown to suppress osteoclast-mediated bone resorption while stimulating osteoblast activity [315]. Icariin was found to augment nitric oxide (NO) production and endothelial NOS (eNOS) expression in human endothelial cells [316]. Icariin also produced endothelium-dependent vasorelaxation through activation of eNOS and the NO-cyclic guanosine monophosphate (cGMP) pathway, reinforcing its potential to improve local perfusion [29,30]. Mechanistically, icariin promotes osteogenic differentiation of rat bone marrow stromal cells via activation of the PI3K/AKT/eNOS/NO/cGMP/dependent protein kinase signaling cascade [317]. Concurrently, icariside II stimulates osteogenesis by enhancing inducible NOS activity and NO synthesis [318]. In vivo study using BALB/c nude mice revealed that epimedin C potently facilitates BMP-2-dependent bone formation by inducing endothelial-like differentiation of C3H/10T1/2 cells [319]. Quercetin induces bone regeneration through upregulated expression of VEGF, angiopoietin-1, basic fibroblast growth factor, and TGF- β [320]. The combination of kaempferol and metformin synergistically promotes osteogenic-angiogenic coupling via the Notch pathway, elevating expression of bone formation markers (e.g., VEGF and Runx2) while improving bone mechanical properties in OVX rats [321]. Further investigations confirmed that the coadministration of these two drugs can stimulate pro-osteogenic factors and VEGF in osteoblasts while inducing angiogenesis in human umbilical vein endothelial cells (HUVECs) through enhanced osteogenic-angiogenic coupling, with Notch pathway involvement [321].

3.5.2. Other Active Constituents

β -sitosterol attenuates joint swelling and bone destruction in collagen-induced arthritis mice by suppressing VEGF-mediated synovial angiogenesis [322]. Caffeic acid phenethyl ester upregulates VEGF production in MC3T3-E1 cells and induces phosphorylation of ERK, p38, MAPK, and stress-activated protein kinase (SAPK)/JNK, all crucial for bone repair [323].

3.6. Antitumor Effects

Reconstruction of oncologic bone defects (e.g., post-osteosarcoma or metastatic tumor resection) requires tumor-free microenvironment verification, as residual malignant cells may disrupt regeneration through aberrant proliferation. Anti-neoplastic interventions—including surgical margin clearance, targeted therapy, or radiation—establish “biological safety zones” for bone tissue engineering. Emerging evidence indicates that certain antitumor modalities concomitantly activate stress-responsive repair mechanisms in osteoprogenitors, achieving therapeutic-regenerative synergy [324].

3.6.1. Flavonoids

Icariin suppresses prostate cancer metastasis and osteolysis via blockade of the tumor-associated macrophage/chemokine (C-C motif) ligand 5/osteoclast pathway [325]. Epithelial-mesenchymal transition (EMT), a pivotal process in osteosarcoma metastasis, is potentiated by miR-194/215 cluster upregulation. Icaritin-doxorubicin combination exerts additive/synergistic antiproliferative effects on MG-63 cells, potentially through Bcl-

2, caspase-3, and p21 gene regulation [326]. Icariside II diminishes osteosarcoma cell metastatic potential by targeting phospho-glycerate kinase 1 and suppressing miR-194/215-mediated EMT [327]. Icariside I and icaricidin counteract cyclophosphamide-induced myelosuppression in mice by regulating hematopoietic cytokines, maintaining Bcl-2/Bax balance, and inhibiting caspase-3 expression [54]. Icariside II also suppresses HIF-1 α in human osteosarcoma cells [27]. Hyperoside significantly inhibits proliferation of U2OS and MG63 osteosarcoma cells by inducing G0/G1 cell cycle arrest while impairing migration and invasion [28]. Hyperoside downregulates Wnt/ β -catenin signaling and suppresses genes associated with multiple myeloma progression [328]. Apigenin inhibits osteosarcoma proliferation and invasion via Wnt/ β -catenin suppression [328,329]. It attenuates EMT, stemness, and Warburg effect in SOSP-9607 cells through PI3K/AKT/mTOR modulation [328,329]. It also induces osteosarcoma apoptosis via caspase-3/8/9, Bax, and apoptosis-inducing factor pathways while suppressing xenograft growth [25].

3.6.2. Other Active Constituents

Magnoflorine enhances cisplatin sensitivity and suppresses malignant phenotypes in osteosarcoma via miR-410-3p/high mobility group box 1/NF- κ B axis modulation [330]. Menadione and protocatechuic acid cotreatment reduces viability, delays migration, and decreases NADPH oxidase 2 expression/ROS generation, enhancing antineoplastic efficacy against murine osteosarcoma [331].

3.7. Chondroprotective Effects

Cartilage plays a pivotal role in bone tissue engineering. As a biological precursor to natural bone, cartilage facilitates new bone formation through a process of staged mineralization. Its porous extracellular matrix not only supports cell migration and vascular ingrowth but also establishes a conducive microenvironment for the directed differentiation of osteoprogenitor cells. During bone regeneration, the mechanical buffering capacity of cartilage effectively distributes interfacial stress between the implant and host bone, thereby minimizing stress concentration and reducing the risk of early implant loosening. Furthermore, chondrocyte-secreted ECM components can activate osteogenic signaling pathways and promote osteoblast proliferation and ECM mineralization via intercellular crosstalk. In the development of composite scaffolds, cartilage tissue functions as a bioactive component that enhances host–tissue integration through strategic surface modifications [332]. Importantly, its degradation profile closely aligns with the rate of bone regeneration, mitigating the need for secondary surgical interventions associated with mismatched degradation kinetics in conventional biomaterials. Clinically, cartilage-based engineered constructs have demonstrated robust osteoinductive potential, offering a promising therapeutic approach for the repair of complex bone defects [333].

3.7.1. Flavonoids

The osteoinductive effect of icariin *in vivo* can be achieved through endochondral ossification [32]. As a safe and potent anabolic agent for chondrocytes, icariin can influence chondrocyte proliferation and reduce ECM degradation [334,335]. Icariin can increase the secretion of Col I and Col II, and the abundant ECM is conducive to the integration of new cartilage and bone [336]. Additionally, Icariin has the potential to induce the proliferation and differentiation of BMSCs into chondrocytes and protect rabbit BMSCs from apoptosis induced by oxygen-glucose deprivation [337]. Interestingly, icariin can accelerate cartilage defect repair by promoting chondrogenic differentiation of BMSCs under oxygen-glucose deprivation conditions [338]. Ziadlou et al. found that epimedin C significantly promoted cartilage regeneration by increasing collagen and non-collagen matrix proteins, demonstrating a clear anabolic effect [339]. Kaempferol has been reported

to induce chondrogenesis in ATDC5 cells through activating the ERK/BMP-2 signaling pathway [340].

3.7.2. Other Active Constituents

Chlorogenic acid can prevent chondrocyte apoptosis caused by thiabendazole by down-regulating the mRNA and protein expression of Bax/caspase-3 and up-regulating the expression of Bcl-2 (under the action of the CD147 signaling axis), thereby treating tibial cartilage dysplasia [341]. And it can positively treat tibial dyschondroplasia caused by mancozeb by regulating the expression of caspase and beclin 1, as well as controlling the degradation of ECM [342]. Caffeic acid has a positive effect on alleviating adjuvant-induced arthritis, which can effectively down-regulate NF- κ B, possibly explaining the observed inhibition of downstream effectors chitinase-3-like protein 1, IL-1 β , and MMP-9, thereby reducing cartilage degeneration and joint damage [74,343,344].

3.8. Antibacterial Effects

Pathogen infection, such as that caused by *Staphylococcus aureus*, remains a critical challenge in the clinical management of nonunion and bone defects, necessitating effective eradication. These pathogens not only directly impair osteoblast function but also trigger inflammatory responses—such as the release of TNF- α —that disrupt bone matrix deposition and vascularization [345]. Infection-induced immune evasion mechanisms, including the formation of polysaccharide-protein complex barriers, along with the growing prevalence of antimicrobial resistance, demand a multifaceted therapeutic approach [346]. This includes surgical debridement, localized sustained-release antibiotic systems, and surface modification of biomaterials (e.g., silver ion coatings) to reestablish a conducive microenvironment for bone regeneration [347,348]. *Epimedium*, a classical traditional Chinese medicinal herb renowned for its kidney-tonifying and bone-strengthening properties, is increasingly being explored in bone tissue engineering beyond its traditional role in osteogenesis promotion, now extending to anti-infective functionalities [167]. Current research focuses on harnessing the multi-modal pharmacological activities of its bioactive constituents—particularly flavonoids such as icaritin—to engineer an integrated “anti-inflammatory, antibacterial, and pro-osteogenic” microenvironment for enhanced bone repair.

Under conditions of *Porphyromonas gingivalis* infection, icariin can downregulate the EphA2-RhoA signaling pathway, thereby enhancing osteogenic differentiation and bone regeneration, with 10 μ M icariin showing the most pronounced effect [167]. However, Liu et al. confirmed icariin exhibits limited antibacterial activity against bacteria associated with peri-implantitis [349]. This research finding was partially corroborated by Peris et al. [350]. They demonstrated that icariin alone exhibited no antimicrobial activity against clinical isolates of *Staphylococcus aureus* or methicillin-resistant *Staphylococcus aureus* [350]. Nevertheless, it is noteworthy that icariin enhanced the antibacterial efficacy of amoxicillin-clavulanate and ampicillin, while no synergistic effect was observed when combined with vancomycin [350]. Moreover, oral administration of luteolin alleviated maxillary alveolar bone resorption in murine periodontitis induced by *Porphyromonas gingivalis* infection [351]. Furthermore, a study of Gao et al. demonstrated that the combination therapy of azithromycin and kaempferol was more effective in countering azithromycin-resistant *Staphylococcus aureus* bacterial growth by suppressing the phosphorylation of ERK 1/2 and SAPK [352]. In fact, the antibacterial activity of flavonoids primarily arises from the multi-target synergistic effects of their C6-C3-C6 skeleton and associated substituents. The hydrophobic core structure can insert into the lipid bilayer of bacterial cell membranes, while the phenolic hydroxyl groups on the B ring—particularly the catechol moiety—can disrupt membrane integrity through hydrogen bonding, resulting in leakage of intracellular contents [353]. Furthermore, the ortho-dihydroxyl groups and the carbonyl group at the

4-position form a potent metal-chelating system that deprives bacteria of essential metal ions and catalyzes the generation of reactive oxygen species, thereby inducing oxidative stress [353]. Additionally, the conjugated planar structure of the molecule—such as the presence of a C2=C3 double bond—enables effective intercalation into and inhibition of the active sites of key bacterial enzymes (e.g., DNA gyrase and fatty acid synthase), interfering with nucleic acid replication and metabolic pathways [353]. Therefore, the antibacterial mechanism of flavonoids is attributed to the combined effects of hydrophobic interactions, membrane disruption, metal ion chelation, and enzymatic inhibition. Figure 3 illustrates the key biological mechanisms by which phytochemicals isolated from *Epimedium* may promote bone regeneration at defect sites.

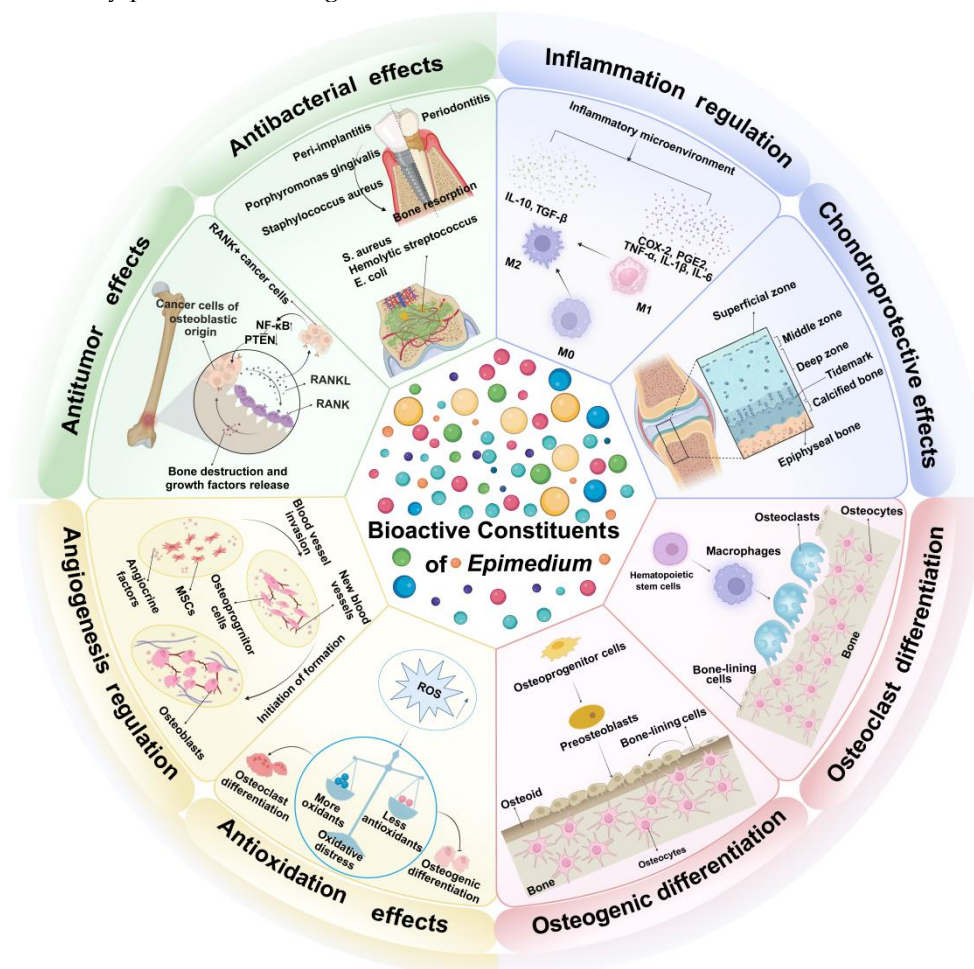


Figure 3. Key molecular mechanisms underlying the bioactive constituents derived from *Epimedium* in relation to bone defect repair and associated biological functions. Several interconnected mechanisms contribute to bone defect repair progression: (1) Osteogenic differentiation regulation: Bioactive constituents promote the differentiation of osteoprogenitor cells into preosteoblasts and mature osteoblasts through activation of osteogenesis-related signaling pathways and transcription factors, leading to osteoid secretion and subsequent initiation of bone matrix mineralization. (2) Inhibition of osteoclast differentiation and bone resorption: Bioactive constituents inhibit osteoclast differentiation by modulating specific signals (e.g., RANKL, HIF-1 α , etc.) and signaling pathways (e.g., MAPK, NF- κ B, etc.), thereby maintaining the balance between osteogenesis and osteoclastogenesis and promoting bone defect repair. (3) Inflammatory modulation: Bioactive constituents modulate macrophage activity, including M0, M1, and M2 phenotypes, thus suppressing the release of pro-inflammatory mediators (e.g., TNF- α , IL-1 β , IL-6, COX-2, PGE2), while promoting anti-inflammatory responses mediated by factors like IL-10 and TGF- β . (4) Antioxidant activity: Bioactive constituents attenuate intracellular reactive oxygen species accumulation and mitigate oxidative stress-

induced damage, thereby preserving the regenerative capacity of osteogenic and endothelial cells. (5) Angiogenesis regulation: In regenerating tissues, constituents of *Epimedium* promote endothelial cell migration and sprouting, leading to neovascularization, which subsequently supports the generation and functional development of osteoblasts. (6) Antitumor effects: Bioactive constituents modulate osteolytic signaling pathways between tumor cells and bone-resorbing cells, thereby mitigating tumor-induced bone destruction. (7) Chondroprotective effects: Bioactive constituents contribute to the mitigation of cartilage degeneration, thereby promoting joint stability and facilitating coordinated bone–cartilage repair. (8) Antibacterial effects: Bioactive constituents inhibit the growth of bone-related pathogens, such as *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Escherichia coli*, and mitigate infection-induced inflammatory responses, thereby preventing secondary bone defects. The circle colors distinguish different *Epimedium*-derived bioactive constituents, whereas the circle sizes indicate their relative molecular weights. Arrowheads indicate the direction of a biological process and denote activation, promotion, secretion, or release, as appropriate, whereas blunt-ended lines denote inhibition.

3.9. Comparative Analysis of Active Constituents' Bone Functions

Icariside is one of the bioactive constituents of *Epimedium*. It is demonstrated that following oral administration of icariin, various metabolites such as icariside I, icariside II, and icaritin are generated through degradation by intestinal flora [54]. These metabolites tend to accumulate at higher concentrations in tissues such as the kidneys, liver, and spleen [54]. Pharmacokinetic studies reveal icariside II's superior bioavailability and absorption kinetics compared to icariin [354,355], with icariside I exhibiting enhanced absorption [54]. Data from rats treated with 30 mg/kg of oral icariside II showed a terminal elimination half-life of 135 min and an absolute bioavailability of 4.1%. Tissue distribution studies in rats show rapid icariside II dispersion to skeletal and muscular systems following intravenous administration, with minimal testicular accumulation and undetectable cerebral penetration [356,357]. Notably, icariside II exhibits both greater biological activity and a longer half-life compared to icariin, and it is also characterized by rapid absorption [354,355]. Furthermore, experimental findings indicate that the absorption efficiency of icariside I surpasses that of icariin. Although the natural abundance of icariside II in *Epimedium* (<0.15%) is lower than icariin, it demonstrates a broader spectrum of pharmacological activities [358,359].

There is still controversy over the strength of the in vivo and in vitro biological activities of the active constituents of *Epimedium*. In the regulation of bone regeneration, Wu et al. reported that although the osteogenic activity of icaritin in vitro is lower than that of recombinant human BMP-2, icaritin at a concentration of 1×10^{-6} M exhibits a stronger effect than icariin [133]. Notably, icariin can achieve a bone-enhancing effect comparable to that of BMP-2 (50 μ M) at a concentration of 30 μ M [360].

Some researchers have pointed out that the effects of various chemical components in *Epimedium* on the treatment of osteoporosis vary in strength, with the following rank order: icariin > icariside II > epimedin A > epimedin B > epimedin C [361]. Huang et al. demonstrated through experimental studies that icaritin and its glycosides can promote the proliferation of osteoblasts in vitro in a dose-dependent manner, with maximal effects observed at a concentration of 1×10^{-6} mol/L [14]. Furthermore, the stimulatory effect of icaritin exceeds that of icariin and icariside II, although it remains lower than that of genistein [14]. Overall, icaritin has the best promoting effect. Research has demonstrated that icaritin and its glycosides can inhibit osteoclast formation. Moreover, at a concentration of 1×10^{-5} mol/L, icaritin exhibits efficacy comparable to that of genistein ($46.1 \pm 7.1\%$ vs. $46.8 \pm 10.3\%$) [14]. Treatment of bone marrow macrophages with icariin and icaritin can inhibit the differentiation of these cells into osteoclasts induced by M-CSF/RANKL. Researchers suggest that osteoclast precursors may represent another cellular target for

icariin and icaritin, whereas icariside II does not exhibit this activity [14]. Moreover, icaritin and its glycosides can reduce the absorption of bone by osteoclasts. In this regard, the three flavonoid glycosides (i.e., icariin, icaritin, and icariside II) are comparable to genistein [14]. In addition, it was observed that icaritin and its glycosides can reduce the number of actin rings in osteoclasts, thereby reducing their size, but have no effect on the number of osteoclasts [14]. The order of inhibitory effect is icaritin > icariside > icariin, and the inhibitory effect of icaritin is stronger than that of genistein, with values representing $35.2 \pm 21.1\%$ and $57.5 \pm 14.3\%$ of the control, respectively [14]. Also, icaritin and its glycosides can reduce the generation of superoxide anions in osteoclasts, with icaritin exhibiting the most significant effect, thereby contributing to the elimination of ROS [23]. This mechanism may attenuate osteoclast formation and reduce bone resorption [14,362–364].

Estrogen plays a pivotal role in bone tissue engineering by regulating bone homeostasis through multiple mechanisms and serves as a critical effector molecule in promoting bone regeneration [365]. The core mechanism of its action can be summarized as follows: (1) Estrogen directly activates osteoblasts by binding to $ESR\alpha$, upregulating the expression of osteogenic transcription factors, promoting collagen synthesis and ECM mineralization, and simultaneously inhibiting osteoblast apoptosis to extend their functional lifespan [366,367]. (2) Estrogen can modulate the RANKL/OPG signaling axis to block osteoclast activation and significantly suppress bone resorption [368]. (3) Estrogen interacts synergistically with the growth factor network, stimulating insulin-like growth factor-1 secretion and activating the Wnt/ β -catenin pathway, which enhances the proliferative and differentiative capacity of osteoblasts and establishes a positive feedback loop for bone formation [369]. (4) Estrogen contributes to the maintenance of bone microstructural integrity through the regulation of calcium metabolism—including enhancing vitamin D activity—thus preventing trabecular bone deterioration associated with postmenopausal osteoporosis [370]. In the context of bone tissue engineering, estrogen-mediated mechanisms offer precise targets for scaffold design and optimization of cell culture protocols, thereby significantly improving bone regeneration efficiency by balancing bone formation and resorption. The estrogenic effects of various flavonoids from *Epimedium* exhibit considerable variability. Studies have demonstrated that the estrogenic activity of *Epimedium* may be mediated by icaritin and demethyl icaritin, with the mechanism of action potentially involving ESRs or other key regulatory proteins [371]. Icariin can be metabolized into icaritin and demethyl icaritin, a metabolic transformation that may account for its selective estrogenic activity via $ER\alpha$ [193,372,373]. Wong et al. evaluated the estrogenic effects in rat serum using $ESR\alpha$ - and $ESR\beta$ -based bioassays and reported a potency order of demethyl icaritin > icaritin > icariside I > icariside II [374]. Additionally, the results indicated that icariin may lack intrinsic estrogenic activity, despite being the primary flavonoid glycoside in *Epimedium* extracts [374]. Liu et al. also reported that icariin exhibited little to no estrogenic activity, as determined by in vitro estrogenic activity assessment using human MCF-7 breast cancer cells in the E-screen assay [375]. Nevertheless, He et al. discovered that when administered peripherally, the central pharmacological effects of icariside II were significantly stronger than those of icaritin [376]. It is evident that following oral administration, icariin may exert its biological effects primarily through its metabolic derivatives. This hypothesis has been further supported by findings from multiple research studies [81,371,374,377,378]. Moreover, icaritin and icariside II display higher musculoskeletal penetration efficiency compared with icariin, with icariside II demonstrating a distinct advantage in tissue permeability [379]. However, with regard to reversing glucocorticoid-induced inhibition of bone formation, icaritin shows superior efficacy compared with icariin and icariside II [380].

3.10. Network Pharmacology

3.10.1. Network Pharmacology Analysis

The structures and identifiers of icariin, icaraside II, and icaritin were obtained from PubChem. We entered the compound names, including icariin, icaritin, and icaraside II into the search bar, then we selected the best compound match for each, and obtained their SMILES codes. Drug-likeness and physicochemical properties were evaluated using SwissADME (accessed on 2 September 2025) [381]. Putative human protein targets were retrieved from SwissTargetPrediction (accessed on 4 September 2025) [382] with the organism restricted to *Homo sapiens*. Targets associated with the 53 bone-related conditions were retrieved from GeneCards (accessed on 4 September 2025) [383], OMIM (accessed on 4 September 2025) [384], and TTD (accessed on 4 September 2025) [385] using the exact disease terms listed in Supplementary Table S1. Genes retrieved from GeneCards with a query-specific Relevance Score ≥ 1.0 were retained. For OMIM and TTD, all gene entries linked to the prespecified disease terms in OMIM and all disease-associated therapeutic targets retrieved from TTD were retained without applying database-specific score or evidence-level cutoffs. The retrieved identifiers were standardized to the corresponding human gene symbols and UniProtKB accession numbers using the UniProt ID Mapping service (accessed on 5 September 2025) [386]. Records that could not be mapped to *Homo sapiens* entries were excluded, and duplicate gene entries were removed before dataset integration. Compound-associated and disease-associated target sets were intersected in Venny 2.1.0 (BioinfoGP, CNB-CSIC; accessed on 7 September 2025) using exact approved gene symbols (Supplementary Table S2). Protein–protein association analysis was performed using STRING v12.0, with the organism restricted to *Homo sapiens* (NCBI taxon ID: 9606). The default full STRING network was used, and associations with a combined confidence score ≥ 0.400 (medium confidence) were retained. No additional interaction partners were added. Disconnected nodes were hidden from the network visualization. The network was visualized using Cytoscape v3.10.3. Betweenness centrality (BC), closeness centrality (CC), and degree centrality (DC) were calculated using the CytoNCA v2.1.6 plugin. Hub targets were defined as nodes whose BC, CC, and DC values were each greater than the corresponding median calculated across all nodes in the original PPI network ($BC > (\text{actual median})$, $CC > (\text{actual median})$, and $DC > (\text{actual median})$) and at the same time all three criteria had to be satisfied simultaneously (Supplementary Table S2). We performed functional enrichment analysis with the DAVID Functional Annotation Tool (DAVID Knowledgebase v2025q1, accessed on 7 September 2025), using the default genome-wide *Homo sapiens* gene set as the background. KEGG pathway annotations were drawn from release 112.0, which was incorporated into this version of the DAVID Knowledgebase. We did not modify the analysis parameters. The Functional Annotation Chart therefore used DAVID's default criteria of at least two genes per term and an EASE score ≤ 0.1 . We applied no additional cutoff based on the Benjamini–Hochberg-adjusted p value or FDR. From the returned KEGG terms, we manually selected those related to bone formation and resorption, skeletal development and remodeling, mineralization, and osteoblast or osteoclast differentiation and function for focused presentation and interpretation (Supplementary Table S3). The heatmap was plotted using <https://www.bioinformatics.com.cn> (accessed on 10 September 2025), an online platform for data analysis and visualization. A dot plot shows the results of the KEGG pathway enrichment analysis. The horizontal axis represents the gene ratio, while the vertical axis represents the enriched pathway name. The color scale indicates different thresholds of the p -value, and the size of the dot indicates the number of genes corresponding to each pathway [387]. We treated these associations as hypothesis-generating rather than as evidence of causal mechanisms.

3.10.2. Molecular Docking

To assess the key interactions predicted by network pharmacology, we performed molecular docking. Hub targets were screened from the PPI network using Cytoscape, retaining only nodes whose NAC, BC, CC, and DC values exceeded the respective median values (Supplementary Table S2). Then, the selected targets were docked with three bioactive compounds: icariin, icaritin, and icariside II. For the “Preparation of ligands and proteins” procedure: the three-dimensional structures of the selected bioactive compounds were downloaded from PubChem in SDF format (Supplementary Table S3). Crystal structures of the hub target proteins were retrieved from the Protein Data Bank (PDB). Protein structures were processed in PyMOL (v2.1.0) by removing water molecules and co-crystallized small-molecule ligands. Hydrogen atoms and charges were then added using AutoDockTools (v1.5.6), and the prepared proteins were saved in PDBQT format. For the “Docking procedure and parameters” procedure: using each processed protein as a receptor and its corresponding active compound as a ligand, molecular docking was performed with the built-in Vina 2.0 engine in PyRx. For each receptor, we defined the docking search space using a grid box centered on the selected binding site. The grid-center coordinates and box dimensions are listed in Supplementary Table S3. Vina generated predicted binding affinities and docking poses for each ligand-receptor pair. We visualized the docking poses in PyMOL (v2.1.0; accessed on 18 September, 2025). We interpreted more negative affinity values (kcal/mol) as indicating stronger predicted ligand-receptor binding.

3.10.3. Network Pharmacology-Based Analysis of Key Flavonoid

Compared with other constituents, flavonoids derived from *Epimedium* have been more extensively studied and applied. Multiple studies have demonstrated a well-established metabolic relationship among icariin, icaritin, and icariside II [48–51,380]. Consequently, we conducted network pharmacology-based predictions for these three flavonoids and analyzed the current research landscape. As shown in Figure 4A, the physicochemical properties of icariin, icaritin, and icariside II were retrieved and evaluated using the SwissADME database [381]. Potential target proteins of these compounds were identified using SwissTargetPrediction database. These targets were subsequently cross-referenced with 53 disease-associated targets related to bone disorders, as predicted by the GeneCards, Online Mendelian Inheritance in Man, and Therapeutic Target Database [383,385,388]. The intersecting targets were imported into the Search Tool for the Retrieval of Interacting Genes/Proteins database to obtain a protein–protein interaction network diagram and visualized using Cytoscape software to further clarify the core targets. Core targets were subsequently subjected to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis using the Database for Annotation, Visualization, and Integrated Discovery [389–391]. Finally, molecular docking studies were performed with AutoDock Vina to evaluate the binding affinities between these flavonoids and their corresponding core targets [392,393].

The relative molecular weights follow the order icariin > icariside II > icaritin (Figure 4E), consistent with gut microbiota-mediated deglycosylation: hydrolysis of the glucose moiety of icariin generates icariside II, which can be further converted to icaritin and desmethylicaritin [79,378]. The partition coefficient reflects lipophilicity, with higher values indicating greater lipophilic character and reduced hydrophilic properties. Optimal gastrointestinal absorption requires a balance between lipophilicity and hydrophilicity, as excessive values in either property are suboptimal. Consequently, icaritin demonstrates the highest gastrointestinal absorption efficiency among the three compounds. Bioavailability predictions further reveal that icaritin and icariside II exhibit substantially higher bioavailability compared with icariin.

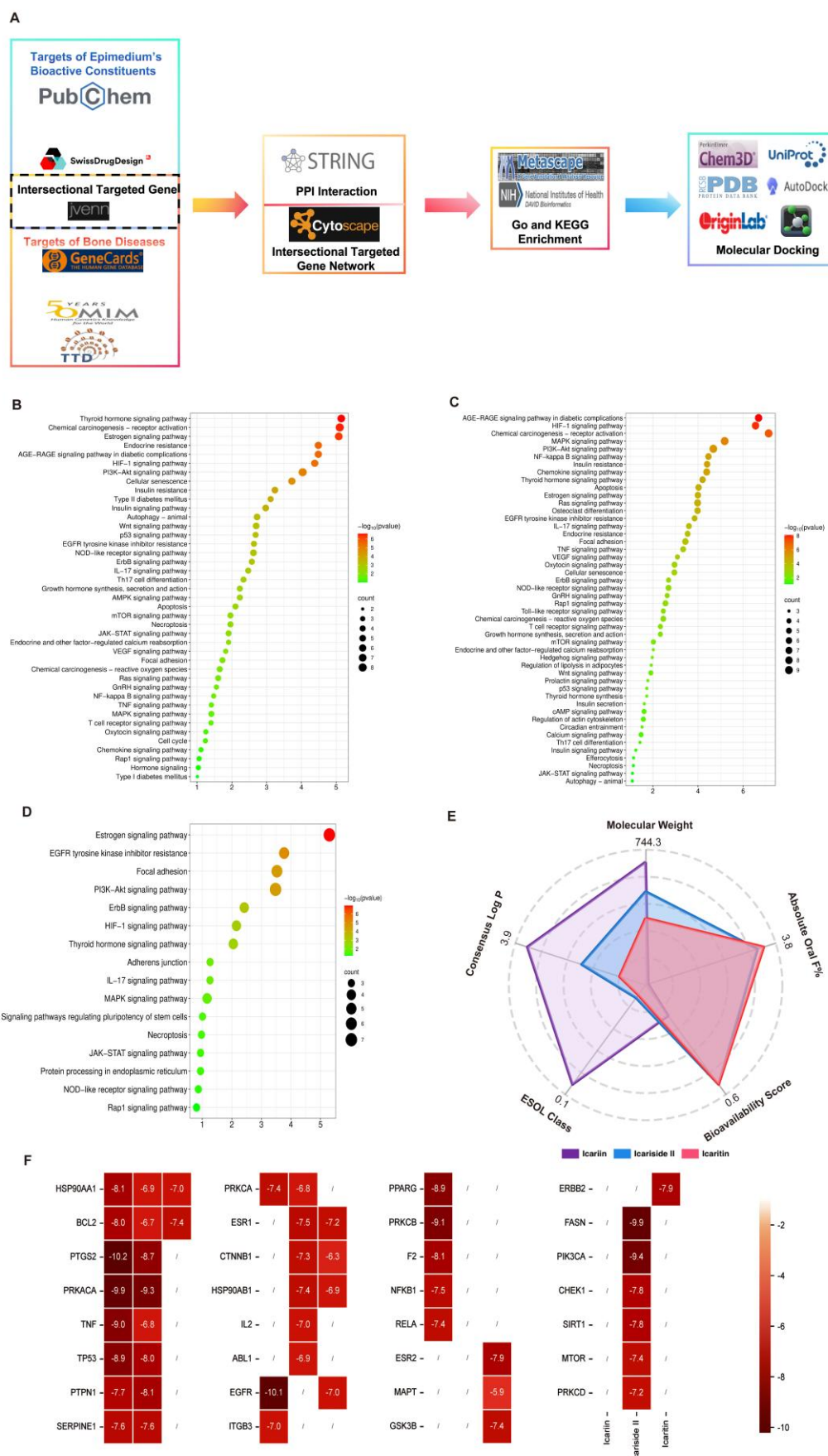


Figure 4. Schematic of the network pharmacology paradigm: (A) A flowchart showing the network pharmacological workflow for the investigation of the 53 bone diseases' healing effects of three *Epimedium* bioactive constituents. (B–D) KEGG pathway enrichment analysis of the 53 bone diseases' healing effects targets of icariside II, icariin, icaritin. (E) ADEM analysis results of icariin, icariside II, icaritin. (F) Heat map of molecular docking binding energy.

The results of the network pharmacology analysis are shown in Figure 4. Given the recognized metabolic interplay among icariin, icaritin, and icariside II, extensive research has explored their distinct effects on bone metabolism, yet their exact molecular mechanisms require further clarification. In vitro evidence demonstrated that icaritin most effectively promoted osteoblast proliferation and differentiation compared to icariin and icariside II [74]. Additionally, the inhibitory effect on osteoclastogenesis ranked in the order of icaritin > icariside II > icariin. Regarding estrogen-like activity, icaritin again surpassed icariside II, while icariin lacked measurable intrinsic estrogenic function [14,133,374,375]. This pharmacological profile aligned perfectly with our network analysis, which pinpointed ESR1 as a core target for icariside II and both ESR1 and ESR2 for icaritin, while excluding these receptors for icariin. These functional discrepancies in estrogen-like activity primarily originate from structural differences (Figure 2), specifically the extent of deglycosylation and spatial steric hindrance, which fundamentally alter receptor binding affinity.

Icariin yielded 25 core targets, and KEGG enrichment analysis revealed 48 bone pathways related to bone metabolism (Figure 4C), among which one core target-F2 and 29 pathways were not identified within the databases or search framework used in this study. Molecular docking demonstrated stable predicted binding (binding energy < −5.0 kcal/mol) between icariin and 17 core targets—AKT1, Bcl-2, HER1, coagulation factor II (F2), heat shock protein 90 kDa alpha A1 (HSP90AA1), integrin beta 3, nuclear factor of kappa light polypeptide gene enhancer in B-cells 1, peroxisome proliferator-activated receptor- γ , protein kinase cAMP-dependent catalytic alpha (PRKACA), protein kinase C alpha (PRKCA), protein kinase C beta, prostaglandin-endoperoxide synthase 2 (PTGS2), protein tyrosine phosphatase non-receptor type 1 (PTPN1), v-rel avian reticuloendotheliosis viral oncogene homolog A, serpin peptidase inhibitor clade E member 1 (SERPINE1), TNF- α , and tumor protein 53 (TP53)—that are enriched in these pathways, suggesting their potential significance in the regulation of icariin-mediated bone metabolism.

Icariside II analysis identified 21 core targets and 41 bone-related pathways (Figure 4B), among which six core target-PTPN1, ABL1, CTNNB1, CHEK1, SIRT1, FASN and 35 pathways were not identified within the databases or search framework used in this study. These core targets exhibited strong docking affinities (binding energy < −5.0 kcal/mol) with icariside II, suggesting their potential involvement in the osteomodulatory effects of this compound. For instance, SIRT1 is a key regulator of cellular-senescence-associated pathways [394]. In osteoblast progenitors, SIRT1 deficiency impairs cortical bone formation, and the age-associated decline in SIRT1 activity may contribute to progressive bone-mass loss [395]. The above analysis suggests that icariside II may be a promising candidate molecule for intervening in age-related osteoporosis compared to icariin, and its specific mechanisms against skeletal aging require further confirmation through future in vitro and in vivo experiments.

Analysis of icaritin identified 17 core targets and 16 bone-related pathways, among which one core target-CTNNB1 and 12 were not identified within the databases or search framework used in this study. Eleven core targets (AKT1, BCL2, CTNNB1, HER1, HER2, ESR1, ESR2, glycogen synthase kinase-3 beta, HSP90AA1, HSP90AB1, and microtubule-associated protein tau) demonstrated high-affinity binding (binding energy < −5.0

kcal/mol) with icaritin, indicating their potential critical role in the regulation of bone metabolism by icaritin. Notably, CTNNB1 was identified as a core target of icariside II and icaritin, but not of icariin. CTNNB1 encodes β -catenin, a key effector molecule in the canonical Wnt signaling pathway involved in regulating osteoblast differentiation and bone formation [395,396]. Therefore, it can be inferred that the regulatory involvement of the Wnt/ β -catenin signaling pathway may provide potential mechanistic insights into why icaritin exhibits stronger osteogenic differentiation activity compared to icariin. Further literature review revealed that icaritin demonstrates the strongest effects among the three compounds in promoting osteogenesis and inhibiting osteoclastogenesis [14]. This is closely associated with its metabolic pathway, specifically the conversion of icariin to icariside II and subsequently to icaritin, which constitutes a progressive deglycosylation process. This process involves the sequential removal of hydrophilic glycosyl groups, a gradual reduction in molecular weight, and the exposure of more active hydroxyl groups. Consequently, this structural modification increases the lipophilicity of icaritin and significantly enhances its cell membrane permeability, thereby enabling it to penetrate cells more easily to exert its biological activities.

The above content systematically reveals the evolutionary process of icariin in terms of “structure-metabolism-target-efficacy.” By identifying novel targets such as SIRT1 and CTNNB1, it provides a microscopic mechanistic hypothesis for how deglycosylation enhances bioavailability and promotes osteogenic activity, overcoming previous limitations that were confined to macro-level efficacy comparisons. This advancement significantly contributes to a comprehensive understanding of the pharmacological basis of icariin. However, these mechanistic hypotheses still require further validation through pharmacokinetic tracking, interventions targeting specific pathways, and functional experiments both in vitro and in vivo.

4. Applications in Bone Tissue Engineering

Epimedium, a traditional Chinese medicinal herb, promotes bone regeneration and plays multifaceted roles in bone tissue engineering. Its core bioactive components facilitate bone metabolic homeostasis through multi-target modulation, enhancing its therapeutic potential. Current applications predominantly rely on biomaterial-based localized delivery systems, with emerging studies reporting synergistic therapeutic effects when its extracts are combined with stem cell therapy, resulting in bone healing rates over 70% in animal models of critical-sized bone defects [381]. Through the in-depth integration of materials science, engineering, and traditional Chinese medicine, *Epimedium* holds promise as a crucial component in innovative “bioactive scaffold + endogenous repair” systems for bone regeneration, potentially advancing the transition of bone tissue engineering from replacement therapy to functional reconstruction (Figure 5 and Table 2).

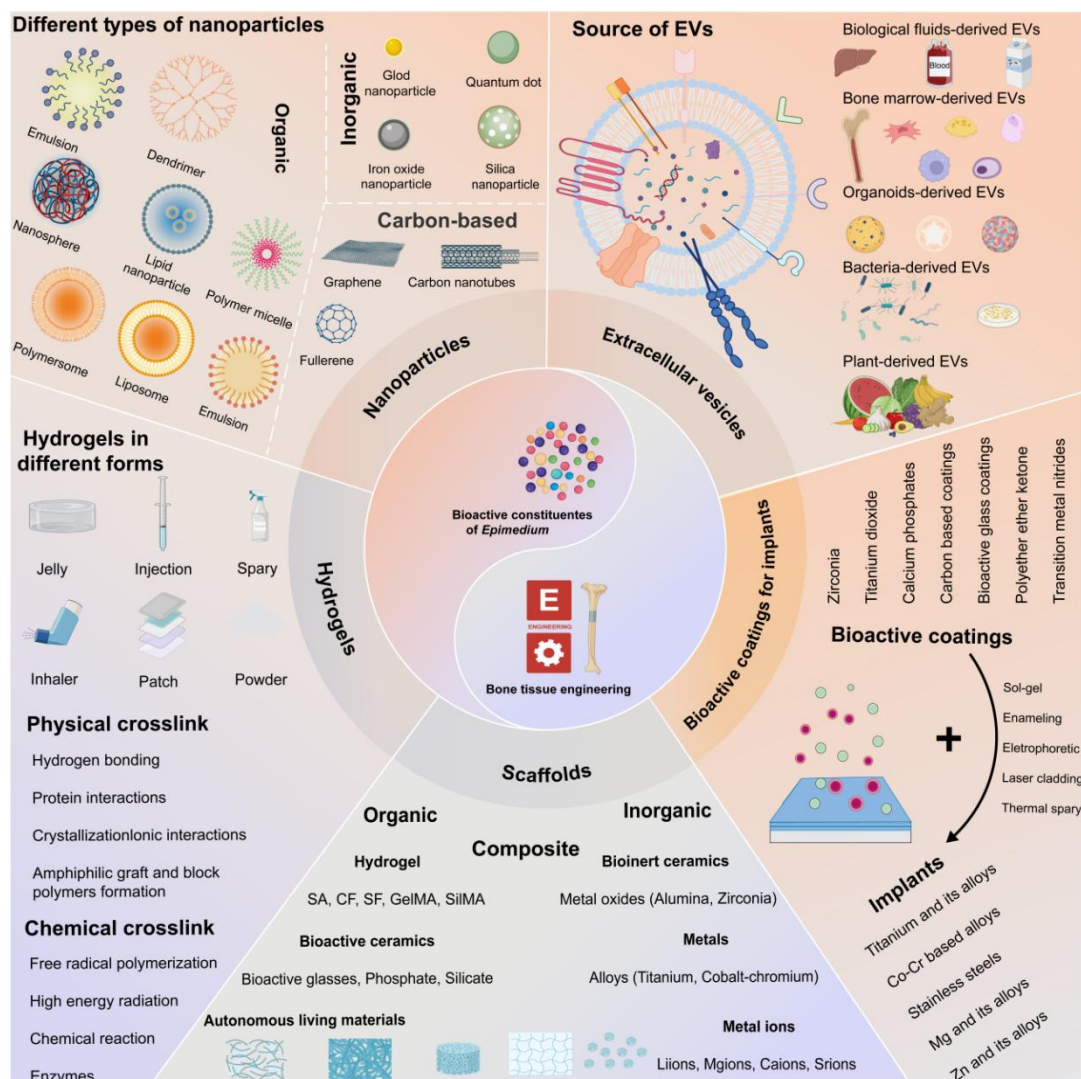


Figure 5. Schematic of five distinct biomaterial delivery systems: Integrating *Epimedium*-derived bioactive constituents (e.g., icariin, icaritin, etc.) with bone tissue engineering to enhance bioavailability and efficacy: (1) Hydrogels: Development of different hydrogel matrices via physical (e.g., hydrogen bonding, protein interactions, etc.) or chemical (e.g., photopolymerization, enzymatic reactions, etc.) crosslinking mechanisms to enhance drug delivery efficacy and minimize adverse effects. (2) Scaffolds: Construction of hierarchical architectures using organic polymers (e.g., hydrogel, bioactive ceramics, autonomous living materials), inorganic polymers (e.g., bioinert ceramics, metals, metal ions), or composites to provide structural support. (3) Nanoparticles: Different types of nanoparticles—such as organic systems (e.g., emulsions, dendrimers, and nanospheres), inorganic systems (e.g., gold nanoparticles and quantum dots), and carbon-based materials (e.g., graphene, silica nanoparticles, and fullerenes) have been engineered to facilitate passive targeting, enabling their extravasation into osseous defect regions. (4) Extracellular vesicles: Utilization of bio-inspired vesicles derived from varying sources including biological fluids, bone marrow, organoids, bacteria and plants for controlled drug release. (5) Bioactive coatings for implants: Functionalization of implant material surfaces through surface modification strategies (e.g., sol–gel method, enameling, etc.) applied to materials (e.g., titanium and its alloys, cobalt–chromium-based alloys, etc.), enabling the construction of bioactive coating composites that enhance the biological performance of the implant surface and more effectively promote bone defect repair.

4.1. Hydrogels

Hydrogels are three-dimensional, crosslinked polymeric networks capable of absorbing and retaining large quantities of water or biological fluids, thereby enabling controlled release of encapsulated therapeutic agents [397–399]. Due to their tunable properties and diverse fabrication methodologies, hydrogels have been widely utilized in the field of biomedical engineering [400]. Among various research efforts aimed at enhancing drug delivery efficacy while minimizing adverse effects, hydrogel-mediated sustained drug release has emerged as a highly promising strategy [401].

Chen et al. encapsulated icariin-loaded zinc–aluminum layered double hydroxide nanosheets into a gallic acid-modified hydroxy butyl chitosan (CTS) hydrogel to develop a tailored hydrogel system [401]. This system achieves sequential antibacterial, anti-inflammatory, and remineralization functions through programmed spatiotemporal dynamic sequences [401]. It synergistically enhances the therapeutic effects of icariin, thereby effectively promoting the regeneration and repair of chronic periodontitis accompanied by bone defects [401]. A novel clinical strategy for the treatment of severe bone and cartilage defects has been developed through the introduction of hydroxyethyl methacrylate (HEMA) groups and the functionalization of thermosensitive hydrogels with HEMA moieties [402]. This system employs supercritical carbon dioxide to achieve controlled release of poly(N-vinyl caprolactam) (PNVCL)@icariin nanoparticles for icariin delivery, while maintaining the optimal compositional characteristics of the ECM [402]. Zhu et al. developed an integrated system that combines a 3D-printed titanium alloy scaffold with a thermosensitive chitosan hydrogel, thereby facilitating localized bone regeneration and enhanced osseointegration through the delivery of icariin [403]. The scaffold exhibits controlled icariin release kinetics, demonstrates exceptional biocompatibility, and effectively promotes the proliferation and osteogenic differentiation of BMSCs [403]. Moreover, Song et al. coated titanium surfaces, which were pretreated with phase transited lysozyme, with icariin-loaded hyaluronic acid (HA)/CTS multilayer films [404]. This configuration exhibits sustained icariin release characteristics, effectively promoting osteogenic differentiation of osteoblasts *in vitro* and enhancing early osseointegration *in vivo* [404]. Fan et al. developed an icariin-loaded CTS/hydroxyapatite composite hydrogel where the release kinetics of icariin were governed by the simultaneous degradation of both CTS and hydroxyapatite matrices [405]. The design enabled the sustained release for over 90 days while effectively stimulating ALP activity in bone marrow stromal cells and promoting mineralized nodule formation [405]. In the study of Shao et al., a thermosensitive icariin/CTS/ β -glycerophosphate hydrogel was engineered to modulate inflammatory responses, angiogenesis, and osteogenic factor expression, demonstrating potent infection control efficacy in a canine model of infectious bone defect [406]. Researchers proposed that the combined application of icariin-conditioned serum with thiolized CTS could mitigate cartilage degeneration and subchondral bone remodeling abnormalities in rabbit models of knee osteoarthritis [407]. Fu et al. constructed an injectable sodium alginate (SA) hydrogel by encapsulating icariin, titanium carbide powder ($\text{Ti}_3\text{C}_2\text{T}_x$), and doxorubicin (DOX) [408]. The incorporation of icariin attenuated DOX-induced inhibition of BMSCs proliferation and differentiation, thereby conferring the hydrogel with robust antitumor efficacy while significantly reducing osteogenic toxicity [408]. Similarly, a composite hydrogel was developed based on a hydrogel matrix composed of Pluronic F127 (PF127) and Pluronic F68 (PF68), co-loaded with icaritin and DOX [409]. The incorporation of icaritin not only decreased the required dosage of DOX and its associated toxic side effects, but also promoted osteogenic differentiation, thereby facilitating osteosarcoma treatment and supporting bone regeneration [409]. Further, Qian et al. developed a unique cell-infiltratable and injectable gelatin hydrogel utilizing a high degree of acryloyl-modified β -cy-

clodextrin (β -CD) [13]. The hydrophobic cavities within β -CD in the hydrogel were employed for the delivery of icaritin [13]. The hydrogel demonstrated sustained release of icaritin over a two-week period, promoting stem cell differentiation while reducing the adverse effects associated with high-dose drug administration [13].

Ginkgetin has been shown to enhance autophagy and suppress inflammatory responses [410,411]. Capitalizing on this advantage, Chen et al. developed a novel MMP13-responsive hydrogel nanoplatfrom capable of orchestrating the controlled release of ginkgetin and rapamycin to target distinct stages in the progression of intervertebral disc degeneration [412]. This strategy effectively modulates inflammation and autophagy in degenerative disc pathology [412]. A magnoflorine-loaded HA hydrogel significantly enhance subchondral bone regeneration by increasing bone volume fraction, trabecular number, and connectivity density while reducing the degree of anisotropy [413]. Furthermore, local administration of magnoflorine-loaded HA hydrogel enables effectively prevented osteoarthritis progression following anterior cruciate ligament rupture [414]. Mohammadi et al. employed a freeze-drying technique to fabricate a composite scaffold through crosslinking of gelatin, CTS, and HA, with the incorporation of diatoms and β -sitosterol [415]. This scaffold enhances cellular adhesion, promotes ECM mineralization, and stimulates osteoblast proliferation, while simultaneously facilitating angiogenesis via β -sitosterol and exerting anti-inflammatory properties [415]. Lee et al. utilized nanoclays as nanoscale crosslinkers to develop a nanoclay-reinforced caffeic acid-modified CTS hydrogel bone sealant, demonstrating an enhanced bone regeneration [416]. Just recently, Gao and colleagues functionalized poly (xylitol succinate) with caffeic acid and citric acid, followed by integration with magnesium oxide to fabricate a multifunctional injectable hydrogel possessing intrinsic antagonistic properties against the TLR4 and myeloid differentiation factor 2 complex [417]. This innovative hydrogel exhibits potent anti-inflammatory and antibacterial properties. And in murine models of periodontitis, the hydrogel not only demonstrated regenerative efficacy but also displayed therapeutic potential in reducing inflammatory cytokine levels, suppressing key periodontopathogenic bacterial species, and maintaining subgingival microbial homeostasis [417]. Palaniraj et al. fabricated a chlorogenic acid-loaded antibacterial porous nanogel based on calcium phosphate-CTS nanocomposites [418]. This nanostructured matrix, formed through ionic gelation of calcium phosphate nanoparticles acting as crosslinking agents, exhibits controlled drug release properties and pronounced antibacterial effects, showing potential in promoting the differentiation of hDPSCs [418]. Furthermore, Fang et al. developed a polymethylacrylamide hydrogel encapsulating neochlorogenic acid and BMSCs [419]. The hydrogel effectively mitigates oxidative stress-induced damage to stem cells, reduces apoptosis rates, and enhances the survival of transplanted stem cells, thereby offering a promising therapeutic strategy for intervertebral disc degeneration [419].

Although hydrogels offer numerous advantages as sustained-release carriers for the active constituents of *Epimedium*, their limitations warrant careful consideration. Firstly, some of *Epimedium*-derived bioactive compounds exhibit poor solubility and low permeability, leading to non-uniform drug loading and compromising the synergistic effects of multi-component formulations due to disruption of the sustained-release mechanism. Secondly, conventional hydrogels are highly sensitive to environmental conditions; swelling of the hydrophilic polymer network in vivo causes drug release kinetics to deviate from zero-order behavior, resulting in an initial burst release or delayed release in later stages, thereby undermining therapeutic efficacy stability. Thirdly, synthetic materials (e.g., polyacrylic acid) may provoke inflammatory responses or immune rejection, whereas natural polymers (e.g., gelatin) often lack sufficient mechanical strength and are susceptible to microbial degradation. Additionally, implantable hydrogel systems typically require surgical intervention, and the long-term toxicity of degradation byproducts

(such as lactic acid) remains unclear. Fourthly, fabrication processes are often complex—requiring techniques such as high-pressure homogenization or graft copolymerization—entailing high production costs and inconsistent batch-to-batch reproducibility. Furthermore, optimizing process parameters for multi-component self-assembly technologies remains challenging, hindering compliance with clinical standardization requirements.

4.2. Organic Scaffolds

In bone tissue engineering, organic scaffold materials demonstrate notable advantages and have consistently garnered significant attention. Their exceptional biocompatibility—exemplified by collagen and CTS, which mimic the structure of the natural ECM—facilitates cell adhesion, proliferation, and differentiation. Additionally, their tunable degradation rates (e.g., those of polylactic acid (PLA) and polycaprolactone (PCL)) can be aligned with the pace of bone regeneration, thereby eliminating the need for secondary surgical interventions. Furthermore, their highly customizable architectures enable precise control over porosity (>70%) through 3D printing, enhancing vascularization and nutrient transport. Mechanical properties can be further improved through crosslinking or the incorporation of inorganic phases such as hydroxyapatite. Finally, their functional versatility supports the incorporation of bioactive agents, including growth factors (e.g., BMP-2) and therapeutic drugs, to achieve synergistic osteogenic and antibacterial effects. Moreover, certain materials, such as HA, possess inherent angiogenic properties, thereby offering multidimensional support for effective bone regeneration. For instance, Xia et al. developed a novel icariin-delivering porous poly (3-hydroxybutyric acid) scaffold using solvent casting and salt leaching methods [420]. The scaffold exhibited a high porosity of 88.80% and possessed appropriate mechanical properties for three-dimensional cell culture [420]. It significantly promoted the proliferation of human osteoblast-like MG-63 cells and preosteoblastic MC3T3-E1 cells, with the formulation containing 0.1% (*w/w*) icariin showing the most pronounced osteogenic effect [420]. Park et al. investigated the osteogenic potential of icariin-loaded 3D-printed PCL scaffolds and demonstrated their efficacy in accelerating alveolar cleft reconstruction [421]. And Gao et al. developed a stem cell-targeted DNA tetrahedron platform (PolyApt) by conjugating Apt19s aptamer to DNA tetrahedrons [422]. Subsequently, icariin and epigallocatechin gallate (EGCG) were intercalated into the double-stranded regions of PolyApt to form PolyApt@icariin and PolyApt@EGCG complexes [422]. A degradable gradient scaffold was then engineered by encapsulating PolyApt@icariin and PolyApt@EGCG into the upper and lower layers of a gelatin methacryloyl and polyethylene glycol diacrylate matrix, respectively [422]. This design synergistically enhances the recruitment, protection, and directed differentiation of endogenous stem cells while promoting accelerated regeneration of osteochondral defects [422]. What is particularly noteworthy is that Li et al. delivered icariin in the form of ECM on a small intestinal submucosa (SIS) scaffold following icariin pre-treatment of osteoblasts [423]. Compared with direct delivery of icariin on SIS scaffold, the SIS scaffold functionalized with icariin-derived ECM exhibited superior osteoconductivity, enhanced cell adhesion, spreading, survival, and proliferation, while not compromising osteoinductivity [423].

4.3. Inorganic Scaffolds

Calcium phosphate cement (CPC) is an outstanding bone substitute material due to its porous structure, favorable mechanical strength, and excellent biocompatibility [424]. They are particularly suitable for clinical applications requiring rapid mechanical stability, such as vertebroplasty. Importantly, by mimicking the inorganic composition of natural bone, CPC can promote mineralization and integration with surrounding bone tissue, serving as a scaffold to support cell adhesion, proliferation, and osteogenic differentiation

[425,426]. Through hydration reactions, they can form a bone-like apatite structure and undergoes gradual resorption and replacement by new bone tissue, thereby avoiding long-term foreign body retention [427]. Moreover, CPC can be combined with biomaterials such as alginate to serve as a stem cell carrier, enhancing cellular protection and collagen synthesis, or functionalized with drugs and growth factors to enable synergistic therapeutic effects [428]. Furthermore, their injectable nature and radiopacity significantly broaden their applicability in minimally invasive surgical procedures [429,430]. These characteristics render CPC a highly effective carrier for the active ingredients derived from Chinese herbal medicines. CPC scaffolds loaded with icariin can accelerate the repair and regeneration of damaged bone tissue in OVX rats [431]. Luo et al. successfully incorporated icarisiide II into CPC scaffolds, which was demonstrated to promote bone regeneration by enhancing the proliferation and survival of BMSCs in vivo [432]. It has also been reported that CPC scaffolds loaded with luteolin and incorporating tantalum nanoparticles significantly enhance osteogenic differentiation in vitro and bone formation in vivo through the synergistic effects of Ta^{2+} and luteolin [433]. More interestingly, Song et al. fabricated a strontium (Sr)-doped biphasic calcium phosphate (BCP) scaffold incorporating a self-crosslinked icariin network [434]. Through the synergistic action of released icariin and Sr^{2+} , the scaffold enhanced osteogenesis and attenuated osteoclast activity [434].

In bone tissue engineering, although β -tricalcium phosphate (β -TCP) and CPC are both classified as calcium phosphate-based biomaterials, they differ significantly in terms of material morphology and the underlying mechanisms of osteogenesis [435]. CPC is an injectable paste that sets in situ via a hydration reaction under physiological conditions upon mixing a powdered component with a liquid phase [435]. Its key advantages include excellent adaptability to irregularly shaped bone defects, a relatively slow initial degradation rate, and its capability to serve as a carrier for therapeutic agents such as drugs or growth factors, thereby enabling precise defect filling and controlled release [435]. In contrast, β -TCP is a pre-sintered porous ceramic scaffold characterized by a stable three-dimensional architecture, which primarily provides mechanical support and facilitates cell migration and infiltration through osteoconductive guidance [435]. It exhibits a faster degradation profile compared with CPC and is primarily designed to promote bone conduction [435]. Naturally, efforts to utilize β -TCP as a carrier for bioactive components derived from Chinese herbal medicines have persisted continuously. Lin et al. innovatively fabricated an icaritin-functionalized β -TCP composite scaffold using cryogenic rapid prototyping technology, which effectively promoted neo-osteogenesis and angiogenesis while enhancing implant-bone integration [436].

Except for the CPC and β -TCP, bioactive glass is another key material in the field of bone tissue engineering, characterized by a well-established and multifaceted mechanism of action. As an excellent osteoconductive scaffold, its three-dimensional interconnected porous structure provides physical pathways for cell migration, vascular ingrowth, and nutrient transport [437]. More importantly, upon implantation in vivo, a layer of bone-like apatite rapidly forms on its surface through reactions with physiological fluids [437]. This enables the material to establish a strong biological bond with host bone, ensuring long-term implant stability, while simultaneously releasing bioactive ions such as silicon (Si^{4+}) and calcium (Ca^{2+}) during gradual degradation [437]. These ions have been shown to upregulate the expression of osteogenesis-related genes, directly stimulate osteoblast proliferation and differentiation, and enhance collagen synthesis and mineralization in the extracellular matrix, thereby actively promoting and accelerating de novo bone formation [437]. Furthermore, bioactive glass can serve as an ideal carrier for stem cells, growth factors (e.g., BMP-2), and active constituents of traditional Chinese medicine, enabling the construction of functionally enhanced tissue engineering composites [438]. By

providing both biochemical cues and structural support, it facilitates a synergistic effect in bone regeneration. It has also been reported that corn protein-coated bioactive glass-based scaffold loaded with kaempferol can enhance the osteogenic activity of BMSCs through the bioactive properties of kaempferol, thereby accelerating the regeneration of critical-sized bone defects [439].

4.4. Organic–Inorganic Composite Scaffolds

Natural bone tissue is a sophisticated composite structure formed through the precise integration of organic components, which impart toughness, and inorganic components, which provide mechanical rigidity. Consequently, an ideal bone scaffold material should overcome the limitations associated with single-phase materials by mimicking the natural composition and hierarchical architecture of bone through the synergistic combination of organic and inorganic phases [440]. The primary advantage of such composite scaffolds lies in their capacity to achieve structural and functional integration. The inorganic phase primarily replicates the mineralized matrix of bone, offering mechanical support, serving as a critical substrate for cell adhesion, and promoting osteogenic differentiation [441]. In contrast, the organic phase constructs a fine three-dimensional porous network that enhances material flexibility, prevents brittle failure, and facilitates the diffusion of nutrients, cells, and biochemical signals [441]. Through precise microscale integration of these two phases, the degradation kinetics of the scaffold can be finely tuned to match the rate of new bone formation, thereby establishing a highly biomimetic microenvironment that effectively supports the repair and regeneration of bone defects [441].

Representatively, poly (lactic-co-glycolic acid) (PLGA)/ β -TCP scaffolds have increasingly attracted significant interest in the field of bone tissue engineering [442,443]. These biodegradable scaffolds exhibit excellent biocompatibility, along with favorable porosity and mechanical properties. They facilitate cell adhesion, provide structural support and resistance to shear forces during the early post-implantation phase, and promote the formation of reparative bone tissue, thereby serving as a promising platform for bone repair applications [444,445]. Lai et al. employed PLGA/ β -TCP/icaritin 3D-printed scaffolds to achieve a synergistic effect between controlled drug release and osteogenic activity [446]. An icaritin-loaded PLGA/TCP scaffold was developed to enhance bone defect repair in steroid-associated osteonecrosis (SAON) [447]. Zhang et al. constructed a PLGA/TCP scaffold co-loaded with icaritin and human fetal mesenchymal stem cells (HFMSCs), which significantly promoted bone regeneration in femoral defects of ovariectomized rats [448]. Their subsequent research demonstrated that, by synergistically combining icaritin with HFMSCs, the PLGA/TCP porous scaffold was endowed with enhanced bioactivity [448]. This created a favorable microenvironment that recruited endogenous MSCs and directed their osteogenic lineage commitment, thereby facilitating osteoporotic bone regeneration [448]. Utilizing low-temperature deposition 3D printing technology, they successfully fabricated PLGA/ β -TCP/icaritin composite scaffolds for the treatment of steroid-associated osteonecrosis [449]. The synergistic effects of PLGA's biodegradability, β -TCP's osteoconductivity, and icaritin's bioactivity significantly enhanced osteogenic differentiation and mineralization in bone defect areas, leading to superior bone repair outcomes [111,449,450]. Also, Chen et al. developed a PLGA/ β -TCP/icaritin composite scaffold incorporating $\text{Ti}_3\text{C}_2\text{T}_x$ MXene, which promotes osteogenesis through near-infrared-mediated mild photothermal therapy [111]. To construct a photoresponsive system for bone repair, Gu et al. developed a PLGA/ β -TCP scaffold functionalized with PLGA/MXene microspheres loaded with icaritin [451]. This design enables the controlled release of icaritin upon near-infrared irradiation, thereby enhancing bone regeneration [451]. As for other PLGA-based composite scaffolds, Fan's research team prepared a gelatin-conjugated caf-

feic acid-coated calcium phosphate/PLGA composite scaffold for the localized and targeted delivery of Tribbles homolog 3 to bone defect sites [452]. This scaffold can direct activate osteogenic differentiation while suppressing adipogenic lineage commitment [452]. Animal studies confirmed the scaffold's efficacy in significantly enhancing bone regeneration within rat mandibular non-union defects [452]. Furthermore, it effectively inhibited the formation of fat-filled cysts [452]. This combined effect demonstrates a novel therapeutic strategy for bone defect repair that integrates mechanical support with precise signal regulation [452].

Furthermore, Shen et al. constructed a silk fibroin/icariin-loaded mesoporous bioactive glass nanoparticle composite scaffold, which achieves long-term drug loading through its mesoporous structure and enhances the proliferation and osteogenic differentiation of BMSCs [453]. Jing et al. utilized icariin-loaded 45S5 bioactive glass in combination with implanted rat ASCs to promote bone healing and angiogenesis in a rat cranial defect model [454]. The results demonstrated that icariin significantly enhanced the bone healing capacity of 45S5 bioactive glass when co-cultured with rat ASCs [454]. And not coincidentally, based on 45S5 bioactive glass, Reiter et al. developed gelatin-coated 3D sponge-like scaffolds loaded with icariin, which expanded the drug delivery capability through the porous structure and provided morphological flexibility for localized drug delivery [455].

Besides PLGA- and bioactive glass-based composite scaffold, Zhao et al. developed a one-step in situ emulsion polymerization strategy to construct a thiolated icariin-functionalized methacrylic anhydride-modified collagen/hydroxyapatite biomimetic scaffold [456]. This methodology enables the in situ encapsulation of PLGA@icariin microspheres, thereby significantly improving the encapsulation efficiency and sustained-release characteristics of icariin [456]. In vitro and in vivo experimental results demonstrate that the scaffold can modulate the immune microenvironment and activate the vascular-bone regeneration cascade [456]. Moreover, Jin et al. fabricated an icariin-loaded silk fibroin/chitosan/nano-hydroxyapatite composite scaffold [457]. It can promote the repair of critical-sized radial bone defects in rabbits by upregulating osteogenic differentiation-and angiogenesis-related genes and proteins [457]. Similar study of Xie et al. reported. an icariin-loaded hydroxyapatite/alginate porous composite scaffold capable of sustained icariin release to enhance bone regeneration by mimicking the bone mineralization microenvironment [458]. In addition, researchers developed a 3D-printed polycaprolactone scaffold that incorporates both strontium-substituted mesoporous bioactive glass nanoparticles and icariin [459]. This composite system enables the sustained release of Sr^{2+} ions and icariin [459]. Together, these agents promote osteoporotic bone regeneration through a dual mechanism: enhancing osteoblast proliferation and differentiation while simultaneously suppressing osteoclastogenesis [459]. Yang et al. developed a functionalized decellularized mushroom stem scaffold composed of naturally aligned channel structures, zinc ion/curcumin metal-organic frameworks, hydroxyapatite minerals, and icariin [460]. This scaffold can control bacterial infection, promote the migration of BMSCs, and further accelerate M2 macrophage polarization and osteogenic differentiation of BMSCs [460].

4.5. Nanoparticles

Nanoparticle-based drug delivery systems exploit size-dependent circulatory properties (10–200 nm) to achieve passive targeting, thereby enabling extravasation into osseous defect regions. Through stimulus-responsive designs, such as pH-and temperature-sensitive mechanisms, these systems allow for controlled drug release within defined microenvironments, including acidic pathological niches. Moreover, the intrinsic bone-targeting affinity of nanomaterials contributes to reduced immunogenicity and cytotoxicity.

There is no doubt that nanoparticles represent one of the most effective delivery systems for bioactive components derived from Chinese herbal medicines.

As for metal or metal oxide nanoparticles, Zhang et al. synthesized dopamine-poly(ethylene glycol)-coated porous hollow iron oxide nanoparticles through thermal decomposition and controlled oxidation techniques, and encapsulated icariin within their porous hollow structures [461]. In vitro studies demonstrated that the nanoparticle system can promote osteogenic differentiation [461]. And in vivo studies revealed that under the application of an external magnetic field, the nanoparticles can accumulate at the fracture site, thereby accelerating fracture healing through the localized release of icariin. Similarly, Wang et al. proposed the incorporation of biocompatible iron oxide nanoparticles and icariin into a nano-scaffold fabricated via coaxial electrospinning technology, which significantly enhanced the osteogenic performance of the scaffolds in vivo [462]. Moreover, Yang et al. successfully constructed a nanohybrid system by combining chlorogenic acid with gold nanorods [463]. These nanohybrids are capable of inducing apoptosis and inhibiting the proliferation of osteosarcoma cells [463]. Furthermore, under controlled and mild near-infrared irradiation, the nanohybrids stimulate osteogenic differentiation by upregulating the expression of Hsp47 and Hsp70 genes and promoting the production of heat shock proteins [463]. Furthermore, Yuan et al. developed a dual-controlled release system for drugs and bioactive ions by loading hydrophobic icariin into MgO/MgCO₃ nanoparticles and subsequently encapsulating the nanoparticles at a fixed ratio (20%) within biodegradable PLGA microspheres, thereby achieving sustained release of both magnesium ions and icariin [464]. This system not only effectively promotes new bone formation but also significantly enhances osteogenic induction capacity [464]. However, metal and metal oxide nanocarrier systems continue to face significant challenges in clinical applications. The primary concern is their biocompatibility and potential for long-term toxicity [465]. Certain metal ions or nanoparticles may induce oxidative stress, provoke inflammatory responses, and lead to organ accumulation, while their metabolic pathways and long-term biodistribution in the body remain poorly understood [465]. Secondly, the inherent chemical properties of these materials can compromise stability, resulting in premature drug leakage, particle aggregation, or degradation under physiological conditions—factors that impair targeting accuracy and therapeutic effectiveness [465]. Finally, there is a risk of off-target effects and immunogenicity, as nanoparticles may be recognized and cleared by the reticuloendothelial system or interact non-specifically with non-target tissues, potentially causing unintended adverse reactions [465]. Collectively, these limitations hinder the clinical translation of such nanocarriers.

For these reasons, various organic nanoparticles and organic/inorganic composite nanoparticles have been explored as delivery vehicles for bioactive components derived from Chinese herbal medicines. Representative examples of successful applications are as follows: Yan et al. designed a novel therapeutic strategy for osteoporosis based on erythrocyte membrane-coated nanoparticles [466]. To enhance biocompatibility, icariin was encapsulated within a poly(D,L-lactide) core, and the surface was functionalized with erythrocyte membranes [466]. Targeting specificity was further improved by synthesizing alendronate-modified 3-phosphoethanolamine-N-methoxy(polyethyleneglycol)-carboxyl bone-targeting polymers, which were incorporated into the membrane coating [466]. This multifunctional nanoparticle system demonstrated significant synergistic effects, promoting both angiogenesis and osteogenesis in vitro [466]. Luo et al. constructed a nanocargo system by loading icariin onto polydopamine-modified covalent organic frameworks [467]. The resulting nanocomplex was shown to synergistically ameliorate the local inflammatory microenvironment via immunomodulation and enhance bone defect repair by promoting osteogenic differentiation [467]. There have also been several attempts to

design delivery systems for other bioactive components derived from *Epimedium*. For instance, Feng et al. developed a pH-responsive HA-modified anti-inflammatory catechin-based metal–organic framework system [468]. It can significantly delay the progression of osteoarthritis by inhibiting Col II degradation and suppressing IL-1 β -stimulated inflammatory mediator production [468]. Also, Li et al. prepared a polyvinylpyrrolidone-modified PLGA-based drug delivery system, which achieves long-term controlled release through co-loading of chlorogenic acid [469]. This system not only efficiently scavenges ROS in the periodontal microenvironment but also disrupts the pathological cycle of inflammation-oxidative stress by suppressing the overexpression of pro-inflammatory cytokines [469]. The scaffold can significantly inhibit alveolar bone resorption and synergistically block the progression of periodontitis through a triple mechanism involving antioxidant activity, anti-inflammatory effects, and bone-protective functions [469]. Tactfully, a novel therapeutic strategy based on calcium-chlorogenic acid nanoparticles was developed [470]. The nanoparticles can promote the differentiation of BMSCs into osteoblasts and induce macrophage polarization toward the M2 phenotype [470]. This dual regulatory effect significantly enhances bone repair [470]. Even more interestingly, a research team fabricated a novel supramolecular nano-agonist through the self-assembly of berberine and chlorogenic acid [471]. When the assembly ratio of chlorogenic acid/berberine changes from 1:1 to 1:4, the nano-agonist can passively target osteoporotic bone tissue after prolonged systemic circulation, modulate the Wnt signaling pathway, and thereby regulate osteogenic differentiation [471]. Moreover, a co-nanoparticle formulation of luteolin and palmitoylethanolamide has been shown to significantly reduce pro-inflammatory cytokines (e.g., IL-6, TNF- α) and oxidative stress markers (e.g., nitrotyrosine, malondialdehyde), thereby alleviating joint erosion [472]. Lee et al. formulated kaempferol-loaded gelatin nanoparticles coated with HA (HA-KM GNPs) [473]. The administration of HA-KM GNPs in the anterior cruciate ligament transection rat models markedly attenuated the progression of knee osteoarthritis (KOA) [473]. The treatment effectively reduced subchondral sclerosis, alleviated inflammation, inhibited extracellular matrix degradation, and restored cartilage thickness, thereby lessening overall KOA severity [473]. Additionally, a novel nano complex composed of CTS, chondroitin sulfate, and daidzein was prepared for the treatment of glucocorticoid-induced osteoporosis through targeted delivery and sustained release of daidzein [474]. This nanocomplex enhanced osteoblast formation by upregulating ALP and OPG expression, while reducing osteoclast activity [474].

Polymer micelles are nanoscale core–shell structures formed through the self-assembly of amphiphilic block copolymers in selective solvents. The hydrophobic core serves as an ideal reservoir for poorly water-soluble drugs, thereby significantly improving drug solubility and stability [475]. And the hydrophilic shell confers excellent aqueous dispersibility, prolonged systemic circulation, and passive targeting capability to the micelles [475]. Furthermore, by surface functionalization, these nanocarriers can achieve efficient and intelligent delivery to specific cells or tissues, demonstrating considerable potential for applications in nanomedicine [475]. It has been reported that polymeric micelles loaded with icaritin can effectively treat various diseases by enhancing its solubility, stability, and bioavailability [476,477]. Sun et al. demonstrated that alendronate-functionalized polymer micelles for targeted delivery of icaritin to osteoporotic bone effectively alleviated osteoporosis in rat models and partially restored bone microarchitecture [478]. Tang et al. employed an innovative acid-base conversion method to prepare spherical polymeric micelles with high drug loading capacity, using Soluplus[®] and Poloxamer 407 as matrix materials for icaritin encapsulation, thereby enhancing both the drug loading capacity and oral bioavailability of icaritin [477]. The fatty acids present in sheep oil (SO) possess long hydrocarbon chains and surfactant-like properties, enabling them to self-assemble with bile salts in vivo to form nanomicelles [479,480]. These nanomicelles can serve

as drug delivery carriers and facilitate drug absorption [481,482]. Jun et al. exploited this characteristic to develop an icaritin-SO-deoxycholate self-assembled nanomicelle system, which reduced the particle size of icaritin, thereby enhancing its absorption, improving bioavailability, and further augmenting its anti-osteoporotic efficacy [483]. Moreover, Akshay et al. synthesized mPEG-b-PCL block copolymers through ring-opening polymerization of ϵ -caprolactone [484]. And they conjugated the hydrophilic compound caffeic acid to mPEG-b-PCL micelles via Steglich esterification and incorporated the therapeutic agent 9-aminoacridine [484]. These nanomicelles were shown to alleviate arthritic symptoms by reducing inflammation, joint damage, bone erosion, and swelling [484].

Both polymer micelles and liposomes are nanocarriers for drug delivery formed through the self-assembly of amphiphilic molecules. However, polymer micelles typically accommodate only hydrophobic drugs, whereas liposomes are capable of encapsulating both hydrophilic and hydrophobic therapeutic agents simultaneously [485,486]. The application of icaritin-loaded liposomes in the treatment of bone-related diseases has also demonstrated promising therapeutic outcomes [487]. The bioavailability of icaritin in this formulation was approximately 28-fold higher than that of free icaritin [487]. Moreover, Chen et al. developed a novel bone-targeted delivery system, i.e., Asp8-liposome-icaritin [487]. Asp8 enhances the specific targeting of the *Epimedium*-derived molecule icaritin, which is encapsulated within liposomes, to bone remodeling sites [487]. This system can promote bone formation by regulating the differentiation of BMSCs into osteoblasts, up-regulating osteoblast function, and inhibiting osteoclast activity [487]. Furthermore, it effectively prevents osteonecrosis by maintaining a high local concentration of icaritin on the bone surface [487].

4.6. Extracellular Vesicles

Extracellular vesicles (EVs) have demonstrated significant potential in the field of controlled drug release, owing to their inherent biological activity and intelligent delivery capabilities [487,488]. As naturally occurring nanoscale carriers, they exhibit excellent biocompatibility, low immunogenicity, and the ability to cross physiological barriers, enabling effective protection of therapeutic cargo and targeted delivery [489]. In the context of controlled release, the lipid bilayer of extracellular vesicles can function as a “switch”, undergoing rupture in response to specific physicochemical stimuli in the pathological microenvironment (such as acidic conditions or the presence of specific enzymes), thus facilitating precise and stimulus-triggered drug release [489]. Furthermore, through surface engineering or hybridization with synthetic materials, their release kinetics can be precisely programmed [489].

In recent years, numerous researchers have sought to engineer EVs as carriers for the bioactive components of *Epimedium* in the context of bone tissue engineering. Yu et al. isolated small EVs (sEVs) from bovine milk and subsequently prepared sEV@icariin complexes [490]. These complexes promote osteogenesis by facilitating the binding of the signal transducer and activator of transcription 5A to the gap junction protein alpha 1 promoter, exhibiting enhanced efficacy in bone repair both in vitro and in vivo [490]. Also, Yan et al. developed an adipose-derived stem cell exosome-based system for icariin delivery [491]. Experimental studies demonstrated that the nanomaterial inhibits glycolysis, thereby modulating macrophage polarization from the M1 to M2 phenotype and alleviating rheumatoid arthritis [491]. Although EV-based carriers have shown considerable potential in bone tissue engineering, the targeting and homing capability of natural vesicles remains limited, and their retention time at the lesion sites is difficult to regulate. Consequently, they are often required to be combined with scaffold materials to enhance therapeutic efficacy. Inspired by mussel-inspired chemistry, Zeng et al. also fabricated a multi-functional hydrogel system capable of co-delivering BMSC-derived exosomes and icariin,

achieving a synergistic therapeutic effect [492]. More importantly, due to its prolonged retention *in vivo*, the system enhances the protective effect on cartilage [492]. Meanwhile, in the osteoarthritis model, the co-delivery hydrogel system effectively alleviated cartilage atrophy and maintained appropriate cartilage thickness [492].

4.7. Bioactive Coatings for Implants

Currently, many soft and hard tissue implant materials used in clinical practice exhibit weak interactions with host tissues due to surface biological inertness [3,4,94,493,494]. The construction of bioactive coatings is widely regarded as a simple, cost-effective, and efficient approach to enhance the biological performance of implant material surfaces. Among them, the sustained-release coating of bioactive substances derived from Chinese herbal medicines, enabled by precise compositional regulation and endowed with programmable functionalities, effectively meets the personalized functional demands of implant surfaces and is currently recognized as a highly promising surface modification strategy [495,496]. First of all, PEEK as well as Ti and its alloys, which are widely used in clinical practice, are taken as examples. He et al. addressed critical challenges in clinical bone implants, including large bone defects and associated infections, by integrating a chlorogenic acid-grafted peptide hydrogel onto sulfonated PEEK surfaces [497]. A study revealed that the surface of a Ti implant coated with quercitrin can effectively reduce the expression of osteoclast functional markers, such as CTSK, H+ATPase, and MMP9, both *in vivo* and *in vitro* [498]. This effect contributes to the maintenance of marginal bone levels and enhances long-term implant stability [498]. The functionalization of the titanium surface with quercetin and paclitaxel was shown to confer excellent osteogenic, anti-inflammatory, and anti-fibrotic properties on the implant, thereby promoting rapid osseointegration between the implant and host bone [498,499]. Yang et al. developed novel caffeic acid nanospheres and deferoxamine-based films coated on Ti implant to modulate ferroptosis. The intelligent Ti implant was capable of alleviating iron overload and oxidative stress in postmenopausal osteoporosis and enhancing osseointegration [500]. Moreover, the porous structure of quercetin-functionalized Ti6Al4V orthopedic implant has been demonstrated to preserve the mechanical integrity of the implant, including its Young's modulus, while exhibiting favorable cytocompatibility [501]. This design also supports the proliferation and differentiation of bone cells, reduces bacterial adhesion and viability, and simultaneously enhances osseointegration and prevents implant-associated infections [501]. Giacomo et al. employed hexamethylenediamine to modify self-oxidized caffeic acid, endowing it with specific film-forming capabilities [502]. The approach enabled the formation of thin, uniform, and stable coatings on polished and chemically pre-treated Ti6Al4V alloys [502]. These coatings exhibited high cytocompatibility with stem cells involved in tissue regeneration and demonstrated effective scavenging activity, thereby protecting cells from apoptosis induced by toxic reactive species in pro-inflammatory microenvironments [502]. As for other types of implants, a thiolated icariin derivative (icariin-SH), characterized by favorable cytocompatibility and biological activity, was immobilized onto a BCP scaffold through intermolecular disulfide bond formation among icariin-SH molecules, forming a thin-film coating (icariin-SH/BCP) [503]. The icariin-SH/BCP scaffold can promote the proliferation, migration, and osteogenic differentiation of osteoporotic rat BMSCs, while also upregulating the expression of angiogenic genes in HUVECs *in vitro* [503]. In addition, the OVX rat femoral condyle defect model demonstrated that the icariin-SH/BCP scaffold significantly enhanced bone regeneration in osteoporotic animals compared to the conventional BCP scaffold [503].

Table 2. Bone tissue engineering strategies for delivering core bioactive components of *Epimedium* in the treatment of bone defects.

Carriers	Active Constituents	Applications	Bone Functions	References
Hydrogel	Icariin	Icariin–zinc–aluminum layered double hydroxide nanosheets-GA-hydroxybutyl CTS hydrogel	Promoted periodontal tissue	[401]
		Poly(N-vinyl caprolactam)@icariin nanoparticles encapsulated in hydroxyethyl methacrylate-functionalized thermosensitive hydrogels	Treated critical bone and cartilage injuries	[402]
		Icariin-loaded 3D-Ti/CTS hydrogel	Promoted osteogenesis and osseointegration	[403]
		Icariin-loaded HA/CTS/Ti composite hydrogel	Improved osteoblast response and osseointegration	[404]
		Icariin-loaded CTS/hydroxyapatite composite hydrogel	Enhanced bone repair and regeneration	[405]
		Icariin/CTS/ β -glycerophosphate hydrogel	Controlled infection and repaired bone tissue	[406]
		Icariin-conditioned serum combined with thiolated CTS	Attenuated cartilage injury in rabbit knees with osteochondral defect	[407]
		Self-assembled injectable icariin@Ti ₃ C ₂ T _x /DOX sodium alginate hydrogel	Significantly improved osteosarcoma's therapeutic efficacy while offered a novel approach to mitigate the osteogenic inhibitory effects of DOX	[408]
	Icaritin	Injectable icaritin@Gelatin/Ac- β -CD hydrogel	Speeded up the in situ bone regeneration in a SAON animal model	[13]
		Icaritin/DOX-loaded PF127/PF68 hydrogel	Contributed to bone regeneration, offering significant potential for enhancing OS treatment outcomes and promoting healing of affected bone tissue	[409]
	Ginkgetin	Ginkgetin/rapamycin-loaded MMP13-responsive nanogel	Suppressed inflammation and rescued the blocked autophagic flux without inducing cell	[412]

			death, demonstrating a notable therapeutic effect in Intervertebral disc degeneration (IVDD)	
	Magnoflorine	A magnoflorine-loaded HA hydrogel	Enhanced subchondral bone regeneration and prevented osteoarthritis progression following anterior cruciate ligament rupture	[413,414]
	β -sitosterol	Gelatin/CTS/HA@Diatoms and β -sitosterol	Promoted osteoblast adhesion, proliferation, and mineralization	[415]
		Nanoclay-reinforced caffeic acid-modified CTS hydrogel	Regulated the Wnt/ β -catenin pathway, along with the addition of osteoinductive signals, resulting in bone regeneration in a non-healing cranial defect	[416]
	Caffeic acid	Caffeic acid/citric acid-functionalized poly (xylitol succinate)@magnesium oxide injectable hydrogel	Exhibited antagonistic properties against TLR4 and Myeloid differentiation factor 2 complex and reduced inflammatory cytokines levels and maintains subgingival microbial homeostasis	[417]
	Chlorogenic acid	Chlorogenic acid-loaded calcium phosphate/CTS porous nanogel	Degraded the biofilm and also be used in the restoration of tooth defects	[418]
	Neochlorogenic acid	A poly-methylacrylamide hydrogel encapsulating neochlorogenic acid and BMSCs	Mitigated oxidative stress and apoptosis, enhancing BMSC survival for IVDD repair	[419]
Organic scaffold	Icariin	Icariin-delivering porous poly (3-hydroxybutyric acid) scaffold	Regenerative repair of femoral head necrosis in rabbits	[420]
		Icariin-loaded 3D-printed PCL scaffolds	Improved the clinical outcome of alveolar cleft reconstruction	[421]

Inorganic scaffold	Gradient gelatin methacryloyl/polyethylene glycol diacrylate matrix scaffold encapsulating PolyApt@icariin and PolyApt@EGCG		Achieved perfect structural and functional regeneration in vivo	[422]
	Icariin-derived ECM-ornamented SIS scaffold		Exhibited superior osteoconductivity, enhanced cell adhesion, spreading, survival, and proliferation, while not compromising osteoinductivity	[423]
	Icariside II	Icariside II-loaded CPC scaffold	Promoted bone regeneration by enhancing the proliferation and survival of BMSCs in vivo	[432]
	Luteolin	Luteolin-loaded biocomposites containing tantalum and polyimide	Facilitated osteogenic differentiation and bone bonding	[433]
	Icariin	Sr-doped BCP/icariin	Enhanced osteogenesis by activating the Wnt/ β -catenin and BMP-2/Smad signaling pathways and attenuated osteoclast activity via suppression of the NF- κ B/p38 MAPK pathway	[434]
	Icaritin	Icaritin-functionalized β -TCP composite scaffold	Regenerative repair of femoral head necrosis in rabbits	[436]
	PLGA/TCP/icariin 3D-printed scaffold		Promoted bone formation	[446]
	A silk fibroin/icariin-loaded mesoporous bioactive glass nanoparticle composite scaffold		Enhanced the proliferation and osteogenic differentiation of BMSCs	[453]
	Icariin-loaded 45S5 bioactive glass/implanted rat ASCs		Enhanced the bone healing capacity	[454]
	Icariin	Icariin-loaded gelatin-coated 3D sponge-like scaffold/45S5 bioactive glass	Expanded icariin delivery capability and provided morphological flexibility for localized drug delivery	[455]
Organic-inorganic composite scaffold	Poly(lactide-co-glycolide)@icariin microspheres encapsulated in thiolated icariin-functionalized methacrylic anhydride-modified collagen/hydroxyapatite biomimetic scaffold		Modulated the immune microenvironment and activated the vascular-bone regeneration cascade	[456]

		An icariin-loaded silk fibroin/chitosan/nano-hydroxyapatite composite scaffold	Promoted the repair of critical-sized radial bone defects in rabbits	[457]
		Icariin-loaded hydroxyapatite/alginate porous composite scaffold	Enhanced bone regeneration	[458]
		Icariin/strontium-substituted mesoporous bioactive glass nanoparticles loaded 3D-printed PCL scaffold	Enhanced osteoblast proliferation and differentiation	[459]
		Icariin/zinc ion/curcumin metal–organic frameworks/hydroxyapatite@Functionalized decellularized mushroom stem scaffold with aligned channel structures	Controlled bacterial infection and promoted bone marrow mesenchymal stem cell migration and accelerated M2 macrophage polarization and osteogenic differentiation	[460]
	Icaritin	An icaritin-loaded PLGA/TCP scaffold	Enhanced bone defect repair in SAON	[447]
		A PLGA/TCP scaffold loaded with icaritin and HFM-SCs	Facilitated osteoporotic bone regeneration	[448]
		PLGA/ β -TCP/icaritin composite scaffolds	Enhanced osteogenic differentiation and mineralization in bone defect areas, leading to superior bone repair outcomes	[449,450]
		A PLGA/ β -TCP/icaritin composite scaffold incorporating $\text{Ti}_3\text{C}_2\text{T}_x$ MXene	Promoted osteogenesis through near-infrared-mediated mild photothermal therapy	[111]
		Icaritin-loaded PLGA/MXene microspheres on PLGA/ β -TCP scaffold	Enhanced bone regeneration	[451]
		A novel gelatin-conjugated caffeic acid-coated apatite/PLGA composite scaffold	Enhanced bone regeneration and inhibited the formation of fat-filled cysts	[452]
	Nanoparticles	Icariin@dopamine-poly (ethylene glycol)-coated porous hollow iron oxide	Promoted osteogenic differentiation and accelerated fracture healing	[461]
		Icariin/iron oxide nanoparticles@ coaxial electrospun nano-scaffold	Enhances the osteogenic performance of the scaffolds in vivo	[462]
		Icariin@MgO/MgCO ₃ /PLGA microspheres	Promoted new bone formation and enhanced osteogenic induction capacity	[464]

Icaritin	Icariin @ alendronate-modified 3-phosphoethanolamine-N-methoxy(polyethyleneglycol)-carboxyl erythrocyte membrane-coated poly(D,L-lactide) nanoparticles	Promoted both angiogenesis and osteogenesis in vitro	[466]
	Icariin@polydopamine-modified covalent organic frameworks	Ameliorated local inflammatory microenvironment and promoted osteogenic differentiation.	[467]
	Icaritin@Soluplus®/Poloxamer 407 micelles	Enhanced both the drug loading capacity and oral bioavailability of icaritin	[477]
	Icaritin@SO-deoxycholate self-assembled nanomicelle	Enhanced the ability to resist osteoporosis.	[483]
	Icaritin@Asp8-liposomes	Promoted bone formation and upregulated osteoblast function and inhibited osteoclast activity and prevents osteonecrosis	[487]
Caffeic acid	Caffeic acid/9-aminoacridine@mPEG-b-PCL	Inhibited articular cartilage deterioration and reduced disease severity in experimental inflammatory arthritis	[484]
Chlorogenic acid	Chlorogenic acid@Gold nanorods (AuNRs)	Inhibited osteosarcoma proliferation and stimulated osteogenic differentiation	[463]
	Chlorogenic acid@polyvinylpyrrolidone-modified PLGA-based drug delivery system	Scavenged ROS and disrupted the pathological cycle of inflammation-oxidative stress and inhibited alveolar bone resorption	[469]
	Calcium-chlorogenic acid nanoparticles	Promoted the differentiation of BMSCs into osteoblasts and induced macrophage polarization toward the M2 phenotype	[470]
	Berberine/chlorogenic acid supramolecular nano-agonist	Regulated osteogenic differentiation	[471]
Catechin	Catechin@pH-responsive HA-modified metal-organic framework	Delayed the progression of osteoarthritis	[468]
Luteolin	Luteolin/palmitoylethanolamide co-nanoparticles	Alleviated joint erosion	[472]

	Kaempferol	Kaempferol@HA-coated gelatin nanoparticles	Attenuated the progression of KOA	[473]
	Daidzein	Daidzein@CTS/chondroitin sulfate nano complex	Enhanced osteoblast formation and reduced osteoclast activity	[474]
Extracellular vesicles	Icariin	Bovine milk derived sEV@icariin	Treated bone defect and improved the osteogenic activity of icariin	[490]
		An adipose-derived stem cell exosome@icariin	Anti-rheumatoid arthritis and inhibition of macrophage function	[491]
		A hydrogel system capable of co-delivering BMSC-derived exosomes and icariin	Alleviated cartilage atrophy and maintained appropriate cartilage thickness	[492]
Bioactive coatings for implants	Icariin	Icariin-SH/BCP	Intended for the repair of bone defects in patients	[503]
	Quercitrin	Ti implant coated with Quercitrin	Reduced the expression of osteoclast functional markers, such as CTSK, H+ATPase, and MMP9, both in vivo and in vitro	[498]
		Quercitrin-functionalized Ti6Al4V orthopedic implant	Promoted the bone integration of orthopedic implants and prevented infections	[501]
	Chlorogenic acid	Chlorogenic acid/grafted peptide hydrogel-modified sulfonated PEEK surfaces	Promoted bone tissue repair and eliminated bacteria	[497]
	Caffeic Acid	Caffeic acid nanospheres and deferoxamine-based films@titanium implant surface	Alleviated iron overload and oxidative stress in postmenopausal osteoporosis and enhanced osseointegration	[500]
		Hexamethylenediamine-modified self-oxidized caffeic acid coating@Ti6Al4V alloy surface	Good cell compatibility and high ability to eliminate reactive oxygen species	[502]

5. Prospects

Although *Epimedium* has been widely used in clinical medicine for a long time and has undergone extensive scientific research, several challenges remain regarding its application in the field of bone tissue engineering. Firstly, studies on the functional and molecular mechanisms of *Epimedium* in regulating bone metabolism remain insufficiently systematic and comprehensive. For example, the cellular metabolic pathways of icaritin,

as well as the intestinal metabolism and tissue distribution of *Epimedium* in bone, have not been adequately investigated. Secondly, insufficient controllability of active ingredients: The composition of *Epimedium* extract is highly complex, and the concentration of its bioactive components is significantly influenced by factors such as geographical origin and extraction methodology, posing challenges to the standardization of its application in scaffold materials or bioinks. Thirdly, poor compatibility of delivery systems: Current delivery carriers exhibit limited sustained-release capacity for active substances, which fails to meet the dynamic demands of bone regeneration. Additionally, the precision of local microenvironment modulation remains inadequate. Fourthly, unclear cross-scale action mechanism: The multi-level mechanisms underlying the transition from molecular effects to tissue regeneration have not yet been fully elucidated, thereby hindering the synergistic design of materials, cells, and host responses. Fifthly, barriers to clinical translation: discrepancies between animal models and the human physiological microenvironment often result in inaccurate efficacy predictions. Furthermore, a robust quality control system for large-scale production is currently lacking.

The following strategic approaches are proposed to effectively overcome the aforementioned challenges. Firstly, researchers are encouraged to integrate single-cell RNA sequencing and spatial transcriptomics to map the spatiotemporal tissue distribution and specific cellular targets of *Epimedium* bioactive constituents, thereby revealing the high-resolution molecular regulatory mechanisms of bone metabolism. Secondly, develop smart systems responsive to stimuli: engineer hydrogels or nanocarriers sensitive to the microenvironment capable of release upon demand (e.g., triggered by pH, reactive oxygen species, or specific enzymes) to achieve precise spatiotemporal modulation during dynamic bone regeneration. Thirdly, biomimetic structural strategies: utilize 3D and 4D bioprinting technology to fabricate dynamic, morphologically adaptable scaffolds that not only mimic the topological architecture of the native bone extracellular matrix but also adapt spatiotemporally to progressive tissue remodeling, thereby enhancing cell recruitment and directed differentiation. Fourthly, AI-driven multi-omics optimization: leverage artificial intelligence algorithms and machine learning to integrate complex metabolomic and spatial omics datasets, constructing a quantitative relationship model among components, structures, and functions to precisely guide the standardization and optimization of protocols. Fifthly, innovative preclinical evaluation methodologies: develop microfluidic organoid platforms and humanized animal models to enable high-throughput, real-time monitoring, effectively bridging interspecies discrepancies and improving the predictive accuracy for clinical translation.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijms27219579/s1>.

Author Contributions: T.X. and R.Z.: formal analysis, investigation, software, writing—original draft, writing—review & editing. J.H., J.J., L.Q., S.D. and X.L.: visualization, writing—review & editing. W.Z.: conceptualization, funding acquisition, methodology, software. Z.Z.: conceptualization, methodology, supervision. K.X.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing—original draft, writing—review & editing. C.M.: conceptualization, funding acquisition, methodology, project administration, supervision, writing—original draft, writing—review & editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Key Project of Natural Science Research in Universities of Anhui Province (Nos. 2024AH050985, 2023AH050708, and 2024AH051031), the Project for Middle-aged and Young Teachers Cultivation in Universities of Anhui Province (No. YQYB2025005),

the National Natural Science Foundation of China (No. 82002272), the Project of High-Level Talents in Anhui University of Chinese Medicine (Nos. 2020rczd002 and DT2300001925), the University-Level Teaching Research Project of Anhui University of Chinese Medicine (No. 2024xjyy_zd036), and the College Students' Innovative Entrepreneurial Training Plan Program (Nos. S202610369050 and S202610369055). Figures 1, 3 and 5 were created using BioRender.com.

Data Availability Statement: The data are provided in the article and Supplementary Materials. The network pharmacology and molecular docking analyses used publicly available databases, as detailed in Section 3.10. Additional information is available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

VEGF	Vascular Endothelial Growth Factor
Ti	Titanium
PEEK	Polyetheretherketone
MSCs	Mesenchymal Stem Cells
MAPK	Mitogen-activated Protein Kinase
NF- κ B	Nuclear Factor kappa-B
RANKL	Receptor Activator of Nuclear Factor kappa-B ligand
TNF- α	Tumor Necrosis Factor-alpha
IL	Interleukin
OVX	Ovariectomy
BMSCs	Bone Marrow-derived MSCs
BMPs	Bone Morphogenetic Proteins
OPN	Osteopontin
RhoA	Ras Homolog Family Member A
ASCs	Adipose-derived Stem Cells
EphA2	Erythropoietin-producing Hepatocellular A2
ERK	Extracellular Signal-regulated Kinase
JNK	c-Jun N-terminal Kinase
CXCL12	C-X-C Motif Chemokine Ligand 12
ALP	Alkaline Phosphatase
ESR	Estrogen Receptor
ESR α	ESR alpha
PI3K	Phosphoinositide 3-Kinase
AKT	PI3K/Protein Kinase B
Smad	Drosophila Mothers Against Decapentaplegic Homolog
ROS	Reactive Oxygen Species
pGSK-3 β	p-Glycogen Synthase Kinase 3 β
FAK	Focal Adhesion Kinase
PPAR	Peroxisome Proliferator-activated Receptor
LPS	Lipopolysaccharide
Nrf2	Nuclear Factor Erythroid 2-related Factor 2
Saos-2 cells	Human Osteoblast-like Cells
OPG	Osteoprotegerin
Bcl-2	B-cell Lymphoma/Leukemia-2
Bax	Bcl-2-associated X Protein
mTOR	Mammalian Target of Rapamycin
AGEs	Advanced Glycation End Products
RAGE	Receptor for AGEs
hDPSCs	Human Dental Pulp Stem Cells
HO-1	Heme Oxygenase-1
HIF-1 α	Hypoxia-inducible Factor-1 α
TRAF6	Tumor Necrosis Factor Receptor-associated Factor 6

RANK	Receptor Activator of Nuclear Factor κ B
NFATc1	Nuclear Factor of Activated T cells c1
AP-1	Activator Protein 1
AMPK	AMP-activated Protein Kinase
TLR4	Toll-like Receptor 4
uPAR	urokinase-type Plasminogen Activator Receptor
c-Fos	Proto-Oncogene c-Fos
CTSK	Cathepsin K
TRAP	Tartrate-Resistant Acid Phosphatase
I κ B α	NF kappa B alpha
M-CSF	Macrophage Colony-stimulating Factor
TGF- β	Transforming Growth Factor- β
cAMP	Cyclic Adenosine Monophosphate
V-ATPase	Vacuolar H-adenosine Triphosphatase
ARE	Antioxidant Response Element
NLRP3	NOD-like Receptor Family Pyrin Domain-containing 3
ECM	Extracellular Matrix
Col	Collagen
SIRT1	Sirtuin 1
CIA	Collagen-Induced Arthritis
STAT3	Signal Transducer and Activator of Transcription 3
iNOS	inducible Nitric Oxide Synthase
NOX	Nicotinamide Adenine Dinucleotide Phosphate Oxidase
RA-FLS	Rheumatoid Arthritis Fibroblast-like Synoviocytes
3D	Three-Dimensional
NO	Nitric Oxide
eNOS	endothelial NOS
cGMP	cyclic Guanosine Monophosphate
HUVECs	Human Umbilical Vein Endothelial Cells
SAPK	Stress-activated Protein Kinase
KEGG	Kyoto Encyclopedia of Genes and Genomes
HER1	Human Epidermal Growth Factor Receptor 1
GnRH	Gonadotropin-releasing Hormone
Rap1	Ras-related Protein 1
F2	Coagulation Factor II
HSP90AA1	Heat Shock Protein 90kDa alpha A1
PRKACA	Protein Kinase cAMP-dependent Catalytic alpha
PRKCA	Protein Kinase C alpha
PTGS2	prostaglandin-endoperoxide synthase 2
PTPN1	Protein Tyrosine Phosphatase Non-Receptor Type 1
SERPINE1	Serpin Peptidase Inhibitor Clade E Member 1
TP53	Tumor Protein 53
CTNNB1	Catenin beta 1
HSP90AB1	Heat Shock Protein 90kDa alpha B1
CTS	Chitosan
HEMA	Hydroxyethyl Methacrylate
PNVCL	poly(N-vinyl caprolactam)
HA	Hyaluronic Acid
SA	Sodium Alginate
DOX	Doxorubicin
PF127	Pluronic F127
PF68	Pluronic F68
β -CD	β -cyclodextrin
PLA	Polylactic Acid
PCL	Polycaprolactone
EGCG	Epigallocatechin Gallate
SIS	Small Intestinal Submucosa
CPC	Calcium Phosphate Cement
Sr	Strontium

BCP	Biphasic Calcium Phosphate
β-TCP	β-tricalcium Phosphate
PLGA	poly (lactic-co-glycolic acid)
SAON	Steroid-associated Osteonecrosis
HFMSCs	Human Fetal Mesenchymal Stem Cells
SO	Sheep Oil
EVs	Extracellular Vesicles
sEVs	small EVs

References

- Roffi, A.; Matteo, B.; Krishnakumar, G.S.; Kon, E.; Filardo, G. Platelet-rich plasma for the treatment of bone defects: From pre-clinical rational to evidence in the clinical practice. A systematic review. *Int. Orthop.* **2017**, *41*, 221–237.
- Roffi, A.; Krishnakumar, G.S.; Gostynska, N.; Kon, E.; Candrian, C.; Filardo, G. The role of three-dimensional scaffolds in treating long bone defects: Evidence from preclinical and clinical literature—a systematic review. *Biomed. Res. Int.* **2017**, *2017*, 8074178.
- Xu, K.; Mu, C.; Zhang, C.; Deng, S.; Lin, S.; Zheng, L. Antioxidative and antibacterial gallium (III)-phenolic coating for enhanced osseointegration of titanium implants via pro-osteogenesis and inhibiting osteoclastogenesis. *Biomaterials* **2023**, *301*, 122268. Erratum in *Biomaterials* **2025**, *317*, 123099.
- Xia, Z.; Sun, X.; Mu, C.; Wang, K.; Ma, W.; Yang, W. An enhanced osseointegration of titanium implants by H₂S sustained-release coating via promoting osteogenesis and inhibiting osteoclastogenesis. *Adv. Healthc. Mater.* **2025**, *14*, e2403125.
- Chen, W.; Xie, G.; Lu, Y.; Wang, J.; Feng, B.; Wang, Q. An improved osseointegration of metal implants by pitavastatin loaded multilayer films with osteogenic and angiogenic properties. *Biomaterials* **2022**, *280*, 121260.
- Xu, Y.; Huang, R.; Shi, W.; Zhou, R.; Xie, X.; Wang, M. ROS-responsive hydrogel delivering METRNL enhances bone regeneration via dual stem cell homing and vasculogenesis activation. *Adv. Healthc. Mater.* **2025**, *14*, e2500060.
- Wang, L.; Wang, X.; Wu, J.; Chen, J.; He, Z.; Wang, J.; Zhang, X. Magnesium ions induce endothelial cell differentiation into tip cell and enhance vascularized bone regeneration. *Adv. Healthc. Mater.* **2025**, *14*, e2500274.
- Locquet, M.A.; Brahmi, M.; Blay, J.Y.; Dutour, A. Radiotherapy in bone sarcoma: The quest for better treatment option. *BMC Cancer* **2017**, *23*, 742.
- Isakoff, M.S.; Bielack, S.S.; Meltzer, P.; Gorlick, R. Osteosarcoma: Current treatment and a collaborative pathway to success. *J. Clin. Oncol.* **2015**, *33*, 3029–3035.
- Zhang, K.; Zhou, Y.; Xiao, C.; Zhao, W.; Wu, H.; Tang, J. Application of hydroxyapatite nanoparticles in tumor-associated bone segmental defect. *Sci. Adv.* **2019**, *5*, eaax6946.
- Aghajanian, P.; Mohan, S. The art of building bone: Emerging role of chondrocyte-to-osteoblast transdifferentiation in endochondral ossification. *Bone Res.* **2018**, *6*, 19.
- Qin, L.; Zhang, G.; Shi, Y.; Lee, K.; Leung, P. Prevention and treatment of osteoporosis with traditional herbal medicine. In *Current Topics in Osteoporosis*; Deng, H.W., Liu, Y.Z., Eds.; World Scientific: Singapore, 2005; pp. 513–531.
- Feng, Q.; Xu, J.; Zhang, K.; Yao, H.; Zheng, N.; Zheng, L.; Wang, J.; Wei, K.; Xiao, X.; Qin, L.; et al. Dynamic and cell-infiltratable hydrogels as injectable carrier of therapeutic cells and drugs for treating challenging bone defects. *ACS Cent. Sci.* **2019**, *5*, 440–450.
- Huang, J.; Yuan, L.; Wang, X.; Zhang, T.-L.; Wang, K. Icaritin and its glycosides enhance osteoblastic, but suppress osteoclastic, differentiation and activity in vitro. *Life Sci.* **2007**, *81*, 832–840.
- Tan, E.M.; Li, L.; Indran, I.R.; Chew, N.; Yong, E.-L. TRAF6 mediates suppression of osteoclastogenesis and prevention of ovariectomy-induced bone loss by a novel prenylflavonoid. *J. Bone Miner. Res.* **2017**, *32*, 846–860.
- Meng, F.-H.; Li, Y.-B.; Xiong, Z.-L.; Jiang, Z.-M.; Li, F.-M. Osteoblastic proliferative activity of *Epimedium brevicornum* Maxim. *Phytomedicine* **2005**, *12*, 189–193.
- Wang, L.; Li, Y.; Guo, Y.; Ma, R.; Fu, M.; Niu, J.; Gao, S.; Zhang, D. Herba Epimedii: An ancient Chinese herbal medicine in the prevention and treatment of osteoporosis. *Curr. Pharm. Des.* **2016**, *22*, 328–349.
- Ye, Y.; Jing, X.; Li, N.; Wu, Y.; Li, B.; Xu, T. Icaritin promotes proliferation and osteogenic differentiation of rat adipose-derived stem cells by activating the RhoA-TAZ signaling pathway. *Biomed. Pharmacother.* **2017**, *88*, 384–394.
- Si, Y.; Li, Y.; Gu, K.; Yin, H.; Ma, Y. Icaritin ameliorates osteoporosis in ovariectomized rats by targeting Cullin 3/Nrf2/OH pathway for osteoclast inhibition. *Biomed. Pharmacother.* **2024**, *173*, 116422.
- Peng, Z.; Zhang, W.; Hong, H.; Liu, L. Effect of luteolin on oxidative stress and inflammation in the human osteoblast cell line hFOB1.19 in an inflammatory microenvironment. *BMC Pharmacol. Toxicol.* **2024**, *25*, 40.

21. Speisky, H.; Arias-Santé, M.F.; Fuentes, J. Oxidation of quercetin and kaempferol markedly amplifies their antioxidant, cyto-protective, and anti-inflammatory properties. *Antioxidants* **2023**, *12*, 155.
22. Liu, J.; Li, D.; Sun, X.; Wang, Y.; Xiao, Q.; Chen, A. Icariin restores LPS-induced bone loss by downregulating miR-34c level. *Inflammation* **2016**, *39*, 1764–1770.
23. Bi, Z.; Zhang, W.; Yan, X. Anti-inflammatory and immunoregulatory effects of icariin and icaritin. *Biomed. Pharmacother.* **2022**, *151*, 113180.
24. Cao, Y. Icariin alleviates MSU-induced rat GA models through NF- κ B/NALP3 pathway. *Cell Biochem. Funct.* **2021**, *39*, 357–366.
25. Lin, C.-C.; Chuang, Y.-J.; Yu, C.-C.; Yang, J.-S.; Lu, C.-C.; Chiang, J.-H.; Lin, J.-P.; Tang, N.-Y.; Huang, A.-C.; Chung, J.-G. Apigenin induces apoptosis through mitochondrial dysfunction in U-2 OS human osteosarcoma cells and inhibits osteosarcoma xenograft tumor growth in vivo. *J. Agric. Food Chem.* **2012**, *60*, 11395–11402.
26. Meng, J.; Zhou, C.; Zhang, W.; Wang, W.; He, B.; Hu, B.; Jiang, G.; Wang, Y.; Hong, J.; Li, S.; et al. Stachydrine prevents LPS-induced bone loss by inhibiting osteoclastogenesis via NF- κ B and Akt signalling. *J. Cell. Mol. Med.* **2019**, *23*, 6730–6743.
27. Choi, H.J.; Eun, J.-S.; Kim, D.K.; Li, R.H.; Shin, T.-Y.; Park, H.; Cho, N.-P.; Soh, Y. Icariside II from *Epimedium koreanum* inhibits hypoxia-inducible factor-1 α in human osteosarcoma cells. *Eur. J. Pharmacol.* **2008**, *579*, 58–65.
28. Zhang, N.; Ying, M.-D.; Wu, Y.-P.; Zhou, Z.-H.; Ye, Z.-M.; Li, H.; Lin, D.-S. Hyperoside, a flavonoid compound, inhibits proliferation and stimulates osteogenic differentiation of human osteosarcoma cells. *PLoS ONE* **2014**, *9*, e98973.
29. Xu, H.-B.; Huang, Z.-Q. Vasorelaxant effects of icariin on isolated canine coronary artery. *J. Cardiovasc. Pharmacol.* **2007**, *49*, 207–213.
30. Yao, W.; Tao, R.; Wang, K.; Ding, X. Icariin attenuates vascular endothelial dysfunction by inhibiting inflammation through GPER/Sirt1/HMGB1 signaling pathway in type 1 diabetic rats. *Chin. J. Nat. Med.* **2024**, *22*, 293–306.
31. Lee, M.; Lim, S.W.; Yang, H.; Sung, S.H.; Lee, H.-S.; Park, M.J.; Kim, Y.C. Osteoblast differentiation stimulating activity of biflavonoids from *Cephalotaxus koreana*. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2850–2854.
32. Zhang, X.; Guo, Y.; Li, D.; Wang, R.; Fan, H.; Xiao, Y. The effect of loading icariin on biocompatibility and bioactivity of porous β -TCP ceramic. *J. Mater. Sci. Mater. Med.* **2011**, *22*, 371–379.
33. Akai, S. Constituents of *Epimedium macranthum* Morr and Decne I. Chemical constitution of a new glucoside of *Epimedium macranthum* Morr and Decne I. *J. Pharm. Soc. Jpn.* **1935**, *55*, 537–599.
34. Chen, X.-L.; Li, S.-X.; Ge, T.; Zhang, D.-D.; Wang, H.-F.; Wang, W.; Li, Y.-Z.; Song, X.-M. *Epimedium* Linn: A comprehensive review of phytochemistry, pharmacology, clinical applications and quality control. *Chem. Biodivers.* **2024**, *21*, e202400846.
35. Sandhar, H.K.; Kumar, B.; Prasher, S.; Tiwari, P.; Salhan, M.; Sharma, P. A review of phytochemistry and pharmacology of flavonoids. *Int. Pharm. Sci.* **2011**, *1*, 25–41.
36. Ma, H.; He, X.; Yang, Y.; Li, M.; Hao, D.; Jia, Z. The genus *Epimedium*: An ethnopharmacological and phytochemical review. *J. Ethnopharmacol.* **2011**, *134*, 519–541.
37. Zhang, H.; Wang, Y.; Liang, Y.; Zhao, X.; Chen, G. One new prenylated flavonoid with cytotoxic activity from *Epimedium brevicornu* Maxim. *Nat. Prod. Res.* **2025**, *39*, 981–984.
38. Xu, Y.; Li, Z.; Yuan, L.; Zhang, X.; Lu, D.; Huang, H.; Wang, Y. Variation of epimedins A-C and icariin in ten representative populations of *Epimedium brevicornu* Maxim., and implications for utilization. *Chem. Biodivers.* **2013**, *10*, 711–721.
39. Lu, Y.; Luo, Q.; Jia, X.; Tam, J.P.; Yang, H.; Shen, Y.; Li, X. Multidisciplinary strategies to enhance therapeutic effects of flavonoids from *Epimedium Folium*: Integration of herbal medicine, enzyme engineering, and nanotechnology. *J. Pharm. Anal.* **2023**, *13*, 239–254.
40. Chen, X.J.; Ji, H.; Zhang, Q.W.; Tu, P.F.; Wang, Y.T.; Guo, B.L.; Li, S.P. A rapid method for simultaneous determination of 15 flavonoids in *Epimedium* using pressurized liquid extraction and ultra-performance liquid chromatography. *J. Pharm. Biomed. Anal.* **2008**, *46*, 226–235.
41. He, C.; Wang, Z.; Shi, J. Pharmacological effects of icariin. *Adv. Pharmacol.* **2020**, *87*, 179–203.
42. Li, B.; Lima, M.R.M.; Nie, Y.; Xu, L.; Liu, X.; Yuan, H.; Chen, C.; Dias, A.C.P.; Zhang, X. HPLC-DAD fingerprints combined with multivariate analysis of *Epimedium folium* from major producing areas in eastern asia: Effect of geographical origin and species. *Front. Pharmacol.* **2021**, *12*, 761551.
43. He, L.; Huang, N.; Yan, Y.; Lin, W.; Gao, L.; Lin, X. Identification and determination of components in five kinds of Yinyanghuo. *Clin. J. Chin. Med.* **2019**, *11*, 139–144.
44. Li, C.; Li, Q.; Mei, Q.; Lu, T. Pharmacological effects and pharmacokinetic properties of icariin, the major bioactive component in *Herba Epimedium*. *Life Sci.* **2015**, *126*, 57–68.

45. Oprita, E.I.; Iosageanu, A.; Craciunescu, O. Progress in composite hydrogels and scaffolds enriched with icariin for osteochondral defect healing. *Gels* **2022**, *8*, 648.
46. Ma, H.-P.; Ming, L.-G.; Ge, B.-F.; Zhai, Y.-K.; Song, P.; Xian, C.J.; Chen, K.-M. Icariin is more potent than genistein in promoting osteoblast differentiation and mineralization in vitro. *J. Cell Biochem.* **2011**, *112*, 916–923.
47. Lin, Y.; Chen, W.; Ding, B.; Guo, M.; Liang, M.; Pang, H.; Wei, Y.; Huang, R.; Du, L. Highly efficient bioconversion of icariin to icaritin by whole-cell catalysis. *Microb. Cell Fact.* **2023**, *22*, 64.
48. Zhang, S.; Luo, J.; Dong, Y.; Wang, Z.; Xiao, W.; Zhao, L. Biotransformation of the total flavonoid extract of *Epimedium* into icaritin by two thermostable glycosidases from *Dictyoglomus thermophilum* DSM3960. *Process Biochem.* **2021**, *105*, 8–18.
49. Gao, L.; Zhang, S.-Q. Antiosteoporosis effects, pharmacokinetics, and drug delivery systems of icaritin: Advances and prospects. *Pharmaceuticals* **2022**, *15*, 397.
50. Zhang, S.Q. Ultra-high performance liquid chromatography-tandem mass spectrometry for the quantification of icaritin in mouse bone. *J. Chromatogr. B* **2015**, *978*, 24–28.
51. Zhang, S.-Q.; Zhang, S.-Z. Oral absorption, distribution, metabolism, and excretion of icaritin in rats by Q-TOF and UHPLC-MS/MS. *Drug Test. Anal.* **2017**, *9*, 1604–1610.
52. Qin, L.; Zhang, G.; Sheng, H.; Wang, X.L.; Wang, Y.X.; Yeung, K.W.; Griffith, J.F.; Li, Z.R.; Leung, K.S.; Yao, X.S. Phytoestrogenic compounds for prevention of steroid-associated osteonecrosis. *J. Musculoskelet. Neuronal Interact.* **2008**, *8*, 18–21.
53. Huang, J.-M.; Wang, Z.; Qi, G.-B.; Lai, Q.; Jiang, A.; Zhang, Y.-Q.; Chen, K.; Wang, X.-H. Icaritin ameliorates RANKL-mediated osteoclastogenesis and ovariectomy-induced osteoporosis. *Aging* **2023**, *15*, 10213.
54. Yang, X.; Lang, S.; Li, S.; Jiang, C.; Han, J. Preparation of icariside I and icariside II, an exploration of their protective mechanism against cyclophosphamide-induced bone marrow suppression in mice, and their regulatory effects on immune function. *Pharmazie* **2022**, *77*, 32–37.
55. Guo, Y.; Wang, X.; Gao, J. Simultaneous preparation and comparison of the osteogenic effects of epimedins A-C and icariin from *Epimedium brevicornu*. *Chem. Biodivers.* **2018**, *15*, e1700578.
56. Chen, X.J.; Guo, B.L.; Li, S.P.; Zhang, Q.W.; Tu, P.F.; Wang, Y.T. Simultaneous determination of 15 flavonoids in *Epimedium* using pressurized liquid extraction and high-performance liquid chromatography. *J. Chromatogr. A* **2007**, *1163*, 96–104.
57. Chen, X.P.; Qian, H.; Wu, J.J.; Ma, X.W.; Gu, Z.X.; Sun, H.Y.; Duan, Y.-Z.; Jin, Z.-L. Expression of vascular endothelial growth factor in cultured human dental follicle cells and its biological roles. *Acta Pharmacol. Sin.* **2007**, *28*, 985–993.
58. Lee, C.-J.; Wu, Y.-T.; Hsueh, T.Y.; Lin, L.-C.; Tsai, T.-H. Pharmacokinetics and oral bioavailability of epimedin C after oral administration of epimedin C and Herba Epimedii extract in rats. *Biomed. Chromatogr.* **2014**, *28*, 630–636.
59. Islam, M.N.; Kim, U.; Kim, D.-H.; Dong, M.-S.; Yoo, H.H. High-performance liquid chromatography-based multivariate analysis to predict the estrogenic activity of an *Epimedium koreanum* extract. *Biosci. Biotechnol. Biochem.* **2012**, *76*, 923–927.
60. Zhang, H.; Wang, H.; Wei, J.; Chen, X.; Sun, M.; Ouyang, H.; Hao, J.; Chang, Y.; Dou, Z.; He, J. Comparison of the active compositions between raw and processed *Epimedium* from different species. *Molecules* **2018**, *23*, 1656.
61. Wang, K.; Zhang, H.; Yuan, L.; Li, X.; Cai, Y. Potential implications of hyperoside on oxidative stress-induced human diseases: A comprehensive review. *J. Inflamm. Res.* **2023**, *16*, 4503–4526.
62. Xu, F.; Ding, Y.; Guo, Y.; Liu, B.; Kou, Z.; Xiao, W.; Zhu, J. Anti-osteoporosis effect of *Epimedium* via an estrogen-like mechanism based on a system-level approach. *J. Ethnopharmacol.* **2016**, *177*, 148–160.
63. Shen, P.; Guo, B.L.; Gong, Y.; Hong, D.Y.Q.; Hong, Y.; Yong, E.L. Taxonomic, genetic, chemical and estrogenic characteristics of *Epimedium* species. *Phytochemistry* **2007**, *68*, 1448–1458.
64. Seelinger, G.; Merfort, I.; Schempp, C.M. Anti-oxidant, anti-inflammatory and anti-allergic activities of luteolin. *Planta Med.* **2008**, *74*, 1667–1677.
65. Chi, A.; Shen, Z.; Zhu, W.; Sun, Y.; Kang, Y.; Guo, F. Characterization of a protein-bound polysaccharide from Herba Epimedii and its metabolic mechanism in chronic fatigue syndrome. *J. Ethnopharmacol.* **2017**, *203*, 241–251.
66. Cheng, H.; Feng, S.; Jia, X.; Li, Q.; Zhou, Y.; Ding, C. Structural characterization and antioxidant activities of polysaccharides extracted from *Epimedium acuminatum*. *Carbohydr. Polym.* **2013**, *92*, 63–68.
67. Wu, Y.; Li, Y.; Liu, C.; Li, E.; Gao, Z.; Liu, C.; Gu, W.; Huang, Y.; Liu, J.; Wang, D.; et al. Structural characterization of an acidic *Epimedium* polysaccharide and its immune-enhancement activity. *Carbohydr. Polym.* **2016**, *138*, 134–142.
68. Xu, L.; Zhang, Y.; Wang, L. Structure characteristics of a water-soluble polysaccharide purified from dragon fruit (*Hylocereus undatus*) pulp. *Carbohydr. Polym.* **2016**, *146*, 224–230.
69. Li, C.; Chen, F.-F.; Jia, X.-B.; Feng, L.; Tan, X.-B. Effects and mechanism of *Epimedium* polysaccharide on solubility of icariin and baohuoside I. *Zhongguo Zhong Yao Za Zhi* **2021**, *46*, 5825–5831.

70. Li, N.; Xie, L.; Yang, N.; Sun, G.; Liu, H.; Bi, C.; Duan, J.; Yuan, Y.; Yu, H.; Xu, Y.; et al. Rapid classification and identification of chemical constituents in *Epimedium koreanum* Nakai by UPLC-Q-TOF-MS combined with data post-processing techniques. *Phytochem. Anal.* **2021**, *32*, 575–591.
71. Tang, X.; Dai, Z.; Wu, Q.; Zeng, J.; Gao, M.; Xiao, H.; Yao, Z.; Dai, Y.; Yao, X. Simultaneous determination of multiple components in rat plasma and pharmacokinetic studies at a pharmacodynamic dose of Xian-Ling-Gu-Bao capsule by UPLC-MS/MS. *J. Pharm. Biomed. Anal.* **2020**, *177*, 112836.
72. Kakkar, S.; Bais, S. A review on protocatechuic acid and its pharmacological potential. *ISRN Pharmacol.* **2024**, *2014*, 952943.
73. Li, W.K.; Xiao, P.G.; Zhang, R.Y. Chemical constituents from the aerial parts of *Epimedium koreanum* Nakai (II). *Nat. Prod. Res. Dev.* **1994**, *6*, 12–18.
74. Fikry, E.M.; Gad, A.M.; Eid, A.H.; Arab, H.H. Caffeic acid and ellagic acid ameliorate adjuvant-induced arthritis in rats via targeting inflammatory signals, chitinase-3-like protein-1 and angiogenesis. *Biomed. Pharmacother.* **2019**, *110*, 878–886.
75. Vari, R.; D'Archivio, M.; Filesi, C.; Carotenuto, S.; Scazzocchio, B.; Santangelo, C.; Giovannini, C.; Masella, R. Protocatechuic acid induces antioxidant/detoxifying enzyme expression through JNK-mediated Nrf2 activation in murine macrophages. *J. Nutr. Biochem.* **2011**, *22*, 409–417.
76. Zhu, J.-J.; Jiang, J.-G. Pharmacological and nutritional effects of natural coumarins and their structure-activity relationships. *Mol. Nutr. Food Res.* **2018**, *62*, 1701073.
77. Xue, J.; Chen, H.; Zhou, Y.; Yuan, J.; Cai, Z.; Wu, N.; Chen, C.; Zou, L.; Yin, S.; Yang, W.; et al. Constituent analysis and quality evaluation of *Epimedium* Folium from different species. *J. Chem.* **2023**, *2023*, 4221158.
78. Zeng, X.; Shao, J.; Pan, D.; Zeng, S.; Wang, Z.; Yao, X.; Li, H.; Xiao, W.; Yu, Y. Quantitative profiling and mechanisms exploration of *Epimedium* total flavonoid capsules in neuroinflammation: An integrated study of pharmacokinetics, network pharmacology, and molecular pathways. *Arab. J. Chem.* **2025**, *18*, 106084.
79. Teo, Y.L.; Cheong, W.F.; Cazenave-Gassiot, A.; Ji, S.; Logan, S.; Lee, Z.X.K.; Li, J.; Seng, K.Y.; Lee, L.S.-U.; Yong, E.L. Pharmacokinetics of prenylflavonoids following oral ingestion of standardized *Epimedium* extract in humans. *Planta Med.* **2019**, *85*, 347–355.
80. Cheng, Q.; Yang, S.; Li, S.; Xiao, M.; Yang, J.; Li, Y.; Cheng, C. Prioritization of germplasm resources for *Epimedium* breeding: Chemotype oriented discovery using cross latitude scale resource evaluation. *Ind. Crops Prod.* **2025**, *238*, 122365.
81. Cheng, T.; Zhang, Y.; Zhang, T.; Lu, L.; Ding, Y.; Zhao, Y. Comparative pharmacokinetics study of icariin and icariside II in rats. *Molecules* **2015**, *20*, 21274–21286.
82. Zhang, L.-B.; Yan, Y.; He, J.; Wang, P.-P.; Chen, X.; Lan, T.-Y.; Guo, Y.-X.; Wang, J.-P.; Luo, J.; Yan, Z.-R.; et al. *Epimedium* Herba: An ancient Chinese herbal medicine in the prevention and treatment of rheumatoid arthritis. *Front. Chem.* **2022**, *10*, 1023779.
83. Sun, M.; Yin, Y.; Wei, J.; Chen, X.; Ouyang, H.; Chang, Y.; Gao, X.; He, J. Development and validation of a HPLC-MS/MS method for simultaneous determination of twelve bioactive compounds in *Epimedium*: Application to a pharmacokinetic study in rats. *Molecules* **2018**, *23*, 1322.
84. Zhang, H.-F.; Yang, T.-S.; Li, Z.-Z.; Wang, Y. Simultaneous extraction of epimedin A, B, C and icariin from *Herba Epimedium* by ultrasonic technique. *Ultrason. Sonochem.* **2008**, *15*, 376–385.
85. Wei, G.; Liang, X.; Wu, F.; Cheng, C.; Huang, S.; Zeng, Y. Ginkgetin attenuates bone loss in OVX mice by inhibiting the NF- κ B/I κ B α signaling pathway. *PeerJ* **2024**, *12*, e17722.
86. Sun, P.; Chen, Y.; Shimizu, N.; Takeda, T. Studies on the constituents of *Epimedium koreanum*. III. *Chem. Pharm. Bull.* **1998**, *46*, 355–358.
87. Hougee, S.; Sanders, A.; Faber, J.; Graus, Y.M.F.; van den Berg, W.B.; Garssen, J.; Smit, H.F.; Hoijer, M.A. Decreased pro-inflammatory cytokine production by LPS-stimulated PBMC upon in vitro incubation with the flavonoids apigenin, luteolin or chrysin, due to selective elimination of monocytes/macrophages. *Biochem. Pharmacol.* **2005**, *69*, 241–248.
88. Chai, S.-W.; Liu, F. Isolation and identification of chemical constituents from *Epimedium brevicornu* Maxim. *Tianjin J. Tradit. Chin. Med.* **2016**, *33*, 114–117.
89. Gao, M.; Fu, C.; Liu, J.; Guo, B.; Yang, X.; Chen, D.; Li, L. Distribution and influencing factors of magnoflorine in *Epimedium*. *Zhongguo Zhong Yao Za Zhi* **2011**, *36*, 2475–2478.
90. Sun, Y.; Gao, L.; Hou, W.; Wu, J. β -Sitosterol alleviates inflammatory response via inhibiting the activation of ERK/p38 and NF- κ B pathways in LPS-exposed BV2 Cells. *Biomed. Res. Int.* **2020**, *2020*, 7532306.
91. Blair, H.C.; Larrouture, Q.C.; Li, Y.; Lin, H.; Beer-Stoltz, D.; Liu, L.; Tuan, R.S.; Robinson, L.J.; Schlesinger, P.H.; Nelson, D.J. Osteoblast differentiation and bone matrix formation in vivo and in vitro. *Tissue Eng. Part B Rev.* **2017**, *23*, 268–280.

92. Zhu, S.; Chen, W.; Masson, A.; Li, Y.-P. Cell signaling and transcriptional regulation of osteoblast lineage commitment, differentiation, bone formation, and homeostasis. *Cell Discov.* **2024**, *10*, 71.
93. Xu, K.; Chen, W.; Mu, C.; Yu, Y.; Cai, K. Strontium folic acid derivative functionalized titanium surfaces for enhanced osteogenic differentiation of mesenchymal stem cells in vitro and bone formation in vivo. *J. Mater. Chem. B* **2017**, *5*, 6811–6826.
94. Xu, K.; Chen, W.; Fu, G.; Mou, X.; Hou, R.; Zhu, Y.; Cai, K. In situ self-assembly of graphene oxide/polydopamine/Sr²⁺ nanosheets on titanium surfaces for enhanced osteogenic differentiation of mesenchymal stem cells. *Carbon* **2019**, *142*, 567–579.
95. Jiao, F.; Tang, W.; Huang, H.; Zhang, Z.; Liu, D.; Zhang, H.; Ren, H. Icariin promotes the migration of BMSCs in vitro and in vivo via the MAPK signaling pathway. *Stem Cells Int.* **2018**, *2018*, 2562105.
96. Tang, D.; Ju, C.; Liu, Y.; Xu, F.; Wang, Z.; Wang, D. Therapeutic effect of icariin combined with stem cells on postmenopausal osteoporosis in rats. *J. Bone Miner. Metab.* **2018**, *36*, 180–188.
97. Wang, W.; Sun, D.-F.; Dong, Z.; Zhang, W.-L. Icariin suppresses osteogenic differentiation and promotes bone regeneration in *Porphyromonas gingivalis*-infected conditions through EphA2-RhoA signaling pathway. *Int. Immunopharmacol.* **2024**, *143*, 113302.
98. Ahmed-Belkacem, A.; Pozza, A.; Munoz-Martinez, F.; Bates, S.E.; Castanys, S.; Gamarro, F.; Di Pietro, A.; Perez-Victoria, J.M. Flavonoid structure-activity studies identify 6-prenylchrysin and tectochrysin as potent and specific inhibitors of breast cancer resistance protein ABCG2. *Cancer Res.* **2005**, *65*, 4852–4860.
99. Zhang, X.; Zhou, C.; Zha, X.; Xu, Z.; Li, L.; Liu, Y.; Xu, L.; Cui, L.; Xu, D.; Zhu, B. Apigenin promotes osteogenic differentiation of human mesenchymal stem cells through JNK and p38 MAPK pathways. *Mol. Cell Biochem.* **2015**, *407*, 41–50.
100. Sheng, H.; Rui, X.; Sheng, C.-J.; Li, W.-J.; Cheng, X.-Y.; Jhummon, N.P.; Yu, Y.-C.; Qu, S.; Zhang, G.; Qin, L. A novel semisynthetic molecule icaritin stimulates osteogenic differentiation and inhibits adipogenesis of mesenchymal stem cells. *Int. J. Med. Sci.* **2013**, *10*, 782.
101. Xu, T.; Wu, X.; Zhou, Z.; Ye, Y.; Yan, C.; Zhuge, N.; Yu, J. Hyperoside ameliorates periodontitis in rats by promoting osteogenic differentiation of BMSCs via activation of the NF- κ B pathway. *FEBS Open Bio* **2020**, *10*, 1843–1855.
102. Liu, H.; Yi, X.; Tu, S.; Cheng, C.; Luo, J. Kaempferol promotes BMSC osteogenic differentiation and improves osteoporosis by downregulating miR-10a-3p and upregulating CXCL12. *Mol. Cell Endocrinol.* **2021**, *520*, 111074.
103. Li, Y.; Wang, Y.; Liu, Q.; Lv, S.; Wang, Y.; Zhang, H.; Zhao, Q.; Shang, L. Kaempferol promotes osteogenic differentiation in bone marrow mesenchymal stem cells by inhibiting CAV-1. *J. Orthop. Surg. Res.* **2024**, *19*, 678.
104. Gómez-Florit, M.; Monjo, M.; Ramis, J.M. Quercitrin for periodontal regeneration: Effects on human gingival fibroblasts and mesenchymal stem cells. *Sci. Rep.* **2015**, *5*, 16593.
105. Napoli, N.; Schwartz, A.V.; Palermo, L.; Jin, J.J.; Wustrack, R.; Cauley, J.A.; Ensrud, K.E.; Kelly, M.; Black, D.M. Risk factors for subtrochanteric and diaphyseal fractures: The study of osteoporotic fractures. *J. Clin. Endocrinol. Metab.* **2013**, *98*, 659–667.
106. Wang, B.Q.; Yang, Y.Q.; Xie, H.F.; Xue, C.Y. Matrix stiffness regulates BMSCs osteogenic differentiation through an autophagy-dependent manner. *Eur. Cell Mater.* **2024**, *47*, 253–264.
107. Rosen, C.J.; Bouxsein, M.L. Mechanisms of disease: Is osteoporosis the obesity of bone? *Nat. Clin. Pract. Rheumatol.* **2006**, *2*, 35–43.
108. Ho, M.-X.; Poon, C.C.-W.; Wong, K.-C.; Qiu, Z.-C.; Wong, M.-S. Icariin, but not genistein, exerts osteogenic and anti-apoptotic effects in osteoblastic cells by selective activation of non-genomic ER α signaling. *Front. Pharmacol.* **2018**, *9*, 474.
109. Luo, Z.; Liu, M.; Sun, L.; Rui, F. Icariin recovers the osteogenic differentiation and bone formation of bone marrow stromal cells from a rat model of estrogen deficiency-induced osteoporosis. *Mol. Med. Rep.* **2015**, *12*, 382–388.
110. Zhang, G.; Qin, L.; Sheng, H.; Yeung, K.W.; Yeung, H.Y.; Cheung, W.H.; Griffith, J.; Chan, C.W.; Lee, K.M.; Leung, K.S. *Epimedium*-derived phytoestrogen exert beneficial effect on preventing steroid-associated osteonecrosis in rabbits with inhibition of both thrombosis and lipid-deposition. *Bone* **2007**, *40*, 685–692.
111. Chen, H.; Xi, H.; Guo, M.; Tan, X.; Xue, P.; He, S.; Sun, G.; Huang, Y.; Jiang, X.; Du, B.; et al. PLGA/ β -TCP/ICT composite scaffold incorporating MXene (Ti₃C₂T_x) promotes osteogenesis through near-infrared-mediated mild photothermal therapy. *Mater. Des.* **2024**, *244*, 113083.
112. Xue, P.; Chen, H.; Xi, H.; He, S.; Sun, G.; Chang, Z.; Tan, X.; Liu, X.; Du, B. Magnesium doped calcium-fluoride/icaritin composite multi-layer coating functionalized 3D printed β -TCP scaffold induces sustained bone regeneration in a rabbit model. *Mater. Des.* **2022**, *223*, 111156.
113. Yao, D.; Xie, X.-H.; Wang, X.-L.; Wan, C.; Lee, Y.-W.; Chen, S.-H.; Pei, D.-Q.; Wang, Y.-X.; Li, G.; Qin, L. Icaritin, an exogenous phytomolecule, enhances osteogenesis but not angiogenesis-an in vitro efficacy study. *PLoS ONE* **2012**, *7*, e41264.
114. Derby, B. Printing and prototyping of tissues and scaffolds. *Science* **2012**, *338*, 921–926.

115. Luo, S.; Chen, J.; Zhong, Z.; Lv, X.; Yang, Y.; Zhang, J.; Cui, L. Salvianolic acid B stimulates osteogenesis in dexamethasone-treated zebrafish larvae. *Acta Pharmacol. Sin.* **2016**, *37*, 1370–1380.
116. Wang, X.; Zhang, G.; Xie, X.; Sheng, H.; Qin, L.; Yao, X. Antagonistic estrogen receptor beta signaling required in promoting peri-osteum bone formation and inhibiting osteoclastic bone resorption by Icaritin-A novel semi-synthetic small molecule. *Bone* **2008**, *43*, S115.
117. Zhang, G.; Wang, X.-L.; Sheng, H.; Xie, X.-H.; He, Y.-X.; Yao, X.-S.; Li, Z.-R.; Lee, K.-M.; He, W.; Leung, K.-S.; et al. Constitutional flavonoids derived from *Epimedium* dose-dependently reduce incidence of steroid-associated osteonecrosis not via direct action by themselves on potential cellular targets. *PLoS ONE* **2009**, *4*, e6419.
118. Xue, Y.; Wang, P.; Qi, Q. The experimental study of the effects of icariin on increasing smad4 mRNA level in MC3T3-E1 cell in vitro. *Chin. J. Orthop.* **2005**, *25*, 119–123.
119. Zhao, J.; Ohba, S.; Shinkai, M.; Chung, U.; Nagamune, T. Icariin induces osteogenic differentiation in vitro in a BMP-and Runx2-dependent manner. *Biochem. Biophys. Res. Commun.* **2008**, *369*, 444–448.
120. Hsieh, T.-P.; Sheu, S.-Y.; Sun, J.-S.; Chen, M.-H.; Liu, M.-H. Icariin isolated from *Epimedium pubescens* regulates osteoblasts anabolism through BMP-2, SMAD4, and Cbfa1 expression. *Phytomedicine* **2010**, *17*, 414–423.
121. Zhang, X.; Chen, Y.; Zhang, C.; Zhang, X.; Xia, T.; Han, J.; Song, S.; Xu, C.; Chen, F. Effects of icariin on the fracture healing in young and old rats and its mechanism. *Pharm. Biol.* **2021**, *59*, 1243–1253.
122. Hu, J.; Mao, Z.; He, S.; Zhan, Y.; Ning, R.; Liu, W.; Yan, B.; Yang, J. Icariin protects against glucocorticoid induced osteoporosis, increases the expression of the bone enhancer DEC1 and modulates the PI3K/Akt/GSK3 β / β -catenin integrated signaling pathway. *Biochem. Pharmacol.* **2017**, *136*, 109–121.
123. Wu, J.-Z.; Liu, P.-C.; Liu, R.; Cai, M. Icariin restores bone structure and strength in a rat model of chronic high-dose alcohol-induced osteopenia. *Cell Physiol. Biochem.* **2018**, *46*, 1727–1736.
124. Yuan, X.; Serra, R.A.; Yang, S. Function and regulation of primary cilia and intraflagellar transport proteins in the skeleton. *Ann. N. Y. Acad. Sci.* **2015**, *1335*, 78–99.
125. Chinipardaz, Z.; Liu, M.; Graves, D.T.; Yang, S. Role of primary cilia in bone and cartilage. *J. Dent. Res.* **2022**, *101*, 253–260.
126. Kawata, K.; Narita, K.; Washio, A.; Kitamura, C.; Nishihara, T.; Kubota, S.; Takeda, S. Odontoblast differentiation is regulated by an interplay between primary cilia and the canonical Wnt pathway. *Bone* **2021**, *150*, 116001.
127. Mittra, E.; Rubin, C.; Gruber, B.; Qin, Y.-X. Evaluation of trabecular mechanical and microstructural properties in human calcaneal bone of advanced age using mechanical testing, μ CT, and DXA. *J. Biomech.* **2008**, *41*, 368–375.
128. Wronski, T.J.; Lowry, P.L.; Walsh, C.C.; Ignaszewski, L.A. Skeletal alterations in ovariectomized rats. *Calcif. Tissue Int.* **1985**, *37*, 324–328.
129. Zhao, B.; Wang, J.; Song, J.; Wang, C.; Gu, J.; Yuan, J.; Zhang, L.; Jiang, J.; Feng, L.; Jia, X. Beneficial effects of a flavonoid fraction of herba epimedii on bone metabolism in ovariectomized rats. *Planta Med.* **2016**, *82*, 322–329.
130. Ouyang, L.; Zhang, Q.; Ruan, X.; Feng, Y.; Wang, X. Treatment effect of Bushen Huayu extract on postmenopausal osteoporosis in vivo. *Exp. Ther. Med.* **2014**, *7*, 1687–1690.
131. Zhang, G.; Qin, L.; Shi, Y. *Epimedium*-derived phytoestrogen flavonoids exert beneficial effect on preventing bone loss in late postmenopausal women: A 24-month randomized, double-blind and placebo-controlled trial. *J. Bone Miner. Res.* **2007**, *22*, 1072–1079.
132. Luo, G.; Xu, B.; Wang, W.; Wu, Y.; Li, M. Study of the osteogenesis effect of icaraside II and icaritin on canine bone marrow mesenchymal stem cells. *J. Bone Miner. Metab.* **2018**, *36*, 668–678.
133. Wu, Z.; Ou, L.; Wang, C.; Yang, L.; Wang, P.; Liu, H.; Xiong, Y.; Sun, K.; Zhang, R.; Zhu, X. Icaritin induces MC3T3-E1 subclone14 cell differentiation through estrogen receptor-mediated ERK1/2 and p38 signaling activation. *Biomed. Pharmacother.* **2017**, *94*, 1–9.
134. Sun, K.; Mei, W.; Mo, S.; Xin, L.; Lei, X.; Huang, M.; Chen, Q.; Han, L.; Zhu, X. Lead exposure inhibits osteoblastic differentiation and inactivates the canonical Wnt signal and recovery by icaritin in MC3T3-E1 subclone 14 cells. *Chem. Biol. Interact.* **2019**, *303*, 7–13.
135. Liao, X.; Lu, S.; Wu, Y.; Xu, W.; Zhuo, Y.; Peng, Q.; Li, B.; Zhang, L.; Wang, Y. The effect of differentiation induction on FAK and Src activity in live HMSCs visualized by FRET. *PLoS ONE* **2013**, *8*, e72233.
136. Liao, X.; Lu, S.; Zhuo, Y.; Winter, C.; Xu, W.; Wang, Y. Visualization of Src and FAK activity during the differentiation process from HMSCs to osteoblasts. *PLoS ONE* **2012**, *7*, e42709.
137. Zheng, W.; Gu, X.; Sun, X.; Wu, Q.; Dan, H. FAK mediates BMP9-induced osteogenic differentiation via Wnt and MAPK signaling pathway in synovial mesenchymal stem cells. *Artif. Cells Nanomed. Biotechnol.* **2019**, *47*, 2641–2649.

138. Wei, Z.; You, F.; Li, H.; Wu, S.; Tang, F.; Wan, X.; Dong, H.; Huang, W.; Gao, S.; Cai, B.; et al. Integrating network pharmacology, IPA, and molecular docking to reveal the anti-osteoporosis effects of EA and EB via the FAK pathway. *Front. Pharmacol.* **2025**, *16*, 1532665.
139. Diao, X.; Wang, L.; Zhou, Y.; Bi, Y.; Zhou, K.; Song, L. The mechanism of Epimedin B in treating osteoporosis as revealed by RNA sequencing-based analysis. *Basic Clin. Pharmacol. Toxicol.* **2021**, *129*, 450–461.
140. Huang, X.; Wang, X.; Zhang, Y.; Shen, L.; Wang, N.; Xiong, X.; Zhang, L.; Cai, X.; Shou, D. Absorption and utilisation of epimedin C and icariin from Epimedium herba, and the regulatory mechanism via the BMP2/Runx2 signalling pathway. *Biomed. Pharmacother.* **2019**, *118*, 109345.
141. Xu, Y.; Chen, S.; Huang, L.; Han, W.; Shao, Y.; Chen, M.; Zhang, Y.; He, R.; Xie, B. Epimedin C alleviates glucocorticoid-induced suppression of osteogenic differentiation by modulating PI3K/AKT/RUNX2 signaling pathway. *Front. Pharmacol.* **2022**, *13*, 894832.
142. Huang, M.; Yu, L.; Wang, Y.; Yang, C. Epimedin C protects dexamethasone-induced osteoblasts through NRF1/RhoA pathway. *Aging* **2023**, *15*, 2033.
143. Xu, M.L.; Bi, C.W.C.; Kong, A.Y.Y.; Dong, T.T.X.; Wong, Y.H.; Tsim, K.W.K. Flavonoids induce the expression of acetylcholinesterase in cultured osteoblasts. *Chem. Biol. Interact.* **2016**, *259*, 295–300.
144. Huang, X.; Su, X.; Ma, Q.; Xie, Y.; Guo, Q.; Liao, L.; Zou, J. FoxO1 agonists promote bone regeneration in periodontitis by protecting the osteogenesis of periodontal ligament stem cells. *Stem Cells Dev.* **2023**, *32*, 491–503.
145. Nash, L.A.; Peters, S.J.; Sullivan, P.J.; Ward, W.E. Supraphysiological levels of quercetin glycosides are required to alter mineralization in Saos2 cells. *Int. J. Environ. Res. Public Health* **2016**, *13*, 460.
146. Seferos, N.; Petrokokkinos, L.; Kotsiou, A.; Rallis, G.; Tesseromatis, C. *Hypericum perforatum* L. treatment restored bone mass changes in swimming stressed rats. *Stomatologija* **2016**, *18*, 9–13.
147. Liu, L.; Wang, D.; Qin, Y.; Xu, M.; Zhou, L.; Xu, W.; Liu, X.; Ye, L.; Yue, S.; Zheng, Q.; et al. Astragalin promotes osteoblastic differentiation in MC3T3-E1 cells and bone formation in vivo. *Front. Endocrinol.* **2019**, *10*, 228.
148. Jia, T.-L.; Wang, H.-Z.; Xie, L.-P.; Wang, X.-Y.; Zhang, R.-Q. Daidzein enhances osteoblast growth that may be mediated by increased bone morphogenetic protein (BMP) production. *Biochem. Pharmacol.* **2003**, *65*, 709–715.
149. Yu, B.; Tang, D.-Z.; Li, S.-Y.; Wu, Y.; Chen, M. Daidzein promotes proliferation and differentiation in osteoblastic OCT1 cells via activation of the BMP-2/Smads pathway. *Pharmazie* **2017**, *72*, 35–40.
150. De Wilde, A.; Lieberherr, M.; Colin, C.; Pointillart, A. A low dose of daidzein acts as an ER β -selective agonist in trabecular osteoblasts of young female piglets. *J. Cell Physiol.* **2004**, *200*, 253–262.
151. Barlet, J.-P.; Picherit, C.; Coxam, V.; Bennetau-Pelissero, C.; Lebecque, P.; Kati-Coulibaly, S.; Davicco, M.-J. Daidzein is more efficient than genistein in preventing ovariectomy-induced bone loss in rats. *J. Nutr.* **2000**, *130*, 1675–1681.
152. Harahap, I.A.; Kuligowski, M.; Cieslak, A.; Kołodziejewski, P.A.; Suliburska, J. Effect of tempeh and daidzein on calcium status, calcium transporters, and bone metabolism biomarkers in ovariectomized rats. *Nutrients* **2024**, *16*, 651.
153. Nayeem, F.; Chen, N.-W.; Nagamani, M.; Anderson, K.E.; Lu, L.-J.W. Daidzein and genistein have differential effects in decreasing whole body bone mineral density but had no effect on hip and spine density in premenopausal women: A 2-year randomized, double-blind, placebo-controlled study. *Nutr. Res.* **2019**, *68*, 70–81.
154. Jung, W.-W. Protective effect of apigenin against oxidative stress-induced damage in osteoblastic cells. *Int. J. Mol. Med.* **2014**, *33*, 1327–1334.
155. Siddiqui, J.A.; Swarnkar, G.; Sharan, K.; Chakravarti, B.; Sharma, G.; Rawat, P.; Kumar, M.; Khan, F.M.; Pierroz, D.; Maurya, R.; et al. 8, 8'-Biapigeninyl stimulates osteoblast functions and inhibits osteoclast and adipocyte functions: Osteoprotective action of 8, 8'-biapigeninyl in ovariectomized mice. *Mol. Cell Endocrinol.* **2010**, *323*, 256–267. Erratum in *Mol. Cell Endocrinol.* **2011**, *333*, 102.
156. Liang, G.; Zhao, J.; Dou, Y.; Yang, Y.; Zhao, D.; Zhou, Z.; Zhang, R.; Yang, W.; Zeng, L. Mechanism and experimental verification of luteolin for the treatment of osteoporosis based on network pharmacology. *Front. Endocrinol.* **2022**, *13*, 866641.
157. Nash, L.A.; Sullivan, P.J.; Peters, S.J.; Ward, W.E. Rooibos flavonoids, orientin and luteolin, stimulate mineralization in human osteoblasts through the Wnt pathway. *Mol. Nutr. Food Res.* **2015**, *59*, 443–453.
158. Arampatzis, A.S.; Pampori, A.; Droutsas, E.; Laskari, M.; Karakostas, P.; Tsalikis, L.; Barmapalexis, P.; Dordas, C.; Assimopoulou, A.N. Occurrence of luteolin in the greek flora, isolation of luteolin and its action for the treatment of periodontal diseases. *Molecules* **2023**, *28*, 7720.
159. Yan, Z.; Zhan, J.; Qi, W.; Lin, J.; Huang, Y.; Xue, X.; Pan, X. The protective effect of luteolin in glucocorticoid-induced osteonecrosis of the femoral head. *Front. Pharmacol.* **2020**, *11*, 1195.

160. Balci Yuce, H.; Toker, H.; Yildirim, A.; Tekin, M.B.; Gevrek, F.; Altunbas, N. The effect of luteolin in prevention of periodontal disease in Wistar rats. *J. Periodontol.* **2019**, *90*, 1481–1489.
161. Quan, H.; Dai, X.; Liu, M.; Wu, C.; Wang, D. Luteolin supports osteogenic differentiation of human periodontal ligament cells. *BMC Oral Health* **2019**, *19*, 229.
162. Yang, H.; Liu, Q.; Ahn, J.H.; Kim, S.B.; Kim, Y.C.; Sung, S.H.; Hwang, B.Y.; Lee, M.K. Luteolin downregulates IL-1 β -induced MMP-9 and-13 expressions in osteoblasts via inhibition of ERK signalling pathway. *J. Enzyme. Inhib. Med. Chem.* **2012**, *27*, 261–266.
163. Lei, S.S.; Li, B.; Huang, X.W.; Wang, X.P.; Xiong, S.; Duan, R.; Li, L.Z. Structural identification of an polysaccharide isolated from *Epimedium brevicornum* and its beneficial effect on promoting osteogenesis in osteoblasts induced by high glucose. *Biomed. Pharmacother.* **2023**, *169*, 115893.
164. Zheng, H.; He, B.; Wu, T.; Cai, J.; Wei, J. Extraction, purification and anti-osteoporotic activity of a polysaccharide from *Epimedium brevicornum* Maxim. in vitro. *Int. J. Biol. Macromol.* **2020**, *156*, 1135–1145.
165. Xing, L.; Ni, H.; Wang, Y. Quercitrin attenuates osteoporosis in ovariectomized rats by regulating mitogen-activated protein kinase (MAPK) signaling pathways. *Biomed. Pharmacother.* **2017**, *89*, 1136–1141.
166. Choi, E.M. Protective effect of quercitrin against hydrogen peroxide-induced dysfunction in osteoblastic MC3T3-E1 cells. *Exp. Toxicol. Pathol.* **2012**, *64*, 211–216.
167. Gan, L.; Leng, Y.; Min, J.; Luo, X.-M.; Wang, F.; Zhao, J. Kaempferol promotes the osteogenesis in rBMSCs via mediation of SOX2/miR-124-3p/PI3K/Akt/mTOR axis. *Eur. J. Pharmacol.* **2022**, *927*, 174954.
168. Sari, D.S.; Pujiastuti, P.; Fatmawati, D.W.A.; Mardiyana, M.A.; Wulandari, A.T.; Arina, Y.M.D. Inhibiting the growth of periopathogenic bacteria and accelerating bone repair processes by using robusta coffee bean extract. *Saudi Dent. J.* **2023**, *35*, 322–329.
169. Kabashima, H.; Miura, N.; Shimizu, M.; Shinoda, W.; Wang, X.; Wang, Z.; Takahashi, S.; Harada, T.; Maruyama, H.; Tashiro, S.; et al. Preventive impact of alkaloids with anti-cancer effect extracted from natural herb and the derivatives. *WebmedCentral Prev. Med.* **2010**, *1*, WMC001309.
170. Yu, W.-X.; Poon, C.C.-W.; Zhou, L.-P.; Wong, K.-Y.; Cao, S.-S.; Lam, C.-Y.; Lee, W.Y.; Wong, M.-S. Oleanolic acid exerts bone anabolic effects via activation of osteoblastic 25-hydroxyvitamin D 1-alpha hydroxylase. *Biomed. Pharmacother.* **2024**, *173*, 116402.
171. Shu, B.; Zhao, Y.; Wang, Y.; Wang, G.; Shang, X.; Britt, M.; Olmedo, M.; Chelly, M.; Morandi, M.M.; Barton, S.; et al. Oleanolic acid enhances mesenchymal stromal cell osteogenic potential by inhibition of notch signaling. *Sci. Rep.* **2017**, *7*, 7002.
172. Wu, Y.; Gao, L.-J.; Fan, Y.-S.; Chen, Y.; Li, Q. Network pharmacology-based analysis on the action mechanism of oleanolic acid to alleviate osteoporosis. *ACS Omega* **2021**, *6*, 28410–28420.
173. Ibrahim, H.-I.M.; Darrag, H.M.; Alhajhoj, M.R.; Khalil, H.E. Biomolecule from *Trigonella stellata* from Saudi flora to suppress osteoporosis via osteostromal regulations. *Plant* **2020**, *9*, 1610.
174. Hu, X.; Wang, L.; He, Y.; Wei, M.; Yan, H.; Zhu, H. Chlorogenic acid promotes osteogenic differentiation of human dental pulp stem cells through Wnt signaling. *Stem Cells Dev.* **2021**, *30*, 641–650.
175. Han, D.; Chen, W.; Gu, X.; Shan, R.; Zou, J.; Liu, G.; Shahid, M.; Gao, J.; Han, B. Cytoprotective effect of chlorogenic acid against hydrogen peroxide-induced oxidative stress in MC3T3-E1 cells through PI3K/Akt-mediated Nrf2/HO-1 signaling pathway. *Onco-target* **2017**, *8*, 14680.
176. Han, D.; Gu, X.; Gao, J.; Wang, Z.; Liu, G.; Barkema, H.W.; Han, B. Chlorogenic acid promotes the Nrf2/HO-1 anti-oxidative pathway by activating p21Waf1/Cip1 to resist dexamethasone-induced apoptosis in osteoblastic cells. *Free Radic. Biol. Med.* **2019**, *137*, 1–12.
177. Zhou, R.P.; Lin, S.J.; Wan, W.B.; Zuo, H.L.; Yao, F.F.; Ruan, H.B.; Xu, J.; Song, W.; Zhou, Y.C.; Wen, S.Y.; et al. Chlorogenic acid prevents osteoporosis by Shp2/PI3K/Akt pathway in ovariectomized rats. *PLoS ONE* **2016**, *11*, e0166751.
178. Zhang, M.; Hu, X. Mechanism of chlorogenic acid treatment on femoral head necrosis and its protection of osteoblasts. *Biomed. Rep.* **2016**, *5*, 57–62.
179. Ho, C.-Y.; Tang, C.-H.; Ho, T.-L.; Wang, W.-L.; Yao, C.-H. Chlorogenic acid prevents ovariectomized-induced bone loss by facilitating osteoblast functions and suppressing osteoclast formation. *Aging* **2024**, *16*, 4832.
180. Kawabata, T.; Otsuka, T.; Fujita, K.; Sakai, G.; Matsushima-Nishiwaki, R.; Kozawa, O.; Tokuda, H. (-)-Epigallocatechin gallate but not chlorogenic acid suppresses EGF-stimulated migration of osteoblasts via attenuation of p38 MAPK activity. *Int. J. Mol. Med.* **2018**, *42*, 3149–3156.
181. Leotoing, L.; Wauquier, F.; Davicco, M.-J.; Lebecque, P.; Gaudout, D.; Rey, S.; Vitrac, X.; Massenat, L.; Rashidi, S.; Wittrant, Y.; et al. The phenolic acids of Agen prunes (dried plums) or Agen prune juice concentrates do not account for the protective action on bone in a rat model of postmenopausal osteoporosis. *Nutr. Res.* **2016**, *36*, 161–173.

182. Yamamoto, N.; Tokuda, H.; Kuroyanagi, G.; Kainuma, S.; Ohguchi, R.; Fujita, K.; Matsushima-Nishiwaki, R.; Kozawa, O.; Otsuka, T. Amplification by (-)-epigallocatechin gallate and chlorogenic acid of TNF- α -stimulated interleukin-6 synthesis in osteoblasts. *Int. J. Mol. Med.* **2015**, *36*, 1707–1712.
183. Kozawa, O.; Suzuki, A.; Kaida, T.; Tokuda, H.; Uematsu, T. Tumor necrosis factor- α autoregulates interleukin-6 synthesis via activation of protein kinase C. *J. Biol. Chem.* **1997**, *272*, 25099–25104.
184. Charles, J.F.; Aliprantis, A.O. Osteoclasts: More than ‘bone eaters’. *Trends Mol. Med.* **2014**, *20*, 449–459.
185. Xiang, Q.; Li, L.; Ji, W.; Gawlitta, D.; Walboomers, X.F.; van den Beucken, J.J.J.P. Beyond resorption: Osteoclasts as drivers of bone formation. *Cell Regen.* **2024**, *13*, 22.
186. Nian, H.; Ma, M.-H.; Nian, S.-S.; Xu, L.-L. Antiosteoporotic activity of icariin in ovariectomized rats. *Phytomedicine* **2009**, *16*, 320–326.
187. Hsieh, T.-P.; Sheu, S.-Y.; Sun, J.-S.; Chen, M.-H. Icariin inhibits osteoclast differentiation and bone resorption by suppression of MAPKs/NF- κ B regulated HIF-1 α and PGE2 synthesis. *Phytomedicine* **2011**, *18*, 176–185.
188. Cui, J.; Zhu, M.; Zhu, S.; Wang, G.; Xu, Y.; Geng, D. Inhibitory effect of icariin on Ti-induced inflammatory osteoclastogenesis. *J. Surg. Res.* **2014**, *192*, 447–453.
189. Zhang, Y.; Lin, Y.; Xiao, L.; Feng, E.; Wang, W.; Lin, L. The effects of icariin concentration on osteoclasts bone resorption induced by titanium particles in vitro. *Regen. Biomater.* **2015**, *2*, 197–202.
190. Kobayashi, N.; Kadono, Y.; Naito, A.; Matsumoto, K.; Yamamoto, T.; Tanaka, S.; Inoue, J. Segregation of TRAF6-mediated signaling pathways clarifies its role in osteoclastogenesis. *EMBO J.* **2001**, *20*, 1271–1280.
191. Zhao, X.; Lin, Y.; Jiang, B.; Yin, J.; Lu, C.; Wang, J.; Zeng, J. Icaritin inhibits lung cancer-induced osteoclastogenesis by suppressing the expression of IL-6 and TNF- α and through AMPK/mTOR signaling pathway. *Anticancer. Drugs* **2020**, *31*, 1004–1011.
192. Wang, C.; Wu, P.; Shi, J.-F.; Jiang, Z.-H.; Wei, X.-Y. Synthesis and cancer cell growth inhibitory activity of icaritin derivatives. *Eur. J. Med. Chem.* **2015**, *100*, 139–150.
193. Wang, Z.; Lou, Y. Proliferation-stimulating effects of icaritin and desmethylicaritin in MCF-7 cells. *Eur. J. Pharmacol.* **2004**, *504*, 147–153.
194. Wang, Z.; Zhang, X.; Wang, H.; Qi, L.; Lou, Y. Neuroprotective effects of icaritin against beta amyloid-induced neurotoxicity in primary cultured rat neuronal cells via estrogen-dependent pathway. *Neuroscience* **2007**, *145*, 911–922.
195. Zhang, D.; Liang, J.; Qu, S.; Xu, C.; Kan, H.; Dong, K.; Wang, Y. Metabolomics and pharmacodynamic analysis unveil the therapeutic role of icaritin on osteoporosis rats. *J. Pharm. Biomed. Anal.* **2024**, *241*, 115979.
196. Lin, X.; Zhou, X.; Liu, X.; Xia, L.; Cai, J.; Huang, N.; Luo, Y.; Wu, W. Icaritin alleviates motor impairment and osteoporosis in Parkinson’s disease mice via the ER-PI3K/Akt pathway. *Sci. Rep.* **2025**, *15*, 3190.
197. Chen, C.; Wu, M.; Lei, H.; Cao, Z.; Wu, F.; Song, Y.; Zhang, C.; Qin, M.; Zhang, C.; Du, R.; et al. A novel prenylflavonoid icaraside I ameliorates estrogen deficiency-induced osteoporosis via simultaneous regulation of osteoblast and osteoclast differentiation. *ACS Pharmacol. Transl. Sci.* **2023**, *6*, 270–280.
198. Lucas, S.; Omata, Y.; Hofmann, J.; Bottcher, M.; Iljazovic, A.; Sarter, K.; Albrecht, O.; Schulz, O.; Krishnacoumar, B.; Kronke, G.; et al. Short-chain fatty acids regulate systemic bone mass and protect from pathological bone loss. *Nat. Commun.* **2018**, *9*, 55.
199. Ma, M.; Fan, A.; Liu, Z.; Yang, L.; Huang, J.; Pang, Z.; Yin, F. Baohuoside I inhibits osteoclastogenesis and protects against ovariectomy-induced bone loss. *Front. Pharmacol.* **2022**, *13*, 874952.
200. Liu, Y.-Q.; Yang, Q.-X.; Cheng, M.-C.; Xiao, H.-B. Synergistic inhibitory effect of Icaraside II with Icaritin from Herba Epimedii on pre-osteoclastic RAW264.7 cell growth. *Phytomedicine* **2014**, *21*, 1633–1637.
201. Zhang, D.; Zhang, J.; Fong, C.; Yao, X.; Yang, M. Herba epimedii flavonoids suppress osteoclastic differentiation and bone resorption by inducing G2/M arrest and apoptosis. *Biochimie* **2012**, *94*, 2514–2522.
202. Li, J.; Wei, J.J.; Wu, C.H.; Zou, T.; Zhao, H.; Huo, T.Q.; Wei, C.J.; Yang, T. Epimedin A inhibits the PI3K/AKT/NF- κ B signalling axis and osteoclast differentiation by negatively regulating TRAF6 expression. *Mol. Med.* **2024**, *30*, 125.
203. Wei, Q.; Ouyang, M.; Guo, X.; Fu, X.; Liu, T.; Luo, Y.; Tang, H.; Yang, Y.; Gao, X.; Mao, H. Effect of hyperoside on osteoporosis in ovariectomized mice through estrogen receptor α /ITG β 3 signaling pathway. *Eur. J. Pharmacol.* **2024**, *977*, 176666.
204. An, H.; Chu, C.; Zhang, Z.; Zhang, Y.; Wei, R.; Wang, B.; Xu, K.; Li, L.; Liu, Y.; Li, G.; et al. Hyperoside alleviates postmenopausal osteoporosis via regulating miR-19a-5p/IL-17A axis. *Am. J. Reprod. Immunol.* **2023**, *90*, e13709.
205. Xing, F.; Geng, L.; Guan, H.; Liu, D.; Li, Y.; Zeng, L.; Chen, Y.; Tian, R.; Li, Z.; Cao, R.; et al. Astragalin mitigates inflammatory osteolysis by negatively modulating osteoclastogenesis via ROS and MAPK signaling pathway. *Int. Immunopharmacol.* **2022**, *112*, 109278.

206. Choi, H.J.; Park, Y.R.; Nepal, M.; Choi, B.-Y.; Cho, N.-P.; Choi, S.H.; Heo, S.R.; Kim, H.S.; Yang, M.-S.; Soh, Y. Inhibition of osteoclastogenic differentiation by Ikariside A in RAW 264.7 cells via JNK and NF- κ B signaling pathways. *Eur. J. Pharmacol.* **2010**, *636*, 28–35.
207. Sun, J.; Sun, W.J.; Li, Z.Y.; Li, L.; Wang, Y.; Zhao, Y.; Wang, C.; Yu, L.R.; Li, L.Z.; Zhang, Y.L. Daidzein increases OPG/RANKL ratio and suppresses IL-6 in MG-63 osteoblast cells. *Int. Immunopharmacol.* **2016**, *40*, 32–40.
208. Tyagi, A.M.; Srivastava, K.; Sharan, K.; Yadav, D.; Maurya, R.; Singh, D. Daidzein prevents the increase in CD4⁺ CD28null T cells and B lymphopoiesis in ovariectomized mice: A key mechanism for anti-osteoclastogenic effect. *PLoS ONE* **2011**, *6*, e21216.
209. Jin, X.; Sun, J.; Yu, B.; Wang, Y.; Sun, W.J.; Yang, J.; Huang, S.H.; Xie, W.L. Daidzein stimulates osteogenesis facilitating proliferation, differentiation, and antiapoptosis in human osteoblast-like MG-63 cells via estrogen receptor-dependent MEK/ERK and PI3K/Akt activation. *Nutr. Res.* **2017**, *42*, 20–30.
210. Kaludjerovic, J.; Ward, W.E. Neonatal exposure to daidzein, genistein, or the combination modulates bone development in female CD-1 mice. *J. Nutr.* **2009**, *139*, 467–473.
211. Kim, T.-H.; Jung, J.W.; Ha, B.G.; Hong, J.M.; Park, E.K.; Kim, H.-J.; Kim, S.-Y. The effects of luteolin on osteoclast differentiation, function in vitro and ovariectomy-induced bone loss. *J. Nutr. Biochem.* **2011**, *22*, 8–15.
212. Lee, J.-W.; Ahn, J.-Y.; Hasegawa, S.; Cha, B.-Y.; Yonezawa, T.; Nagai, K.; Seo, H.-J.; Jeon, W.-B.; Woo, J.-T. Inhibitory effect of luteolin on osteoclast differentiation and function. *Cytotechnology* **2009**, *61*, 125–134.
213. Tavares, S.J.S.; Lima, V. Bone anti-resorptive effects of coumarins on RANKL downstream cellular signaling: A systematic review of the literature. *Fitoterapia* **2021**, *150*, 104842.
214. Lee, E.-J.; Kang, M.-K.; Kim, Y.-H.; Kim, D.Y.; Oh, H.; Kim, S.-I.; Oh, S.Y.; Na, W.; Kang, Y.-H. Coumarin ameliorates impaired bone turnover by inhibiting the formation of advanced glycation end products in diabetic osteoblasts and osteoclasts. *Biomolecule* **2020**, *10*, 1052.
215. Lee, W.-S.; Lee, E.-G.; Sung, M.-S.; Yoo, W.-H. Kaempferol inhibits IL-1 β -stimulated, RANKL-mediated osteoclastogenesis via downregulation of MAPKs, c-Fos, and NFATc1. *Inflammation* **2014**, *37*, 1221–1230.
216. Sun, Z.; Zeng, J.; Wang, W.; Jia, X.; Wu, Q.; Yu, D.; Mao, Y. Magnoflorine suppresses MAPK and NF- κ B signaling to prevent inflammatory osteolysis induced by titanium particles in vivo and osteoclastogenesis via RANKL in vitro. *Front. Pharmacol.* **2020**, *11*, 389.
217. Okumo, T.; Takayama, Y.; Maruyama, K.; Kato, M.; Sunagawa, M. Senso-immunologic prospects for complex regional pain syndrome treatment. *Front. Immunol.* **2022**, *12*, 786511.
218. Kim, E.H.; Jo, C.S.; Ryu, S.Y.; Kim, S.H.; Lee, J.Y. Anti-osteoclastogenic diacetylenic components of *Dendropanax moribifera*. *Arch. Pharm. Res.* **2018**, *41*, 506–512.
219. Zhang, W.; Xue, K.; Gao, Y.; Huai, Y.; Wang, W.; Miao, Z.; Dang, K.; Jiang, S.; Qian, A. Systems pharmacology dissection of action mechanisms of Dipsaci Radix for osteoporosis. *Life Sci.* **2019**, *235*, 116820.
220. Guo, H.; Chen, X.; Li, J.; Mo, G.; Li, Y.; Tang, Y.; Kai, Y.; Zhang, S. β -Sitosterol inhibits osteoclast activity and reduces ovariectomy-induced bone loss by regulating the cAMP and NF- κ B signaling pathways. *Cell Signal* **2025**, *130*, 111672.
221. Wang, T.; Li, S.; Yi, C.; Wang, X.; Han, X. Protective role of β -sitosterol in glucocorticoid-induced osteoporosis in rats via the RANKL/OPG Pathway. *Altern. Ther. Health Med.* **2022**, *28*, 18–25.
222. Komakech, R.; Shim, K.-S.; Yim, N.-H.; Song, J.H.; Yang, S.; Choi, G.; Lee, J.; Kim, Y.; Omujal, F.; Okello, D.; et al. GC-MS and LC-TOF-MS profiles, toxicity, and macrophage-dependent in vitro anti-osteoporosis activity of *Prunus africana* (Hook f.) Kalkman Bark. *Sci. Rep.* **2022**, *12*, 7044.
223. Xie, B.; Shi, L.; Li, J.; Zeng, Y.; Liu, W.; Tang, S.; Jia, L.; Zhang, J.; Gan, G. Oleanolic acid inhibits RANKL-induced osteoclastogenesis via ER α /miR-503/RANK signaling pathway in RAW264.7 cells. *Biomed. Pharmacother.* **2019**, *117*, 109045.
224. Zhao, D.; Shu, B.; Wang, C.; Zhao, Y.; Cheng, W.; Sha, N.; Li, C.; Wang, Q.; Lu, S.; Wang, Y. Oleanolic acid exerts inhibitory effects on the late stage of osteoclastogenesis and prevents bone loss in osteoprotegerin knockout mice. *J. Cell Biochem.* **2020**, *121*, 152–164.
225. Wu, Y.-X.; Wu, T.; Xu, B.; Xu, X.; Chen, H.; Li, X.; Wang, G. Protocatechuic acid inhibits osteoclast differentiation and stimulates apoptosis in mature osteoclasts. *Biomed. Pharmacother.* **2016**, *82*, 399–405.
226. Jang, S.-A.; Song, H.S.; Kwon, J.E.; Baek, H.J.; Koo, H.J.; Sohn, E.-H.; Lee, S.R.; Kang, S.C. Protocatechuic acid attenuates trabecular bone loss in ovariectomized mice. *Oxid. Med. Cell. Longev.* **2018**, *2018*, 7280342.
227. Park, S.-H.; Kim, J.-Y.; Cheon, Y.-H.; Baek, J.M.; Ahn, S.-J.; Yoon, K.-H.; Lee, M.S.; Oh, J. Protocatechuic acid attenuates osteoclastogenesis by downregulating JNK/c-Fos/NFATc1 signaling and prevents inflammatory bone loss in mice. *Phytother. Res.* **2016**, *30*, 604–612.

228. Tang, Q.Y.; Kukita, T.; Ushijima, Y.; Kukita, A.; Nagata, K.; Sandra, F.; Watanabe, T.; Toh, K.; Okuma, Y.; Kawasaki, S.; et al. Regulation of osteoclastogenesis by Simon extracts composed of caffeic acid and related compounds: Successful suppression of bone destruction accompanied with adjuvant-induced arthritis in rats. *Histochem. Cell Biol.* **2006**, *125*, 215–225.
229. Shen, J.; Zhang, S.; Zhang, J.; Wei, X.; Wang, Z.; Han, B. Osteogenic mechanism of chlorogenic acid and its application in clinical practice. *Front. Pharmacol.* **2024**, *15*, 1396354.
230. Kwak, S.C.; Lee, C.; Kim, J.-Y.; Oh, H.M.; So, H.-S.; Lee, M.S.; Rho, M.C.; Oh, J. Chlorogenic acid inhibits osteoclast differentiation and bone resorption by down-regulation of receptor activator of nuclear factor kappa-B ligand-induced nuclear factor of activated T cells c1 expression. *Biol. Pharm. Bull.* **2013**, *36*, 1779–1786.
231. Chen, X.; Zhu, X.; Wei, A.; Chen, F.; Gao, Q.; Lu, K.; Jiang, Q.; Cao, W. Nrf2 epigenetic derepression induced by running exercise protects against osteoporosis. *Bone Res.* **2021**, *9*, 15.
232. Rousselle, A.-V.; Heymann, D. Osteoclastic acidification pathways during bone resorption. *Bone* **2002**, *30*, 533–540.
233. Lee, S.-H.; Lee, J.-Y.; Kwon, Y.-I.; Jang, H.-D. Anti-osteoclastic activity of artemisia capillaris thunb. Extract depends upon attenuation of osteoclast differentiation and bone resorption-associated acidification due to chlorogenic acid, hyperoside, and scoparone. *Int. J. Mol. Sci.* **2017**, *18*, 322.
234. Pantoom, S.; Konstantinidis, G.; Voss, S.; Han, H.; Hofnagel, O.; Li, Z.; Wu, Y.-W. RAB33B recruits the ATG16L1 complex to the phagophore via a noncanonical RAB binding protein. *Autophagy* **2021**, *17*, 2290–2304.
235. Fujiwara, T.; Ye, S.; Castro-Gomes, T.; Winchell, C.G.; Andrews, N.W.; Voth, D.E.; Varughese, K.I.; Mackintosh, S.G.; Feng, Y.; Pavlos, N.; et al. PLEKHM1/DEF8/RAB7 complex regulates lysosome positioning and bone homeostasis. *JCI Insight* **2016**, *1*, e86330.
236. Hiura, F.; Kawabata, Y.; Aoki, T.; Mizokami, A.; Jimi, E. Inhibition of the ATG4-LC3 pathway suppressed osteoclast maturation. *Biochem. Biophys. Res. Commun.* **2022**, *632*, 40–47.
237. Headland, S.E.; Norling, L.V. The resolution of inflammation: Principles and challenges. *Semin. Immunol.* **2015**, *27*, 149–160.
238. El-Jawhari, J.J.; Jones, E.; Giannoudis, P.V. The roles of immune cells in bone healing; what we know, do not know and future perspectives. *Injury* **2016**, *47*, 2399–2406.
239. Bai, L.; Du, Z.; Du, J.; Yao, W.; Zhang, J.; Weng, Z.; Liu, S.; Zhao, Y.; Liu, Y.; Zhang, X.; et al. A multifaceted coating on titanium dictates osteoimmunomodulation and osteo/angio-genesis towards ameliorative osseointegration. *Biomaterials* **2018**, *162*, 154–169.
240. Li, H.; Zhang, S.; Huo, S.; Tang, H.; Nie, B.; Qu, X.; Yue, B. Effects of staphylococcal infection and aseptic inflammation on bone mass and biomechanical properties in a rabbit model. *J. Orthop. Translat.* **2020**, *21*, 66–72.
241. Bai, L.; Liu, Y.; Zhang, X.; Chen, P.; Hang, R.; Xiao, Y.; Wang, J.; Liu, C. Osteoporosis remission via an anti-inflammaging effect by icariin activated autophagy. *Biomaterials* **2023**, *297*, 122125.
242. Shao, H.; Shen, J.; Wang, M.; Cui, J.; Wang, Y.; Zhu, S.; Zhang, W.; Yang, H.; Xu, Y.; Geng, D. Icariin protects against titanium particle-induced osteolysis and inflammatory response in a mouse calvarial model. *Biomaterials* **2015**, *60*, 92–99.
243. Zuo, S.; Zou, W.; Wu, R.-M.; Yang, J.; Fan, J.-N.; Zhao, X.-K.; Li, H.-Y. Icariin alleviates IL-1 β -induced matrix degradation by activating the Nrf2/ARE pathway in human chondrocytes. *Drug Des. Devel. Ther.* **2019**, *13*, 3949–3961.
244. Zu, Y.; Mu, Y.; Li, Q.; Zhang, S.-T.; Yan, H.-J. Icariin alleviates osteoarthritis by inhibiting NLRP3-mediated pyroptosis. *J. Orthop. Surg. Res.* **2019**, *14*, 307.
245. Sun, P.; Liu, Y.; Deng, X.; Yu, C.; Dai, N.; Yuan, X.; Chen, L.; Yu, S.; Si, W.; Wang, X.; et al. An inhibitor of cathepsin K, icariin suppresses cartilage and bone degradation in mice of collagen-induced arthritis. *Phytomedicine* **2013**, *20*, 975–979.
246. Ma, Y.; Yang, H.; Huang, J. Icariin ameliorates dexamethasone-induced bone deterioration in an experimental mouse model via activation of microRNA-186 inhibition of cathepsin K. *Mol. Med. Rep.* **2018**, *17*, 1633–1641.
247. Liu, N.; Zhang, T.; Cao, B.-R.; Luan, F.-Y.; Liu, R.-X.; Yin, H.-R.; Wang, W.-B. Icariin possesses chondroprotective efficacy in a rat model of dexamethasone-induced cartilage injury through the activation of miR-206 targeting of cathepsin K. *Int. J. Mol. Med.* **2018**, *41*, 1039–1047.
248. Zhang, J.; Fan, F.; Liu, A.; Zhang, C.; Li, Q.; Zhang, C.; He, F.; Shang, M. Icariin: A potential molecule for treatment of knee osteoarthritis. *Front. Pharmacol.* **2022**, *13*, 811808.
249. Liu, Y.; Yang, J.; Luo, Z.; Li, D.; Lu, J.; Wang, Q.; Xiao, Y.; Zhang, X. Development of an injectable thiolated icariin functionalized collagen/hyaluronic hydrogel to promote cartilage formation in vitro and in vivo. *J. Mater. Chem. B* **2019**, *7*, 2845–2854.
250. Zhou, X.; Lian, K.; Jia, J.; Zhao, X.; Duan, P.; Huang, J.; Shi, Y. Functions of Epimedin C in a zebrafish model of glucocorticoid-induced osteoporosis. *J. Cell. Mol. Med.* **2024**, *28*, e18569.

251. Aryal, Y.P.; Yeon, C.-Y.; Kim, T.-Y.; Lee, E.-S.; Sung, S.; Pokharel, E.; Kim, J.-Y.; Choi, S.-Y.; Yamamoto, H.; Sohn, W.-J.; et al. Facilitating reparative dentin formation using apigenin local delivery in the exposed pulp cavity. *Front. Physiol.* **2021**, *12*, 773878.
252. Yu, M.; Gu, Q.; Xu, J. Discovering new PI3K α inhibitors with a strategy of combining ligand-based and structure-based virtual screening. *J. Comput. Aided Mol. Des.* **2018**, *32*, 347–361.
253. Lien, E.C.; Dibble, C.C.; Toker, A. PI3K signaling in cancer: Beyond AKT. *Curr. Opin. Cell Biol.* **2017**, *45*, 62–71.
254. Ligresti, G.; Militello, L.; Steelman, L.S.; Cavallaro, A.; Basile, F.; Nicoletti, F.; Stivala, F.; McCubrey, J.A.; Libra, M. PIK3CA mutations in human solid tumors: Role in sensitivity to various therapeutic approaches. *Cell Cycle* **2009**, *8*, 1352–1358.
255. Si, H.-B.; Zeng, Y.; Liu, S.-Y.; Zhou, Z.-K.; Chen, Y.-N.; Cheng, J.-Q.; Lu, Y.-R.; Shen, B. Intra-articular injection of microRNA-140 (miRNA-140) alleviates osteoarthritis (OA) progression by modulating extracellular matrix (ECM) homeostasis in rats. *Osteoarthritis. Cartil.* **2017**, *25*, 1698–1707.
256. Guo, H.; Yin, W.; Zou, Z.; Zhang, C.; Sun, M.; Min, L.; Yang, L.; Kong, L. Quercitrin alleviates cartilage extracellular matrix degradation and delays ACLT rat osteoarthritis development: An in vivo and in vitro study. *J. Adv. Res.* **2021**, *28*, 255–267.
257. Jin, X.; Yan, E.; Wang, H.; Sui, H.; Liu, Z.; Gao, W.; Jin, Y. Hyperoside exerts anti-inflammatory and anti-arthritis effects in LPS-stimulated human fibroblast-like synoviocytes in vitro and in mice with collagen-induced arthritis. *Acta Pharmacol. Sin.* **2016**, *37*, 674–686.
258. Xie, T.; Yuan, J.; Mei, L.; Li, P.; Pan, R. Hyperoside ameliorates TNF- α -induced inflammation, ECM degradation and ER stress-mediated apoptosis via the SIRT1/NF- κ B and Nrf2/ARE signaling pathways in vitro. *Mol. Med. Rep.* **2022**, *260*, 1–10.
259. Jia, Q.; Wang, T.; Wang, X.; Xu, H.; Liu, Y.; Wang, Y.; Shi, Q.; Liang, Q. Astragalin suppresses inflammatory responses and bone destruction in mice with collagen-induced arthritis and in human fibroblast-like synoviocytes. *Front. Pharmacol.* **2019**, *10*, 94.
260. Estakhri, F.; Panjehshahin, M.R.; Tanideh, N.; Gheisari, R.; Mahmoodzadeh, A.; Azarpira, N.; Gholijani, N. The effect of kaempferol and apigenin on allogenic synovial membrane-derived stem cells therapy in knee osteoarthritic male rats. *Knee* **2020**, *27*, 817–832.
261. Zhuang, Z.; Ye, G.; Huang, B. Kaempferol alleviates the interleukin-1 β -induced inflammation in rat osteoarthritis chondrocytes via suppression of NF- κ B. *Med. Sci. Monit.* **2017**, *23*, 3925.
262. Xiao, Y.; Liu, L.; Zheng, Y.; Liu, W.; Xu, Y. Kaempferol attenuates the effects of XIST/miR-130a/STAT3 on inflammation and extracellular matrix degradation in osteoarthritis. *Future Med. Chem.* **2021**, *13*, 1451–1464.
263. Wang, L.; Li, P.; Zhou, Y.; Gu, R.; Lu, G.; Zhang, C. Magnoflorine ameliorates collagen-induced arthritis by suppressing the inflammation response via the NF- κ B/MAPK signaling pathways. *J. Inflamm. Res.* **2023**, *16*, 2271–2296.
264. Shen, Y.; Fan, X.; Qu, Y.; Tang, M.; Huang, Y.; Peng, Y.; Fu, Q. Magnoflorine attenuates inflammatory responses in RA by regulating the PI3K/Akt/NF- κ B and Keap1-Nrf2/HO-1 signalling pathways in vivo and in vitro. *Phytomedicine* **2022**, *104*, 154339.
265. Valerio, M.; Awad, A.B. β -Sitosterol down-regulates some pro-inflammatory signal transduction pathways by increasing the activity of tyrosine phosphatase SHP-1 in J774A. 1 murine macrophages. *Int. Immunopharmacol.* **2011**, *11*, 1012–1017.
266. Choi, J.N.; Choi, Y.-H.; Lee, J.-M.; Noh, I.C.; Park, J.W.; Choi, W.S.; Choi, J.H. Anti-inflammatory effects of β -sitosterol- β -D-glucoside from *Trachelospermum jasminoides* (Apocynaceae) in lipopolysaccharide-stimulated RAW 264.7 murine macrophages. *Nat. Prod. Res.* **2012**, *26*, 2340–2343.
267. Kim, K.-A.; Lee, I.-A.; Gu, W.; Hyam, S.R.; Kim, D.-H. β -Sitosterol attenuates high-fat diet-induced intestinal inflammation in mice by inhibiting the binding of lipopolysaccharide to toll-like receptor 4 in the NF- κ B pathway. *Mol. Nutr. Food Res.* **2014**, *58*, 963–972.
268. Zhang, J.; Fu, B.; Chen, X.; Chen, D.; Yang, H. Protocatechuic acid attenuates anterior cruciate ligament transection-induced osteoarthritis by suppressing osteoclastogenesis. *Exp. Ther. Med.* **2020**, *19*, 232–240.
269. Hwang, S.J.; Kim, Y.-W.; Park, Y.; Lee, H.-J.; Kim, K.-W. Anti-inflammatory effects of chlorogenic acid in lipopolysaccharide-stimulated RAW 264.7 cells. *Inflamm. Res.* **2014**, *63*, 81–90.
270. López-Otín, C.; Blasco, M.A.; Partridge, L.; Serrano, M.; Kroemer, G. The hallmarks of aging. *Cell* **2013**, *153*, 1194–1217.
271. Gu, H.; Liu, N.; Lin, F.; Yin, J. Nrf2 signaling pathway: Focus on oxidative stress in osteoporosis. *Osteoporos. Int.* **2025**, *36*, 1837–1854.
272. Horwood, N.J. Macrophage polarization and bone formation: A review. *Clin. Rev. Allergy Immunol.* **2016**, *51*, 79–86.
273. Song, Z.; Cheng, Y.; Chen, M.; Xie, X. Macrophage polarization in bone implant repair: A review. *Tissue Cell* **2023**, *82*, 102112.
274. Woo, H.A.; Yim, S.H.; Shin, D.H.; Kang, D.; Yu, D.-Y.; Rhee, S.G. Inactivation of peroxiredoxin I by phosphorylation allows localized H₂O₂ accumulation for cell signaling. *Cell* **2010**, *140*, 517–528.
275. Sies, H.; Jones, D.P. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nat. Rev. Mol. Cell Biol.* **2020**, *21*, 363–383.

276. Lee, N.K.; Choi, Y.G.; Baik, J.Y.; Han, S.Y.; Jeong, D.; Bae, Y.S.; Kim, N.; Lee, S.Y. A crucial role for reactive oxygen species in RANKL-induced osteoclast differentiation. *Blood* **2005**, *106*, 852–859.
277. Sies, H. Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: Oxidative eustress. *Redox Biol.* **2017**, *11*, 613–619.
278. Sies, H.; Berndt, C.; Jones, D.P. Oxidative stress. *Annu. Rev. Biochem.* **2017**, *86*, 715–748.
279. Sanchis-Gomar, F.; Garcia-Gimenez, J.L.; Perez-Quilis, C.; Gomez-Cabrera, M.C.; Pallardo, F.V.; Lippi, G. Physical exercise as an epigenetic modulator: Eustress, the “positive stress” as an effector of gene expression. *J. Strength. Cond. Res.* **2012**, *26*, 3469–3472.
280. Yan, L.J. Positive oxidative stress in aging and aging-related disease tolerance. *Redox Biol.* **2014**, *2*, 165–169.
281. Niki, E. Oxidative stress and antioxidants: Distress or eustress? *Arch. Biochem. Biophys.* **2016**, *595*, 19–24.
282. Sies, H. Oxidative eustress: On constant alert for redox homeostasis. *Redox Biol.* **2021**, *41*, 101867.
283. Mandal, C.C.; Ganapathy, S.; Gorin, Y.; Mahadev, K.; Block, K.; Abboud, H.E.; Harris, S.E.; Ghosh-Choudhury, G.; Ghosh-Choudhury, N. Reactive oxygen species derived from Nox4 mediate BMP2 gene transcription and osteoblast differentiation. *Biochem. J.* **2011**, *433*, 393–402.
284. Arakaki, N.; Yamashita, A.; Niimi, S.; Yamazaki, T. Involvement of reactive oxygen species in osteoblastic differentiation of MC3T3-E1 cells accompanied by mitochondrial morphological dynamics. *Biomed. Res.* **2013**, *34*, 161–166.
285. Jackson, S.H.; Gallin, J.I.; Holland, S.M. The p47phox mouse knock-out model of chronic granulomatous disease. *J. Exp. Med.* **1995**, *182*, 751–758.
286. Craige, S.M.; Chen, K.; Pei, Y.; Li, C.; Huang, X.; Chen, C.; Shibata, R.; Sato, K.; Walsh, K.; Keaney, J.J.F. NADPH oxidase 4 promotes endothelial angiogenesis through endothelial nitric oxide synthase activation. *Circulation* **2011**, *124*, 731–740.
287. Ristow, M.; Zarse, K.; Oberbach, A.; Klötting, N.; Birringer, M.; Kiehnopf, M.; Stumvoll, M.; Kahn, C.R.; Blüher, M. Antioxidants prevent health-promoting effects of physical exercise in humans. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 8665–8670.
288. Zhong, Z.M.; Bai, L.; Chen, J.T. Advanced oxidation protein products inhibit proliferation and differentiation of rat osteoblast-like cells via NF- κ B pathway. *Cell Physiol. Biochem.* **2009**, *24*, 105–114.
289. Garrett, I.R.; Boyce, B.F.; O Oreffo, R.; Bonewald, L.; Poser, J.; Mundy, G.R. Oxygen-derived free radicals stimulate osteoclastic bone resorption in rodent bone in vitro and in vivo. *J. Clin. Investig.* **1990**, *85*, 632–639.
290. Park, J.H.; Koh, E.-B.; Seo, Y.-J.; Oh, H.-S.; Won, J.-Y.; Hwang, S.-C.; Byun, J.-H. Tiron has negative effects on osteogenic differentiation via mitochondrial dysfunction in human periosteum-derived cells. *Int. J. Mol. Sci.* **2022**, *23*, 14040.
291. Sun, H.; Xu, J.; Wang, Y.; Shen, S.; Xu, X.; Zhang, L.; Jiang, Q. Bone microenvironment regulative hydrogels with ROS scavenging and prolonged oxygen-generating for enhancing bone repair. *Bioact. Mater.* **2023**, *24*, 477–496.
292. Chen, G.-H.; Song, C.-C.; Pantopoulos, K.; Wei, X.-L.; Zheng, H.; Luo, Z. Mitochondrial oxidative stress mediated Fe-induced ferroptosis via the NRF2-ARE pathway. *Free Radic. Biol. Med.* **2022**, *180*, 95–107.
293. Liu, P.; Wang, W.; Li, Z.; Li, Y.; Yu, X.; Tu, J.; Zhang, Z. Ferroptosis: A new regulatory mechanism in osteoporosis. *Oxid. Med. Cell. Longev.* **2022**, *2022*, 2634431.
294. Park, C.K.; Lee, Y.; Kim, K.H.; Lee, Z.H.; Joo, M.; Kim, H.-H. Nrf2 is a novel regulator of bone acquisition. *Bone* **2014**, *63*, 36–46.
295. Xiang, S.; Zhao, L.; Tang, C.; Ling, L.; Xie, C.; Shi, Y.; Liu, W.; Li, X.; Cao, Y. Icaritin inhibits osteoblast ferroptosis via Nrf2/HO-1 signaling and enhances healing of osteoporotic fractures. *Eur. J. Pharmacol.* **2024**, *965*, 176244.
296. Jing, X.; Du, T.; Chen, K.; Guo, J.; Xiang, W.; Yao, X.; Sun, K.; Ye, Y.; Guo, F. Icaritin protects against iron overload-induced bone loss via suppressing oxidative stress. *J. Cell Physiol.* **2019**, *234*, 10123–10137.
297. Yan, C.-Y.; Gu, X.-Y.; Tan, S.-Y.; Mei, A.-Y.; Mao, J.-H.; Dai, Y.; Niu, J.; Li, W.-X.; Kurihara, H.; Li, Y.-F.; et al. Lipid peroxidation inhibition by icaritin and its glycosides as a strategy to combat iron overload-induced osteoporosis in zebrafish. *Food Res. Int.* **2025**, *203*, 115900.
298. Bedard, K.; Krause, K.-H. The NOX family of ROS-generating NADPH oxidases: Physiology and pathophysiology. *Physiol. Rev.* **2007**, *87*, 245–313.
299. Ji, R.; Wu, D.; Liu, Q. Icaritin inhibits RANKL-induced osteoclastogenesis in RAW264.7 cells via inhibition of reactive oxygen species production by reducing the expression of NOX1 and NOX4. *Biochem. Biophys. Res. Commun.* **2022**, *600*, 6–13.
300. Bai, X.; Lu, D.; Bai, J.; Zheng, H.; Ke, Z.; Li, X.; Luo, S. Oxidative stress inhibits osteoblastic differentiation of bone cells by ERK and NF- κ B. *Biochem. Biophys. Res. Commun.* **2004**, *314*, 197–207.
301. Qi, X.-C.; Li, B.; Wu, W.-L.; Liu, H.-C.; Jiang, Y.-P. Protective effect of hyperoside against hydrogen peroxide-induced dysfunction and oxidative stress in osteoblastic MC3T3-E1 cells. *Artif. Cells Nanomed. Biotechnol.* **2020**, *48*, 377–383.

302. Satué, M.; del Mar Arriero, M.; Monjo, M.; Ramis, J.M. Quercitrin and taxifolin stimulate osteoblast differentiation in MC3T3-E1 cells and inhibit osteoclastogenesis in RAW 264.7 cells. *Biochem. Pharmacol.* **2013**, *86*, 1476–1486.
303. Shin, G.-C.; Kim, C.; Lee, J.-M.; Cho, W.-S.; Lee, S.-G.; Jeong, M.; Cho, J.; Lee, K. Apigenin-induced apoptosis is mediated by reactive oxygen species and activation of ERK1/2 in rheumatoid fibroblast-like synoviocytes. *Chem. Biol. Interact.* **2009**, *182*, 29–36.
304. Sun, Q.; Jiang, S.; Yang, K.; Zheng, J.; Zhang, L.; Xu, W. Apigenin enhances the cytotoxic effects of tumor necrosis factor-related apoptosis-inducing ligand in human rheumatoid arthritis fibroblast-like synoviocytes. *Mol. Biol. Rep.* **2012**, *39*, 5529–5535.
305. Chen, C.-Y.; Peng, W.-H.; Tsai, K.-D.; Hsu, S.-L. Luteolin suppresses inflammation-associated gene expression by blocking NF- κ B and AP-1 activation pathway in mouse alveolar macrophages. *Life Sci.* **2007**, *81*, 1602–1614.
306. Li, Y.; Liu, F.; Li, S.; Huang, W.; Zhou, S.; Han, Y.; Liu, L.; Li, Y.; Yin, Z. Luteolin regulating synthesis and catabolism of osteoarthritis chondrocytes via activating autophagy. *Heliyon* **2024**, *10*, e31028.
307. Van Rensburg, S.J.; Daniels, W.M.U.; Van Zyl, J.M.; Taljaard, J.J.F. A Comparative study of the effects of cholesterol, beta-sitosterol, beta-sitosterol glucoside, dehydro-epiandrosterone sulphate and melatonin on in vitro lipid peroxidation. *Metab. Brain Dis.* **2000**, *15*, 257–265.
308. Kumatia, E.K.; Ocloo, A.; Tung, N.H. Triterpenoid esters from *Capparis erythrocarpos* (Isert), Capparaceae, root bark ameliorates CFA-induced arthritis. *Saudi Pharm. J.* **2023**, *31*, 698–705.
309. Seo, S.-J.; Park, J.-Y.; Park, H.-J.; Hwang, J.-T. Protocatechuic acid impacts rotator cuff healing and reduces fatty degeneration in a chronic rotator cuff tear model in rats. *Clin. Shoulder Elb.* **2022**, *25*, 5.
310. Nugraha, A.P.; Ardani, I.G.A.W.; Sitalaksmi, R.M.; Ramadhani, N.F.; Rachmayanti, D.; Kumala, D.; Kharisma, V.D.; Rahmadani, D.; Puspitaningrum, M.S.; Rizqianti, Y.; et al. Anti-Peri-implantitis Bacteria's Ability of Robusta Green Coffee Bean (*Coffea Canephora*) Ethanol Extract: An In Silico and In Vitro Study. *Eur. J. Dent.* **2023**, *17*, 649–662.
311. Chen, W.; Li, W.; Xu, K.; Li, M.; Dai, L.; Shen, X.; Hu, Y.; Cai, K. Functionalizing titanium surface with PAMAM dendrimer and human BMP2 gene via layer-by-layer assembly for enhanced osteogenesis. *J. Biomed. Mater. Res. A* **2018**, *106*, 706–717.
312. Mont, M.A.; Cherian, J.J.; Sierra, R.J.; Jones, L.C.; Lieberman, J.R. Nontraumatic Osteonecrosis of the femoral head: Where do we stand today?: A ten-year update. *JBJS* **2015**, *97*, 1604–1627.
313. Ye, C.; Zhang, W.; Wang, S.; Jiang, S.; Yu, Y.; Chen, E.; Xue, D.; Chen, J.; He, R. Icariin promotes tendon-bone healing during repair of rotator cuff tears: A biomechanical and histological study. *Int. J. Mol. Sci.* **2016**, *17*, 1780.
314. Yao, Z.; Huang, W.; Yang, Y.; Zou, L.; Zhang, Y.; Zhang, J.; Luo, G. Investigation of the osteogenic effects of ICA and ICSII on rat bone marrow mesenchymal stem cells. *Sci. Rep.* **2025**, *15*, 3060.
315. Wimalawansa, S.J. Nitric oxide and bone. *Ann. N. Y. Acad. Sci.* **2010**, *1192*, 391–403.
316. Chung, B.-H.; Kim, J.-D.; Kim, C.-K.; Kim, J.H.; Won, M.-H.; Lee, H.-S.; Dong, M.-S.; Ha, K.-S.; Kwon, Y.-G.; Kim, Y.-M. Icariin stimulates angiogenesis by activating the MEK/ERK-and PI3K/Akt/eNOS-dependent signal pathways in human endothelial cells. *Biochem. Biophys. Res. Commun.* **2008**, *376*, 404–408. Erratum in *Biochem. Biophys. Res. Commun.* **2009**, *383*, 522.
317. Zhai, Y.-K.; Guo, X.-Y.; Ge, B.-F.; Zhen, P.; Ma, X.-N.; Zhou, J.; Ma, H.-P.; Xian, C.J.; Chen, K.-M. Icariin stimulates the osteogenic differentiation of rat bone marrow stromal cells via activating the PI3K-AKT-eNOS-NO-cGMP-PKG. *Bone* **2014**, *66*, 189–198.
318. Zhai, Y.-K.; Chen, K.-M.; Ge, B.-F.; Ma, H.-P.; Ming, L.-G.; Cheng, G.-Z. The changes of iNOS and NO in the osteogenic differentiation process of rat bone marrow stromal cells promoted by icaraside II. *Yao Xue Xue Bao* **2011**, *46*, 383–389.
319. Shui, Y.M.; Lv, G.Y.; Shan, L.T.; Fan, C.L.; Tian, N.; Zhang, L.; He, T.-C.; Gao, J.-L. Epimedin C promotes vascularization during BMP2-induced osteogenesis and tumor-associated angiogenesis. *Am. J. Chin. Med.* **2017**, *45*, 1093–1111.
320. Wong, S.K.; Chin, K.-Y.; Ima-Nirwana, S. Quercetin as an agent for protecting the bone: A review of the current evidence. *Int. J. Mol. Sci.* **2020**, *21*, 6448.
321. Zhang, Z.; Xu, W.; Zhang, Z.; Chen, X.; Jin, H.; Jiang, N.; Xu, H. The bone-protective benefits of kaempferol combined with metformin by regulation of osteogenesis-angiogenesis coupling in OVX rats. *Biomed. Pharmacother.* **2024**, *173*, 116364.
322. Qian, K.; Zheng, X.-X.; Wang, C.; Huang, W.-G.; Liu, X.-B.; Xu, S.-D.; Liu, D.-K.; Liu, M.-Y.; Lin, C.-S. β -Sitosterol inhibits rheumatoid synovial angiogenesis through suppressing VEGF signaling pathway. *Front. Pharmacol.* **2022**, *12*, 816477.
323. Kuramoto, H.; Nakanishi, T.; Yumoto, H.; Takegawa, D.; Mieda, K.; Hosaka, K. Caffeic Acid Phenethyl Ester Enhances Bone Repair-related Factors in MC3T3-E1 Cells. *Cell Biochem. Biophys.* **2024**, *83*, 2323–2331.
324. Da Costa, A.A.B.A.; Chowdhury, D.; Shapiro, G.I.; D'Andrea, A.D.; Konstantinopoulos, P.A. Targeting replication stress in cancer therapy. *Nat. Rev. Drug Discov.* **2023**, *22*, 38–58.

325. Chen, C.; Wang, S.; Wang, N.; Zheng, Y.; Zhou, J.; Hong, M.; Chen, Z.; Wang, S.; Wang, Z.; Xiang, S. Icaritin inhibits prostate cancer bone metastasis and destruction via suppressing TAM/CCL5-mediated osteoclastogenesis. *Phytomedicine* **2023**, *120*, 155076.
326. Lin, S.W.; Li, X.Q.; Liu, S.Y.; Shi, J.M.; Xu, J.H.; Mao, L.H.; Yin, M. Inhibition of combination of Icaritin and doxorubicin on human osteosarcoma MG-63 cells in vitro. *Zhongguo Zhong Xi Yi Jie He Za Zhi* **2016**, *36*, 729–734.
327. Hu, J.; Chen, J.; Zhao, C.; Yu, P.; Xu, W.; Yin, Y.; Yang, L.; Zhang, Z.; Kong, L.; Zhang, C. Icariside II inhibits Epithelial-Mesenchymal transition in metastatic osteosarcoma by antagonizing the miR-194/215 cluster via PGK1. *Biochem. Pharmacol.* **2025**, *236*, 116838.
328. Hou, J.; Qian, J.; Li, Z.; Gong, A.; Zhong, S.; Qiao, L.; Qian, S.; Zhang, Y.; Dou, R.; Li, R.; et al. Bioactive compounds from *Abelmoschus manihot* L. alleviate the progression of multiple myeloma in mouse model and improve bone marrow microenvironment. *Onco. Targets Ther.* **2020**, *13*, 959–973.
329. Shi, Y.; Lian, K.; Jia, J. Apigenin suppresses the Warburg effect and stem-like properties in SOSP-9607 cells by inactivating the PI3K/Akt/mTOR signaling pathway. *Evid. Based Complement. Alternat. Med.* **2022**, *2022*, 3983637.
330. Wang, Y.; Shang, G.; Wang, W.; Qiu, E.; Pei, Y.; Zhang, X. Magnoflorine inhibits the malignant phenotypes and increases cisplatin sensitivity of osteosarcoma cells via regulating miR-410-3p/HMGB1/NF- κ B pathway. *Life Sci.* **2020**, *256*, 117967.
331. Inacio, K.K.; de Souza Pessoa, A.; Tokuhara, C.K.; Pagnan, A.L.; Sanches, M.L.R.; Fakhoury, V.S.; de Oliveira, G.S.N.; de Oliveira, F.A.; Ximenes, V.F.; de Oliveira, R.C. Menadione and protocatechuic acid: A drug combination with antitumor effects in murine osteosarcoma cells. *Arch. Biochem. Biophys.* **2024**, *751*, 109840.
332. Moutos, F.T.; Guilak, F. Composite scaffolds for cartilage tissue engineering. *Biorheology* **2008**, *45*, 501–512.
333. Liu, Y.; Zhou, G.; Cao, Y. Recent progress in cartilage tissue engineering—our experience and future directions. *Engineering* **2017**, *3*, 28–35.
334. Liu, M.-H.; Sun, J.-S.; Tsai, S.-W.; Sheu, S.-Y.; Chen, M.-H. Icaritin protects murine chondrocytes from lipopolysaccharide-induced inflammatory responses and extracellular matrix degradation. *Nutr. Res.* **2010**, *30*, 57–65.
335. Xu, C.Q.; Liu, B.-J.; Wu, J.-F.; Xu, Y.-C.; Duan, X.-H.; Cao, Y.-X.; Dong, J.-C. Icaritin attenuates LPS-induced acute inflammatory responses: Involvement of PI3K/Akt and NF- κ B signaling pathway. *Eur. J. Pharmacol.* **2010**, *642*, 146–153.
336. Li, D.; Yuan, T.; Zhang, X.; Xiao, Y.; Wang, R.; Fan, Y. Icaritin: A potential promoting compound for cartilage tissue engineering. *Osteoarthr. Cartil.* **2012**, *20*, 1647–1656.
337. Liu, D.; Tang, W.; Zhang, H.; Huang, H.; Zhang, Z.; Tang, D.; Jiao, F. Icaritin protects rabbit BMSCs against OGD-induced apoptosis by inhibiting ERs-mediated autophagy via MAPK signaling pathway. *Life Sci.* **2020**, *253*, 117730.
338. Tang, W.; Zhang, H.; Liu, D.; Jiao, F. Icaritin accelerates cartilage defect repair by promoting chondrogenic differentiation of BMSCs under conditions of oxygen-glucose deprivation. *J. Cell Mol. Med.* **2022**, *26*, 202–215.
339. Ziadlou, R.; Barbero, A.; Martin, I.; Wang, X.; Qin, L.; Alini, M.; Grad, S. Anti-inflammatory and chondroprotective effects of vanillic acid and epimedin C in human osteoarthritic chondrocytes. *Biomolecule* **2020**, *10*, 932.
340. Nepal, M.; Li, L.; Cho, H.K.; Park, J.K.; Soh, Y. Kaempferol induces chondrogenesis in ATDC5 cells through activation of ERK/BMP-2 signaling pathway. *Food Chem. Toxicol.* **2013**, *62*, 238–245.
341. Kulyar, M.F.-A.; Yao, W.; Ding, Y.; Du, H.; Li, K.; Zhang, L.; Li, A.; Huachun, P.; Waqas, M.; Mehmood, K.; et al. Cluster of differentiation 147 (CD147) expression is linked with thiram induced chondrocyte's apoptosis via Bcl-2/Bax/Caspase-3 signaling in tibial growth plate under chlorogenic acid reperussion. *Ecotoxicol. Environ. Saf.* **2021**, *213*, 112059.
342. Zhang, J.; Huang, S.; Tong, X.; Zhang, L.; Jiang, X.; Zhang, H.; Mehmood, K.; Li, J. Chlorogenic acid alleviates thiram-induced tibial dyschondroplasia by modulating caspases. BECN1 expression and ECM degradation. *Int. J. Mol. Sci.* **2019**, *20*, 3160.
343. Huang, X.; Xi, Y.; Pan, Q.; Mao, Z.; Zhang, R.; Ma, X.; You, H. Caffeic acid protects against IL-1 β -induced inflammatory responses and cartilage degradation in articular chondrocytes. *Biomed. Pharmacother.* **2018**, *107*, 433–439.
344. Wang, W.; Sun, W.; Jin, L. Caffeic acid alleviates inflammatory response in rheumatoid arthritis fibroblast-like synoviocytes by inhibiting phosphorylation of I κ B kinase α/β and I κ B α . *Int. Immunopharmacol.* **2017**, *48*, 61–66.
345. Josse, J.; Velard, F.; Gangloff, S.C. Staphylococcus aureus vs. osteoblast: Relationship and consequences in osteomyelitis. *Front. Cell Infect. Microbiol.* **2015**, *5*, 85.
346. Arciola, C.R.; Campoccia, D.; Montanaro, L. Implant infections: Adhesion, biofilm formation and immune evasion. *Nat. Rev. Microbiol.* **2018**, *16*, 397–409.
347. Nandi, S.K.; Mukherjee, P.; Roy, S.; Kundu, B.; De, D.K.; Basu, D. Local antibiotic delivery systems for the treatment of osteomyelitis—A review. *Mater. Sci. Eng. C* **2009**, *29*, 2478–2485.
348. Huang, D.N.; Wang, J.; Ren, K.F.; Ji, J. Functionalized biomaterials to combat biofilms. *Biomater. Sci.* **2020**, *8*, 4052–4066.

349. Liu, N.; Huang, S.; Guo, F.; Zhai, S.; Wang, D.; Li, F.; Liu, C. Calcium phosphate cement with icariin-loaded gelatin microspheres as a local drug delivery system for bone regeneration. *Biomed. Eng. Online* **2022**, *21*, 89.
350. Peris, M.C.; Martínez, A.; Ortíz, M.P.; Sheth, C.C.; Veses, V. Icariin in combination with amoxycillin-clavulanate and ampicillin, but not vancomycin, increases antibiotic sensitivity and growth inhibition against methicillin-resistant *Staphylococcus aureus*. *Antibiotic* **2022**, *11*, 233.
351. Kariu, T.; Hamada, N.; Lakshmyya, K. Luteolin inhibits *Porphyromonas gingivalis* growth and alleviates alveolar bone destruction in experimental murine periodontitis. *Biosci. Biotechnol. Biochem.* **2024**, *88*, 37–43.
352. Gao, L.; Tang, Z.; Li, T.; Wang, J. Combination of kaempferol and azithromycin attenuates *Staphylococcus aureus*-induced osteomyelitis via anti-biofilm effects and by inhibiting the phosphorylation of ERK1/2 and SAPK. *Pathog. Dis.* **2021**, *79*, ftab048.
353. Xie, Y.; Yang, W.; Tang, F.; Chen, X.; Ren, L. Antibacterial activities of flavonoids: Structure-activity relationship and mechanism. *Curr. Med. Chem.* **2015**, *22*, 132–149.
354. Zhai, Y.-K.; Ge, B.-F.; Chen, K.-M.; Ma, H.-P.; Ming, L.-G.; Li, Z.-F. Comparative study on the osteogenic differentiation of rat bone marrow stromal cells effected by icariin and icariside II. *Zhong Yao Cai* **2010**, *33*, 1896–1900.
355. Xu, W.; Zhang, Y.; Yang, M.; Shen, Z.; Zhang, X.; Zhang, W.; Li, H. LC-MS/MS method for the simultaneous determination of icariin and its major metabolites in rat plasma. *J. Pharm. Biomed. Anal.* **2007**, *45*, 667–672.
356. Wang, C.-G.; Sun, J.-B.; Wang, J.-C.; Zhang, Q.; Zheng, Y. LC determination of icariside II in rat plasma and tissues: Application to a tissue distribution study. *Chromatographia* **2011**, *74*, 251–258.
357. Li, Y.; Zhang, J.; Liu, A.; Zhang, C.; Li, Q.; Fan, F. Icariin: A potential lipid metabolism regulator in osteoarthritis. *Nat. Prod. Commun.* **2022**, *17*, 1934578X221126046.
358. Lu, S.; Zou, K.; Guo, B.; Pei, J.; Wang, Z.; Xiao, W.; Zhao, L. One-step purification and immobilization of thermostable β -glucosidase on Na-Y zeolite based on the linker and its application in the efficient production of baohuoside I from icariin. *Bioorg. Chem.* **2022**, *121*, 105690.
359. Xie, J.; Xu, H.; Jiang, J.; Zhang, N.; Yang, J.; Zhao, J.; Wei, M. Characterization of a novel thermostable glucose-tolerant GH1 β -glucosidase from the hyperthermophile *Ignisphaera aggregans* and its application in the efficient production of baohuoside I from icariin and total *Epimedium* flavonoids. *Bioorg. Chem.* **2020**, *104*, 104296.
360. Xie, L.; Liu, N.; Xiao, Y.; Liu, Y.; Yan, C.; Wang, G.; Jing, X. In vitro and in vivo osteogenesis induced by icariin and bone morphogenetic protein-2: A dynamic observation. *Front. Pharmacol.* **2020**, *11*, 1058.
361. Zhang, J.; Chen, Y.; Tang, Y.; Guo, S.; Ye, C.; Li, W. Quality differentiation of *Epimedium* from different harvesting periods based on LC-MS and network pharmacology. *New J. Chem.* **2024**, *48*, 9020–9029.
362. Nesbitt, S.A.; Horton, M.A. Trafficking of matrix collagens through bone-resorbing osteoclasts. *Science* **1997**, *276*, 266–269.
363. Suda, N.; Morita, I.; Kuroda, T.; Murota, S. Participation of oxidative stress in the process of osteoclast differentiation. *Biochim. Biophys. Acta-Gen. Subj.* **1993**, *1157*, 318–323.
364. Ha, H.; Kwak, H.B.; Lee, S.W.; Jin, H.M.; Kim, H.-M.; Kim, H.-H.; Lee, Z.H. Reactive oxygen species mediate RANK signaling in osteoclasts. *Exp. Cell Res.* **2004**, *301*, 119–127.
365. Lara-Castillo, N. Estrogen signaling in bone. *Appl. Sci.* **2021**, *11*, 4439.
366. Manolagas, S.C.; O'Brien, C.A.; Almeida, M. The role of estrogen and androgen receptors in bone health and disease. *Nat. Rev. Endocrinol.* **2013**, *9*, 699–712.
367. Haffner-Luntzer, M.; Kovtun, A.; Lackner, I.; Mödinger, Y.; Hacker, S.; Liedert, A.; Tuckermann, J.; Ignatius, A. Estrogen receptor α (ER α), but not ER β -signaling, is crucially involved in mechanostimulation of bone fracture healing by whole-body vibration. *Bone* **2018**, *110*, 11–20.
368. Cheng, C.-H.; Chen, L.-R.; Chen, K.-H. Osteoporosis due to hormone imbalance: An overview of the effects of estrogen deficiency and glucocorticoid overuse on bone turnover. *Int. J. Mol. Sci.* **2022**, *23*, 1376.
369. Sunter, A.; Armstrong, V.J.; Zaman, G.; Kypta, R.M.; Kawano, Y.; Lanyon, L.E.; Price, J.S. Mechano-transduction in osteoblastic cells involves strain-regulated estrogen receptor α -mediated control of insulin-like growth factor (IGF) I receptor sensitivity to ambient IGF, leading to phosphatidylinositol 3-kinase/AKT-dependent Wnt/LRP5 receptor-independent activation of β -catenin signaling. *J. Biol. Chem.* **2010**, *285*, 8743–8758.
370. Saki, F.; Sadeghian, F.; Kasaei, S.R.; Talezadeh, P.; Omrani, G.H.R. The effect of prolactin itself and in combination with estrogen on bone mineral density in female rats. *Gynecol. Endocrinol.* **2019**, *35*, 539–543.
371. Shen, P.; Wong, S.P.; Yong, E. Sensitive and rapid method to quantify icaritin and desmethylicaritin in human serum using gas chromatography-mass spectrometry. *J. Chromatogr. B* **2007**, *857*, 47–52.

372. Cao, Y.-F.; He, R.-R.; Cao, J.; Chen, J.-X.; Huang, T.; Liu, Y. Drug-drug interactions potential of icariin and its intestinal metabolites via inhibition of intestinal UDP-Glucuronosyltransferases. *Evid. Based Complement. Alternat. Med.* **2012**, *2012*, 395912.
373. Xiao, H.-H.; Fung, C.-Y.; Mok, S.-K.; Wong, K.-C.; Ho, M.-X.; Wang, X.-L.; Yao, X.-S.; Wong, M.-S. Flavonoids from *Herba epimedii* selectively activate estrogen receptor alpha (ER α) and stimulate ER-dependent osteoblastic functions in UMR-106 cells. *J. Steroid Biochem. Mol. Biol.* **2014**, *143*, 141–151.
374. Wong, S.P.; Shen, P.; Lee, L.; Li, J.; Yong, E.L. Pharmacokinetics of prenylflavonoids and correlations with the dynamics of estrogen action in sera following ingestion of a standardized *Epimedium* extract. *J. Pharm. Biomed. Anal.* **2009**, *50*, 216–223.
375. Liu, J.; Ye, H.; Lou, Y. Determination of rat urinary metabolites of icariin in vivo and estrogenic activities of its metabolites on MCF-7 cells. *Pharmazie* **2005**, *60*, 120–125.
376. Liu, J.; Lou, Y. Determination of icariin and metabolites in rat serum by capillary zone electrophoresis: Rat pharmacokinetic studies after administration of icariin. *J. Pharm. Biomed. Anal.* **2004**, *36*, 365–370.
377. Xu, S.; Yu, J.; Zhan, J.; Yang, L.; Guo, L.; Xu, Y. Pharmacokinetics, tissue distribution, and metabolism study of icariin in rat. *Biomed. Res. Int.* **2017**, *2017*, 4684962.
378. Wu, H.; Kim, M.; Han, J. Icariin metabolism by human intestinal microflora. *Molecules* **2016**, *21*, 1158.
379. Jiang, J.; He, J.; Xiao, S.; Shenyuan, J.; Chen, T.; Pei, D. Screening of superior anti-osteoporotic flavonoids from *Epimedium Folium* with dual effects of reversing iron overload and promoting osteogenesis. *Biomed. Chromatogr.* **2023**, *37*, e5686.
380. Wang, Z.; Liu, C.; Yu, H.; Wu, B.; Huai, B.; Zhuang, Z.; Sun, C.; Xu, L.; Jin, F. Icaritin preparation from icariin by a special *Epimedium* flavonoid-glycosidase from *Aspergillus* sp. y848 strain. *J. Microbiol. Biotechnol.* **2022**, *32*, 437.
381. Daina, A.; Michielin, O.; Zoete, V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci. Rep.* **2017**, *7*, 42717.
382. Daina, A.; Michielin, O.; Zoete, V. SwissTargetPrediction: Updated data and new features for efficient prediction of protein targets of small molecules. *Nucleic Acids Res.* **2019**, *47*, W357–W364.
383. Stelzer, G.; Rosen, N.; Plaschkes, I.; Zimmerman, S.; Twik, M.; Fishilevich, S.; Stein, T.I.; Nudel, R.; Lieder, I.; Mazor, Y.; et al. The GeneCards suite: From gene data mining to disease genome sequence analyses. *Curr. Protoc. Bioinform.* **2016**, *54*, 1.30.1–1.30.33.
384. Amberger, J.S.; Hamosh, A. Searching Online Mendelian Inheritance in Man (OMIM): A knowledgebase of human genes and genetic phenotypes. *Curr. Protoc. Bioinform.* **2017**, *58*, 1.2.1–1.2.12.
385. Zhou, Y.; Zhang, Y.; Zhao, D.; Yu, X.; Shen, X.; Zhou, Y.; Wang, S.; Qiu, Y.; Chen, Y.; Zhu, F. TTD: Therapeutic Target Database describing target druggability information. *Nucleic Acids Res.* **2024**, *52*, D1465–D1477.
386. UniProt Consortium. UniProt: The Universal Protein Knowledgebase in 2025. *Nucleic Acids Res.* **2025**, *53*, D609–D617.
387. Tang, D.; Chen, M.; Huang, X.; Zhang, G.; Zeng, L.; Zhang, G.; Wu, S.; Wang, Y. SRplot: A free online platform for data visualization and graphing. *PLoS ONE* **2023**, *18*, e0294236.
388. Amberger, J.S.; Bocchini, C.A.; Scott, A.F.; Hamosh, A. OMIM.org: Leveraging knowledge across phenotype-gene relationships. *Nucleic Acids Res.* **2019**, *47*, D1038–D1043.
389. Szklarczyk, D.; Gable, A.L.; Lyon, D.; Junge, A.; Wyder, S.; Huerta-Cepas, J.; Simonovic, M.; Doncheva, N.T.; Morris, J.H.; Bork, P.; et al. STRING v11: Protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res.* **2019**, *47*, D607–D613.
390. Shannon, P.; Markiel, A.; Ozier, O.; Baliga, N.S.; Wang, J.T.; Ramage, D.; Amin, N.; Schwikowski, B.; Ideker, T. Cytoscape: A software environment for integrated models of biomolecular interaction networks. *Genome Res.* **2003**, *13*, 2498–2504.
391. Huang, D.W.; Sherman, B.T.; Lempicki, R.A. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat. Protoc.* **2009**, *4*, 44–57.
392. Trott, O.; Olson, A.J. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* **2010**, *31*, 455–461.
393. Guo, B.; Zhao, C.; Zhang, C.; Xiao, Y.; Yan, G.; Liu, L.; Pan, H. Elucidation of the anti-inflammatory mechanism of Er Miao San by integrative approach of network pharmacology and experimental verification. *Pharmacol. Res.* **2022**, *175*, 106000.
394. Hwang, J.-W.; Yao, H.; Caito, S.; Sundar, I.K.; Rahman, I. Redox regulation of SIRT1 in inflammation and cellular senescence. *Free Radic. Biol. Med.* **2013**, *61*, 95–110.
395. Iyer, S.; Han, L.; Bartell, S.M.; Kim, H.-N.; Gubrij, I.; de Cabo, R.; O'Brien, C.A.; Manolagas, S.C.; Almeida, M. Sirtuin1 (Sirt1) promotes cortical bone formation by preventing β -catenin sequestration by FoxO transcription factors in osteoblast progenitors. *J. Biol. Chem.* **2014**, *289*, 24069–24078.

396. Xu, C.; Xu, Z.; Zhang, Y.; Evert, M.; Calvisi, D.F.; Chen, X. β -Catenin signaling in hepatocellular carcinoma. *J. Clin. Investig.* **2022**, *132*, e154515.
397. Xu, K.; Deng, S.; Zhu, Y.; Yang, W.; Chen, W.; Huang, L.; Zhang, C.; Li, M.; Ao, L.; Jiang, Y.; et al. Platelet rich plasma loaded multifunctional hydrogel accelerates diabetic wound healing via regulating the continuously abnormal microenvironments. *Adv. Healthc. Mater.* **2023**, *12*, e2301370.
398. Shi, Y.; Wang, S.; Wang, K.; Yang, R.; Liu, D.; Liao, H.; Qi, Y.; Qiu, K.; Hu, Y.; Wen, H.; et al. Relieving macrophage dysfunction by inhibiting SREBP2 activity: A hypoxic mesenchymal stem cells-derived exosomes loaded multifunctional hydrogel for accelerated diabetic wound healing. *Small* **2024**, *20*, 2309276.
399. Deng, Z.H.; Yang, Z.L.; Yi, S.; Liu, Z.Q. Hydrogel-exosome systems in the treatment of refractory diabetic ulcers: Mechanisms, progress, and prospects. *Eur. Cell Mater.* **2025**, *53*, 82–97.
400. Liu, D.; Lu, G.; Shi, B.; Ni, H.; Wang, J.; Qiu, Y.; Yang, L.; Zhu, Z.; Yi, X.; Du, X.; et al. ROS-scavenging hydrogels synergize with neural stem cells to enhance spinal cord injury repair via regulating microenvironment and facilitating nerve regeneration. *Adv. Healthc. Mater.* **2023**, *12*, e2300123. Erratum in *Adv. Healthc. Mater.* **2025**, *14*, e2501967.
401. Chen, J.; Guan, X.; Chen, L.; Zheng, B.; Li, F.; Fang, C.; Fu, Y.; Li, X.; Wang, H.; Zhou, Y. Customized Hydrogel System for the Spatiotemporal Sequential Treatment of Periodontitis Propelled by ZEB1. *Adv. Sci.* **2025**, *12*, 2503338.
402. García-Sobrino, R.; Casado-Losada, I.; Caltagirone, C.; García-Crespo, A.; García, C.; Rodríguez-Hernández, J.; Reinecke, H.; Gallardo, A.; Elvira, C.; Martínez-Campos, E. Osteoblastic cell sheet engineering using P(VCL-HEMA)-based thermosensitive hydrogels doped with pVCL@icariin nanoparticles obtained with supercritical CO₂-SAS. *Pharmaceutics* **2024**, *16*, 1063.
403. Zhu, C.; Jia, Y.; Tang, Y.; Guo, C.; Xi, J.; Sun, C.; Li, H.; Wang, W.; Zhai, Y.; Zhu, Y.; et al. Functionalized chitosan hydrogel promotes osseointegration at the interface of 3D printed titanium alloy scaffolds. *Int. J. Biol. Macromol.* **2024**, *266*, 131169.
404. Song, Y.; Ma, A.; Ning, J.; Zhong, X.; Zhang, Q.; Zhang, X.; Hong, G.; Li, Y.; Sasaki, K.; Li, C. Loading icariin on titanium surfaces by phase-transited lysozyme priming and layer-by-layer self-assembly of hyaluronic acid/chitosan to improve surface osteogenesis ability. *Int. J. Nanomed.* **2018**, *13*, 6751–6767.
405. Fan, J.; Bi, L.; Wu, T.; Cao, L.; Wang, D.; Nan, K.; Chen, J.; Jin, D.; Jiang, S.; Pei, G. A combined chitosan/nano-size hydroxyapatite system for the controlled release of icariin. *J. Mater. Sci. Mater. Med.* **2012**, *23*, 399–407.
406. Shao, B.; Fu, Y.; Li, B.; Huo, S.; Du, J.; Zhang, X.; Yin, X.; Li, Y.; Cao, Z.; Song, M. Icariin-loaded chitosan/ β -glycerophosphate thermosensitive hydrogel enhanced infection control and bone regeneration in canine with infectious bone defects. *J. Biomater. Appl.* **2025**, *39*, 696–713.
407. Zhang, J.; Fan, F.; Zhang, C.; Liu, A.; Shang, M.; Meng, L. Icariin-conditioned serum combined with chitosan attenuates cartilage injury in rabbit knees with osteochondral defect. *J. Orthop. Surg. Res.* **2023**, *18*, 125.
408. Fu, J.; Chen, H.; Zhao, Y.; Xi, H.; Huang, Y.; Liu, C.; Wu, Y.; Song, W.; Liu, X.; Du, B.; et al. Self-assembled injectable Icariin@Ti₃C₂T_x/doxorubicin hydrogel preserving osteogenesis while synergizing photodynamic and chemodynamic therapy for osteosarcoma. *J. Mater. Sci. Mater. Med.* **2025**, *36*, 28.
409. Jin, Q.R.; Zhou, T.J.; Yao, Y.T.; Yu, H.Q.; Che, H.B.; Zhang, M.M.; Cui, C.-H.; Wang, Y.; Li, C.-J.; Xing, L.; et al. Icaritin-incorporated dual-sensitizing hydrogel for osteosarcoma doxorubicin treatment and bone regeneration. *Chin. Chem. Lett.* **2025**, *36*, 111539.
410. Yu, H.; Teng, Y.; Ge, J.; Yang, M.; Xie, H.; Wu, T.; Yan, Q.; Jia, M.; Zhu, Q.; Shen, Y.; et al. Isoginkgetin-loaded reactive oxygen species scavenging nanoparticles ameliorate intervertebral disc degeneration via enhancing autophagy in nucleus pulposus cells. *J. Nanobiotechnology* **2023**, *21*, 99.
411. Hu, B.; Lin, S.; Lin, S.; Rui, G. Ginkgetin alleviates intervertebral disc degeneration by inhibiting apoptosis, inflammation, and disturbance of extracellular matrix synthesis and catabolism via inactivation of NLRP3 inflammasome. *Immunol. Investig.* **2023**, *52*, 546–560.
412. Chen, J.; Gan, X.; Su, S.; Jiao, S.; Gong, Z.; Liu, Z.; Wu, X.; Jiang, J.; Zhang, J.; Wan, J. Conditional sequential delivery of ginkgetin and rapamycin orchestrates inflammation and autophagy to alleviate intervertebral disc degeneration. *J. Control. Release* **2025**, *381*, 113556.
413. Cai, Z.; Feng, Y.; Li, C.; Yang, K.; Sun, T.; Xu, L.; Chen, Y.; Yan, C.-H.; Lu, W.W.; Chiu, K.-Y. Magnoflorine with hyaluronic acid gel promotes subchondral bone regeneration and attenuates cartilage degeneration in early osteoarthritis. *Bone* **2018**, *116*, 266–278.
414. Cai, Z.; Hong, M.; Xu, L.; Yang, K.; Li, C.; Sun, T.; Feng, Y.; Zeng, H.; Lu, W.W.; Chiu, K.-Y. Prevent action of magnoflorine with hyaluronic acid gel from cartilage degeneration in anterior cruciate ligament transection induced osteoarthritis. *Biomed. Pharmacother.* **2020**, *126*, 109733.

415. Mohammadi, M.; Abbaszadeh, S.; Nosrati-Siahmazgi, V.; Akbari, M.; Rezaei, S.; Musaie, K.; Eskandari, M.R.; Santos, H.A.; Poursina, N.; Shahbazi, M.-A. Diatom-guided bone healing via a hybrid natural scaffold. *Heliyon* **2024**, *10*, e25878.
416. Lee, C.-S.; Hwang, H.S.; Kim, S.; Fan, J.; Aghaloo, T.; Lee, M. Inspired by nature: Facile design of nanoclay-organic hydrogel bone sealant with multifunctional properties for robust bone regeneration. *Adv. Funct. Mater.* **2020**, *30*, 2003717.
417. Gao, S.; Li, H.; Li, Z.; Wang, H.; Li, X.; Yang, S.; Huang, L.; Zhang, B.; Zhang, K.; Tsoi, J.K.H.; et al. Multifunctional injectable bioadhesive with Toll-like Receptor 4 and Myeloid Differentiation Factor 2 antagonistic anti-inflammatory potential for periodontal regeneration. *ACS Nano* **2025**, *19*, 7098–7116.
418. Palaniraj, S.; Murugesan, R.; Narayan, S. Chlorogenic acid-loaded calcium phosphate chitosan nanogel as biofilm degradative materials. *Int. J. Biochem. Cell Biol.* **2019**, *114*, 105566.
419. Fang, Y.; Hu, J.; Zou, Y.; Wang, Z.; Ye, Y.; Zhang, C. Neochlorogenic acid combined with bone marrow mesenchymal stem cells encapsulated into GelMA hydrogel for transplantation to repair intervertebral disk degeneration. *Biomacromolecule* **2024**, *25*, 729–740.
420. Xia, L.; Li, Y.; Zhou, Z.; Dai, Y.; Liu, H.; Liu, H. Icaritin delivery porous PHBV scaffolds for promoting osteoblast expansion in vitro. *Mater. Sci. Eng. C* **2013**, *33*, 3545–3552.
421. Park, S.; Pascal, A.B.; Eisig, S.B.; Feng, M.; Jeong, H.J.; Zhu, E.; Zhang, E.; Lee, C.H. Icaritin-Releasing 3-Dimensionally Printed Scaffolds for Alveolar Cleft Reconstruction. *Biomater. Res.* **2025**, *29*, 199.
422. Gao, F.; Zhang, Q.; Yi, W.; Zhang, T.; Wang, Z.; Ge, S.; Ma, B. Tetrahedral DNA Nanoframework-Based Multivalent Aptamers Functionalized Biomimetic Hydrogel Scaffold Enhances Osteochondral Regeneration by Recruitment and Protection of Endogenous Stem Cells. *Adv. Funct. Mater.* **2025**, *35*, 2502871.
423. Li, M.; Zhang, C.; Zhong, Y.; Zhao, J. A novel approach to utilize icaritin as icaritin-derived ECM on small intestinal submucosa scaffold for bone repair. *Ann. Biomed. Eng.* **2017**, *45*, 2673–2682.
424. Zhang, J.T.; Tancrét, F.; Bouler, J.-M. Fabrication and mechanical properties of calcium phosphate cements (CPC) for bone substitution. *Mater. Sci. Eng. C* **2011**, *31*, 740–747.
425. Reitmaier, S.; Kovtun, A.; Schuelke, J.; Kanter, B.; Lemm, M.; Hoess, A.; Heinemann, S.; Nies, B.; Ignatius, A. Strontium (II) and mechanical loading additively augment bone formation in calcium phosphate scaffolds. *J. Orthop. Res.* **2018**, *36*, 106–117.
426. Luo, G.; Huang, Y.; Gu, F. rhBMP2-loaded calcium phosphate cements combined with allogenic bone marrow mesenchymal stem cells for bone formation. *Biomed. Pharmacother.* **2017**, *92*, 536–543.
427. Ramirez, J.E.V.; Sharpe, L.A.; Peppas, N.A. Current state and challenges in developing oral vaccines. *Adv. Drug Deliv. Rev.* **2017**, *114*, 116–131. Erratum in *Adv Drug Deliv Rev.* **2019**, *139*, 158.
428. Xin, D.; Li, T.; Chu, X.; Ke, H.; Yu, Z.; Cao, L.; Bai, X.; Liu, D.; Wang, Z. Mesenchymal stromal cell-derived extracellular vesicles modulate microglia/macrophage polarization and protect the brain against hypoxia-ischemic injury in neonatal mice by targeting delivery of miR-21a-5p. *Acta Biomater.* **2020**, *113*, 597–613.
429. Åberg, J.; Pankotai, E.; Billström, G.H.; Weszl, M.; Larsson, S.; Forster-Horváth, C.; Lacza, Z.; Engqvist, H. In vivo evaluation of an injectable premixed radiopaque calcium phosphate cement. *Int. J. Biomater.* **2011**, *2011*, 232574.
430. Åberg, J.; Henriksson, H.B.; Engqvist, H.; Palmquist, A.; Brantsing, C.; Lindahl, A.; Thomsen, P.; Brisby, H. Biocompatibility and resorption of a radiopaque premixed calcium phosphate cement. *J. Biomed. Mater. Res. A* **2012**, *100*, 1269–1278.
431. Wu, Y.; Cao, L.; Xia, L.; Wu, Q.; Wang, J.; Wang, X.; Xu, L.; Zhou, Y.; Xu, Y.; Jiang, X. Evaluation of osteogenesis and angiogenesis of icaritin in local controlled release and systemic delivery for calvarial defect in ovariectomized rats. *Sci. Rep.* **2017**, *7*, 5077.
432. Luo, G.; Yao, Z.; Yang, Y.; Chai, J.; Fu, L. Icaritin II-loaded calcium phosphate cement scaffolds combined with bone marrow mesenchymal stem cells for osteogenesis. *J. Biomater. Tissue Eng.* **2023**, *13*, 364–370.
433. Wu, Z.; Kaewmanee, R.; Yang, Q.; Wang, Z.; Xie, E.; Wei, J.; Zhang, C. Luteolin-loaded biocomposites containing tantalum and polyimide with antibacterial effects for facilitating osteogenic differentiation and bone bonding. *J. Mater. Chem. B* **2023**, *11*, 10218–10233.
434. Song, T.; Yang, J.; Liu, P.; Liu, M.; Li, D.; Xiao, Y.; Wang, Y.; Zhang, X. Icaritin self-crosslinked network functionalized strontium-doped bioceramic scaffolds synergistically enhanced the healing of osteoporotic bone defects. *Compos. B Eng.* **2022**, *235*, 109759.
435. LeGeros, R.Z. Calcium phosphate-based osteoinductive materials. *Chem. Rev.* **2008**, *108*, 4742–4753.
436. Lin, Z.; Liu, J.; Zhong, L.; Niu, W.; Hu, X. Icaritin-based bone scaffold for treating steroid-induced necrosis of femoral head by restoring angiogenesis and promoting osteogenesis. *Mater. Des.* **2025**, *253*, 113913.
437. Jones, J.R. Review of bioactive glass: From Hench to hybrids. *Acta Biomater.* **2013**, *9*, 4457–4486.

438. Liu, X.; Xie, Z.; Zhang, C.; Pan, H.; Rahaman, M.N.; Zhang, X.; Fu, Q.; Huang, W. Bioactive borate glass scaffolds: In vitro and in vivo evaluation for use as a drug delivery system in the treatment of bone infection. *J. Mater. Sci. Mater. Med.* **2010**, *21*, 575–582.
439. Ranjbar, F.E.; Farzad-Mohajeri, S.; Samani, S.; Saremi, J.; Khademi, R.; Dehghan, M.M.; Azami, M. Kaempferol-loaded bioactive glass-based scaffold for bone tissue engineering: In vitro and in vivo evaluation. *Sci. Rep.* **2023**, *13*, 12375.
440. Jiang, S.; Wang, M.; He, J. A review of biomimetic scaffolds for bone regeneration: Toward a cell-free strategy. *Bioeng. Transl. Med.* **2021**, *6*, e10206.
441. Shin, H.; Jo, S.; Mikos, A.G. Biomimetic materials for tissue engineering. *Biomaterial* **2003**, *24*, 4353–4364.
442. Shi, G.-S.; Li, Y.-Y.; Luo, Y.-P.; Jin, J.-F.; Sun, Y.-X.; Zheng, L.-Z.; Lai, Y.-X.; Li, L.; Fu, G.; Qin, L.; et al. Bioactive PLGA/tricalcium phosphate scaffolds incorporating phytomolecule icaritin developed for calvarial defect repair in rat model. *J. Orthop. Translat.* **2020**, *24*, 112–120.
443. Won, J.Y.; Park, C.Y.; Bae, J.H.; Ahn, G.; Kim, C.; Lim, D.H.; Cho, D.W.; Yun, W.S.; Shim, J.H.; Huh, J.B. Evaluation of 3D printed PCL/PLGA/ β -TCP versus collagen membranes for guided bone regeneration in a beagle implant model. *Biomed. Mater.* **2016**, *11*, 55013.
444. Kim, H.D.; Amirthalingam, S.; Kim, S.L.; Lee, S.S.; Rangasamy, J.; Hwang, N.S. Biomimetic materials and fabrication approaches for bone tissue engineering. *Adv. Healthc. Mater.* **2017**, *6*, 1700612.
445. Cheng, W.X.; Liu, Y.Z.; Meng, X.B.; Zheng, Z.T.; Li, L.L.; Ke, L.Q.; Li, L.; Huang, C.-S.; Zhu, G.-Y.; Pan, H.-D.; et al. PLGA/ β -TCP composite scaffold incorporating cucurbitacin B promotes bone regeneration by inducing angiogenesis. *J. Orthop. Translat.* **2021**, *31*, 41–51.
446. Lai, Y.; Cao, H.; Wang, X.; Chen, S.; Zhang, M.; Wang, N.; Yao, Z.; Dai, Y.; Xie, X.; Zhang, P.; et al. Porous composite scaffold incorporating osteogenic phytomolecule icariin for promoting skeletal regeneration in challenging osteonecrotic bone in rabbits. *Biomaterials* **2018**, *153*, 1–13.
447. Wang, X.-L.; Xie, X.-H.; Zhang, G.; Chen, S.-H.; Yao, D.; He, K.; Wang, X.-H.; Yao, X.-S.; Leng, Y.; Fung, K.-P.; et al. Exogenous phytoestrogenic molecule icaritin incorporated into a porous scaffold for enhancing bone defect repair. *J. Orthop. Res.* **2013**, *31*, 164–172.
448. Zhang, X.; Wang, X.; Lee, Y.; Feng, L.; Wang, B.; Pan, Q.; Meng, X.; Cao, H.; Li, L.; Wang, H.; et al. Bioactive scaffold fabricated by 3D printing for enhancing osteoporotic bone regeneration. *Bioengineering* **2022**, *9*, 525.
449. Qin, L.; Yao, D.; Zheng, L.; Liu, W.-C.; Liu, Z.; Lei, M.; Huang, L.; Xie, X.; Wang, X.; Chen, Y.; et al. Phytomolecule icaritin incorporated PLGA/TCP scaffold for steroid-associated osteonecrosis: Proof-of-concept for prevention of hip joint collapse in bipedal emus and mechanistic study in quadrupedal rabbits. *Biomaterial* **2015**, *59*, 125–143.
450. Chen, S.-H.; Lei, M.; Xie, X.-H.; Zheng, L.-Z.; Yao, D.; Wang, X.-L.; Li, W.; Zhao, Z.; Kong, A.; Xiao, D.-M.; et al. PLGA/TCP composite scaffold incorporating bioactive phytomolecule icaritin for enhancement of bone defect repair in rabbits. *Acta Biomater.* **2013**, *9*, 6711–6722.
451. Gu, C.; Chen, H.; Zhao, Y.; Xi, H.; Tan, X.; Xue, P.; Sun, G.; Jiang, X.; Du, B.; Liu, X. Ti₃C₂T_x@ PLGA/Icaritin microspheres-modified PLGA/ β -TCP scaffolds modulate Icaritin release to enhance bone regeneration through near-infrared response. *Biomed. Mater.* **2024**, *19*, 55038.
452. Fan, J.; Lee, C.-S.; Kim, S.; Zhang, X.; Pi-Anfruns, J.; Guo, M.; Chen, C.; Rahnama, M.; Li, J.; Wu, B.M.; et al. Trb3 controls mesenchymal stem cell lineage fate and enhances bone regeneration by scaffold-mediated local gene delivery. *Biomaterials* **2021**, *264*, 120445.
453. Shen, X.; Yu, P.; Chen, H.; Wang, J.; Lu, B.; Cai, X.; Gu, C.; Liang, G.; Hao, D.; Ma, Q.; et al. Icariin controlled release on a silk fibroin/mesoporous bioactive glass nanoparticles scaffold for promoting stem cell osteogenic differentiation. *RSC Adv.* **2020**, *10*, 12105–12112.
454. Jing, X.; Yin, W.; Tian, H.; Chen, M.; Yao, X.; Zhu, W.; Guo, F.; Ye, Y. Icariin doped bioactive glasses seeded with rat adipose-derived stem cells to promote bone repair via enhanced osteogenic and angiogenic activities. *Life Sci.* **2018**, *202*, 52–60.
455. Reiter, T.; Panick, T.; Schuhlarden, K.; Roether, J.; Hum, J.; Boccaccini, A. Bioactive Glass Based Scaffolds Coated with Gelatin for the Sustained Release of Icariin. *Bioact. Mater.* **2019**, *4*, 1–7. Erratum in *Bioact. Mater.* **2020**, *6*, 1810.
456. Zhao, F.; Chen, F.; Song, T.; Tian, L.; Guo, H.; Li, D.; Yang, J.; Zhang, K.; Xiao, Y.; Zhang, X. One-step strategy for fabricating icariin-encapsulated biomimetic Scaffold: Orchestrating immune, angiogenic, and osteogenic cascade for enhanced bone regeneration. *Bioact. Mater.* **2025**, *52*, 271–286.
457. Jin, D.-S.; Zhao, Z.-H.; Ruan, S.-Q.; Huang, W.-L.; Tian, R.-Y.; Wan, Y.; Deng, J. Icariin-loaded composite scaffold promotes osteogenic differentiation and bone regeneration. *BMC Musculoskelet. Disord.* **2025**, *26*, 548.

458. Xie, Y.; Sun, W.; Yan, F.; Liu, H.; Deng, Z.; Cai, L. Icariin-loaded porous scaffolds for bone regeneration through the regulation of the coupling process of osteogenesis and osteoclastic activity. *Int. J. Nanomed.* **2019**, *14*, 6019–6033.
459. Kong, C.H.; Steffi, C.; Cai, Y.; Wang, W. E-jet printed polycaprolactone with strontium-substituted mesoporous bioactive glass nanoparticles for bone tissue engineering. *Biomater. Adv.* **2025**, *169*, 214173.
460. Yang, G.; Pan, H.; Wei, Y.; Yang, J.; Zhang, Z.; Chen, S.; Wan, W. Directional mushroom-derived scaffold for microenvironment regulation in infected bone defects. *Adv. Mater.* **2025**, *37*, e2407730.
461. Zhang, C.; Wang, X.; Zhang, C. Icaritin inhibits CDK2 expression and activity to interfere with tumor progression. *iScience* **2022**, *25*, 104991.
462. Wang, P.; Wang, Q.; Wu, D.; Zhang, Y.; Kang, S.; Wang, X.; Gu, J.; Wu, H.; Xu, Z.; Jiang, Q. Enhancing osteogenic bioactivities of coaxial electrospinning nano-scaffolds through incorporating iron oxide nanoparticles and icaritin for bone regeneration. *Nano Res.* **2024**, *17*, 6430–6442.
463. Yang, Y.; Zheng, Y.; Liu, J.; Chang, Z.; Wang, Y.; Shao, Y.; Hou, R.; Zhang, X. Natural Chlorogenic Acid Planted Nanohybrids with Steerable Hyperthermia for Osteosarcoma Suppression and Bone Regeneration (Adv. Healthcare Mater. 23/2023). *Adv. Healthc. Mater.* **2023**, *12*, 2370141.
464. Yuan, Z.; Wan, Z.; Wei, P.; Lu, X.; Mao, J.; Cai, Q.; Zhang, X.; Yang, X. Dual-controlled release of icariin/Mg²⁺ from biodegradable microspheres and their synergistic upregulation effect on bone regeneration. *Adv. Healthc. Mater.* **2020**, *9*, 2000211.
465. Radulescu, D.-M.; Surdu, V.-A.; Fica, A.; Fica, D.; Grumezescu, A.-M.; Andronescu, E. Green synthesis of metal and metal oxide nanoparticles: A review of the principles and biomedical applications. *Int. J. Mol. Sci.* **2023**, *24*, 15397.
466. Yan, H.; Liao, Y.; Chen, W.; Luo, J.; Li, D.; Wang, J.; Liu, F. Bone-targeted Nanomedicine Based on Red Blood Cell Membrane-coated Polymeric Carriers for Effective Osteoporosis Reversal. *J. Drug Deliv. Sci. Technol.* **2025**, *110*, 107063.
467. Luo, M.; Lin, X.; Wang, N.; Xie, Q.; Zeng, S.; Li, S.; He, Z.; Lan, X. Icariin delivery system based on covalent organic framework materials: Dual effects of immune modulation and osteogenesis promotion. *RSC Adv.* **2025**, *15*, 23783–23800.
468. Xiong, F.; Qin, Z.; Chen, H.; Lan, Q.; Wang, Z.; Lan, N.; Yang, Y.; Zheng, L.; Zhao, J.; Kai, D. pH-responsive and hyaluronic acid-functionalized metal-organic frameworks for therapy of osteoarthritis. *J. Nanobiotechnology* **2020**, *18*, 139.
469. Li, H.; Xu, J.; Hu, J.-F.; Hu, Q.-Y.; Fang, X.; Sun, Z.-J.; Xu, Z.; Zhang, L. Sustained release of chlorogenic acid-loaded nanomicelles alleviates bone loss in mouse periodontitis. *Biomater. Sci.* **2022**, *10*, 5583–5595.
470. Zhou, R.-P.; Deng, M.-T.; Chen, L.-Y.; Fang, N.; Du, C.; Chen, L.-P.; Zou, Y.-Q.; Dai, J.-H.; Zhu, M.-L.; Wang, W.; et al. Shp2 regulates chlorogenic acid-induced proliferation and adipogenic differentiation of bone marrow-derived mesenchymal stem cells in adipogenesis. *Mol. Med. Rep.* **2015**, *11*, 4489–4495.
471. Chang, Z.; Chen, D.; Peng, J.; Liu, R.; Li, B.; Kang, J.; Guo, L.; Hou, R.; Xu, X.; Lee, M.; et al. Bone-targeted supramolecular nanoagonist assembled by accurate ratiometric herbal-derived therapeutics for osteoporosis reversal. *Nano Lett.* **2024**, *24*, 5154–5164.
472. Impellizzeri, D.; Esposito, E.; Di Paola, R.; Ahmad, A.; Campolo, M.; Peli, A.; Morittu, V.M.; Britti, D.; Cuzzocrea, S. Palmitoylethanolamide and luteolin ameliorate development of arthritis caused by injection of collagen type II in mice. *Arthritis Res. Ther.* **2013**, *15*, 4055. Erratum in *Arthritis Res. Ther.* **2016**, *18*, 101.
473. Lee, C.-Y.; Chang, Y.-C.; Yang, K.-C.; Lin, Y.; Wu, A.T.H.; Tseng, C.-L. Development and functional evaluation of a hyaluronic acid coated nano-formulation with kaempferol as a novel intra-articular agent for Knee Osteoarthritis treatment. *Biomed. Pharmacother.* **2024**, *175*, 116717. Erratum in *Biomed Pharmacother.* **2024**, *177*, 116944.
474. Priya, P.S.; Suriseti, R.; Gopi, S.; Pachaiappan, R.; Pasupuleti, M.; Rajagopal, R.; Alfarhan, A.; Guru, A.; Arockiaraj, J. Chitosan-chondroitin sulfate-daidzein nanoconjugate ameliorates glucocorticoid induced osteoporosis in vivo. *Int. J. Biol. Macromol.* **2024**, *280*, 135662.
475. Ghezzi, M.; Pescina, S.; Padula, C.; Santi, P.; Del Favero, E.; Cantù, L.; Nicoli, S. Polymeric micelles in drug delivery: An insight of the techniques for their characterization and assessment in biorelevant conditions. *J. Control. Release* **2021**, *332*, 312–336.
476. Yang, J.-G.; Zhang, J.; Chen, X.-J.; Zhou, G. Stable loading and delivery of icaritin using PEG-PCL micelles for effective treatment of oral squamous cell carcinoma. *Curr. Drug Deliv.* **2021**, *18*, 975–983.
477. Tang, C.; Chen, X.; Yao, H.; Yin, H.; Ma, X.; Jin, M.; Lu, X.; Wang, Q.; Meng, K.; Yuan, Q. Enhanced oral absorption of icaritin by using mixed polymeric micelles prepared with a creative acid-base shift method. *Molecules* **2021**, *26*, 3450.
478. Sun, X.; Lin, Y.; Zhong, X.; Fan, C.; Liu, Z.; Chen, X.; Luo, Z.; Wu, J.; Tima, S.; Zhang, Z.; et al. Alendronate-functionalized polymeric micelles target icaritin to bone for mitigating osteoporosis in a rat model. *J. Control. Release* **2024**, *376*, 37–51.
479. Narayanan, V.S.; Storch, J. Fatty acid transfer in taurodeoxycholate mixed micelles. *Biochemistry* **1996**, *35*, 7466–7473.

480. Turner, D.C.; Yin, F.; Kindt, J.T.; Zhang, H. Molecular dynamics simulations of glycocholate- oleic acid mixed micelle assembly. *Langmuir* **2010**, *26*, 4687–4692.
481. Van Hasselt, P.M.; Janssens, G.; Rijcken, C.J.F.; van Nostrum, C.F. Influence of bile on the oral bioavailability of vitamin K-loaded polymeric micelles. *J. Control. Release* **2008**, *132*, e29–e31.
482. Jiang, L.; Wang, K.; Deng, M.; Wang, Y.; Huang, J. Bile salt-induced vesicle-to-micelle transition in catanionic surfactant systems: Steric and electrostatic interactions. *Langmuir* **2008**, *24*, 4600–4606.
483. Jiang, J.; Li, J.; Zhang, Z.; Sun, E.; Feng, L.; Jia, X. Mechanism of enhanced antiosteoporosis effect of circinal-icaritin by self-assembled nanomicelles in vivo with suet oil and sodium deoxycholate. *Int. J. Nanomed.* **2015**, *10*, 2377–2389.
484. Vyawahare, A.; Prakash, R.; Jori, C.; Ali, A.; Raza, S.S.; Khan, R. Caffeic acid modified nanomicelles inhibit articular cartilage deterioration and reduce disease severity in experimental inflammatory arthritis. *ACS Nano* **2022**, *16*, 18579–18591.
485. Sercombe, L.; Veerati, T.; Moheimani, F.; Wu, S.Y.; Sood, A.K.; Hua, S. Advances and challenges of liposome assisted drug delivery. *Front. Pharmacol.* **2015**, *6*, 286.
486. Blanco, E.; Shen, H.; Ferrari, M. Principles of nanoparticle design for overcoming biological barriers to drug delivery. *Nat. Biotechnol.* **2015**, *33*, 941–951.
487. Sun, Z.; Li, J.; Wu, Z.; Zhang, J.; Wang, T.; Zheng, Y.; Xu, X.; Ding, W.; Qian, H.; Tan, Y. Extracellular vesicles and their mimetics: Clinical application prospects in medical aesthetics. *Burns Trauma* **2025**, *13*, tkaf033.
488. Shi, Y.; Wang, S.; Liu, D.; Wang, Z.; Zhu, Y.; Li, J.; Xu, K.; Li, F.; Wen, H.; Yang, R. Exosomal miR-4645-5p from hypoxic bone marrow mesenchymal stem cells facilitates diabetic wound healing by restoring keratinocyte autophagy. *Burns Trauma*. **2024**, *12*, tkad058.
489. Elsharkasy, O.M.; Nordin, J.Z.; Hagey, D.W.; de Jong, O.G.; Schiffelers, R.M.; Andaloussi, S.E.L.; Vader, P. Extracellular vesicles as drug delivery systems: Why and how? *Adv. Drug Deliv. Rev.* **2020**, *159*, 332–343.
490. Yu, X.; Dong, M.; Wang, L.; Yang, Q.; Wang, L.; Han, W.; Dong, J.; Liu, T.; Kong, Y.; Niu, W. Nanotherapy for bone repair: Milk-derived small extracellular vesicles delivery of icariin. *Drug Deliv.* **2023**, *30*, 2169414.
491. Yan, Q.; Liu, H.; Sun, S.; Yang, Y.; Fan, D.; Yang, Y.; Zhao, Y.; Song, Z.; Chen, Y.; Zhu, R.; et al. Adipose-derived stem cell exosomes loaded with icariin alleviates rheumatoid arthritis by modulating macrophage polarization in rats. *J. Nanobiotechnol.* **2024**, *22*, 423.
492. Zeng, J.; Sun, P.; Zhao, Y.; Fang, X.; Wu, Z.; Qi, X. Bone mesenchymal stem cell-derived exosomes involved co-delivery and synergism effect with icariin via mussel-inspired multifunctional hydrogel for cartilage protection. *Asian J. Pharm. Sci.* **2023**, *18*, 100799.
493. Montoya, C.; Du, Y.; Gianforcaro, A.L.; Orrego, S.; Yang, M.; Lelkes, P.I. On the road to smart biomaterials for bone research: Definitions, concepts, advances, and outlook. *Bone Res.* **2021**, *9*, 12.
494. Xu, K.; Zhou, M.; Chen, W.; Zhu, Y.; Wang, X.; Zhang, Y.; Zhang, Q. Bioinspired polydopamine/graphene oxide/collagen nanofilms as a controlled release carrier of bioactive substances. *Chem. Eng. J.* **2021**, *405*, 126930.
495. Yu, M.; You, D.; Zhuang, J.; Lin, S.; Dong, L.; Weng, S.; Zhang, B.; Cheng, K.; Weng, W.; Wang, H. Controlled release of naringin in metal-organic framework-loaded mineralized collagen coating to simultaneously enhance osseointegration and antibacterial activity. *ACS Appl. Mater. Interfaces* **2017**, *9*, 19698–19705.
496. Sang, S.; Wang, S.; Yang, C.; Geng, Z.; Zhang, X. Sponge-inspired sulfonated polyetheretherketone loaded with polydopamine-protected osthole nanoparticles and berberine enhances osteogenic activity and prevents implant-related infections. *Chem. Eng. J.* **2022**, *437*, 135255.
497. He, X.; Deng, Y.; Yu, Y.; Lyu, H.; Liao, L. Drug-loaded/grafted peptide-modified porous PEEK to promote bone tissue repair and eliminate bacteria. *Colloids Surf. B Biointerfaces* **2019**, *181*, 767–777.
498. Chen, Y.; Zhang, J.; Feng, J.; Chen, W.; Liu, W.; Chen, J.; Ye, J.; Li, W. Holistic quality evaluation method of *Epimedii Folium* based on NIR spectroscopy and chemometrics. *Phytochem. Anal.* **2024**, *35*, 771–785.
499. Wu, H.; Lien, E.J.; Lien, L.L. Chemical and pharmacological investigations of *Epimedium* species: A survey. In *Progress in Drug Research*; Birkhäuser: Basel, Switzerland, 2003; pp. 1–57.
500. Yang, Y.; Zhang, X.; Yang, Y.; Gao, P.; Fan, W.; Zheng, T.; Yang, W.; Tang, Y.; Cai, K. A two-pronged approach to inhibit ferroptosis of MSCs caused by the iron overload in postmenopausal osteoporosis and promote osseointegration of titanium implant. *Bioact. Mater.* **2024**, *41*, 336–354.
501. Llopis-Grimalt, M.A.; Arbós, A.; Gil-Mir, M.; Mosur, A.; Kulkarni, P.; Salito, A.; Ramis, J.M.; Monjo, M. Multifunctional properties of quercitrin-coated porous Ti-6Al-4V implants for orthopaedic applications assessed in vitro. *J. Clin. Med.* **2020**, *9*, 855.

502. Alfieri, M.L.; Riccucci, G.; Ferraris, S.; Cochis, A.; Scalia, A.C.; Rimondini, L.; Panzella, L.; Spriano, S.; Napolitano, A. Deposition of antioxidant and cytocompatible caffeic acid-based thin films onto Ti6Al4V alloys through hexamethylenediamine-mediated crosslinking. *ACS Appl. Mater. Interfaces* **2023**, *15*, 29618–29635.
503. Luo, Z.; Chen, X.; Liu, M.; Wang, Y.; Li, D.; Li, X.; Xiao, Y.; Wang, Y.; Zhang, X. The controlled release of a novel thiolated icariin for enhanced osteoporotic bone regeneration. *Mater. Des.* **2021**, *200*, 109468. Erratum in *Mater. Des.* **2021**, *205*, 109735.

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