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Review Article

Scaffold-Guided Local Bone Regeneration via Receptor Activator of NF-κB Ligand (RANKL) Reverse Signaling: from Gelatin Hydrogels to Injectable Microparticles and Cholesteryl-Bearing Pullulan (CHP) Nanogels

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Abstract: Although systemic therapies for bone loss are well established, localized bone augmentation approaches remain less mature overall. This unmet need is driving a shift toward localized, minimally invasive strategies. In receptor activator of NF-κB ligand (RANKL) reverse signaling—a distinct, osteoanabolic pathway compared with the canonical receptor activator of NF-κB (RANK)–RANKL–osteoprotegerin (OPG) resorptive axis—RANKL, normally a ligand, acts as a receptor on osteoblast-lineage cells, where membrane accumulation and clustering trigger pro-osteogenic signaling. Peptide activators of this pathway (OP3-4, W9) are effective, but their performance depends on scaffold properties that sustain local release and provide multivalent presentation to drive RANKL membrane accumulation and clustering. This review synthesizes recent advances in scaffold platforms engineered to enable and amplify RANKL reverse signaling, with emphasis on gelatin hydrogels, cholesteryl-bearing pullulan (CHP) nanogels, and injectable microparticle systems. We articulate design principles—release kinetics, porosity/architecture, and chemical modification—that govern local bioavailability and effective ligand valency, and we outline how these principles extend to RANK-bearing extracellular vesicles. Finally, we outline practical criteria for pairing RANKL-binding peptides with scaffolds compatible with non-surgical injectable delivery and discuss translational considerations for future clinical use.

Keywords: RANKL reverse signaling, scaffold, gelatin hydrogels, CHP nanogels, injectable microparticle systems, bone regeneration

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1. Introduction

Bone tissue undergoes continuous remodeling through a tightly regulated balance between bone resorption by osteoclasts and bone formation by osteoblasts. In both medical and dental fields, maintaining this balance is essential for skeletal integrity, implant stability, and functional recovery following trauma, disease, or surgical intervention. Disruption of this equilibrium leads to disorders such as osteoporosis, periodontitis, inflammatory bone resorption, and localized bone defects [1]. Systemic bone anabolic agents, including bisphosphonates and parathyroid hormone analogs, have been widely used to enhance bone mass but are not approved for localized bone regeneration [2–4]. Current local bone augmentation techniques invariably involve invasive surgical procedures, imposing a substantial burden on patients [5,6]. Therefore, less-invasive, site-specific, and locally controlled bone-regenerative approaches are increasingly sought, particularly in orthopedic, craniofacial, and implant dentistry applications.

To address site-specific bone defects, various bioactive molecules have been developed for local delivery using scaffolds, hydrogels, or carrier systems. Fibroblast growth factor-

2 (FGF-2) plays a pivotal role in osteogenic differentiation, cell proliferation, and angiogenesis, particularly in craniofacial bone regeneration [7]. Bone morphogenetic protein-2 (BMP-2) is among the most potent osteoinductive molecules, capable of driving mesenchymal stem cells toward osteoblastic lineage commitment [8,9]. Enamel matrix derivatives (EMDs), particularly amelogenin, originally studied for enamel and periodontal regeneration, have recently attracted attention for their osteogenic and mineralization-promoting properties beyond dental tissues [10–12]. Emerging evidence indicates that amelogenin-derived peptides promote osteogenic responses in vitro and enhance bone repair in preclinical craniofacial models, with preliminary indications in orthopedic settings [13].

Recently, receptor activator of NF-κB ligand (RANKL) reverse signaling has emerged as a novel anabolic mechanism in bone remodeling. Although RANKL was initially characterized for its essential role in osteoclast differentiation, it has been shown to deliver reverse signals to osteoblastic-lineage cells, thereby stimulating osteogenesis [14]. Harnessing this mechanism requires pharmacological activators such as RANKL-binding peptides. The development of such molecules depends not only on ligand binding but also on their ability (i) to promote the accumulation of membrane-bound RANKL and (ii) to avoid disrupting RANKL clustering at the osteoblast membrane, which is crucial for signal amplification[14–16].

As a brief mechanistic overview, the canonical receptor activator of NF-κB (RANK)–RANKL–osteoprotegerin (OPG) axis primarily regulates bone resorption through RANK on osteoclast precursors, with OPG acting as a decoy receptor for RANKL [17]. By contrast, in RANKL reverse signaling, RANKL on osteoblastic-lineage cells functions as the signal-receiving molecule (a reverse-signaling receptor) and requires membrane-level accumulation and clustering to initiate downstream osteogenic programs [14,18]. This mechanistic distinction directly links efficacy to scaffold-controlled delivery through (i) release kinetics that sustain local exposure above an activation threshold without systemic spillover and (ii) ligand valency/spatial presentation that promotes RANKL membrane accumulation and clustering for signal amplification [15,19].

Consistent with this mechanistic view, successful local bone regeneration depends not only on biological signaling molecules but also on biocompatible scaffolds that provide structural support and regulate cellular behavior. Acting as a temporary extracellular matrix mimic, these scaffolds promote cell adhesion, migration, proliferation, and osteogenic differentiation. Among existing materials, atelocollagen, a purified low-antigenicity collagen, has been widely used clinically due to its excellent biocompatibility and ability to sustain bone tissue formation [20–22]. It often serves as a carrier for growth factors such as BMP-2 and FGF-2, enabling controlled release and enhanced regenerative efficacy [23–25].

In addition to collagen-based scaffolds, other platforms have been developed to meet distinct mechanical and biological requirements, including hydroxyapatite composites, gelatin-based hydrogels, synthetic polymers, octacalcium phosphate–collagen hybrids, and carbonate apatite materials [26–29]. The efficiency of bone regeneration is also influenced by scaffold morphology, emphasizing that the integration of scaffold architecture with local delivery systems is indispensable for achieving predictable and functional bone regeneration in both orthopedic and dental contexts. In this review, we integrate recent advances in scaffold-guided bone regeneration mediated by RANKL reverse signaling, highlighting the evolution from gelatin hydrogels to cholesteryl-bearing pullulan (CHP) nanogels and injectable systems. We further discuss why gelatin hydrogels outperform collagen sponges for the delivery of RANKL-binding peptides such as W9, and how macroporous CHP nanogels modulate osteoinductive processes. Finally, we present a critical comparison between non-surgical injectable systems and conventional surgically assisted "injectable" formulations, defining their respective potentials in future minimally invasive bone regeneration strategies. We summarize the state of scaffold-guided activation of RANKL reverse signaling—linking material choice, release profiles, and osteogenic efficacy—and outline key considerations for clinical translation.

2. RANKL Reverse Signaling and Pharmacological Activators

RANKL is originally recognized as a key regulator of osteoclast differentiation and bone resorption. However, subsequent research has revealed that RANKL also transmits "reverse signals" into osteoblast-lineage cells, promoting their differentiation and enhancing bone formation [14]. This duality challenges the classical understanding of RANKL solely as a bone-resorptive factor, positioning it as a pivotal molecule in coupling resorption and formation (Figure 1).

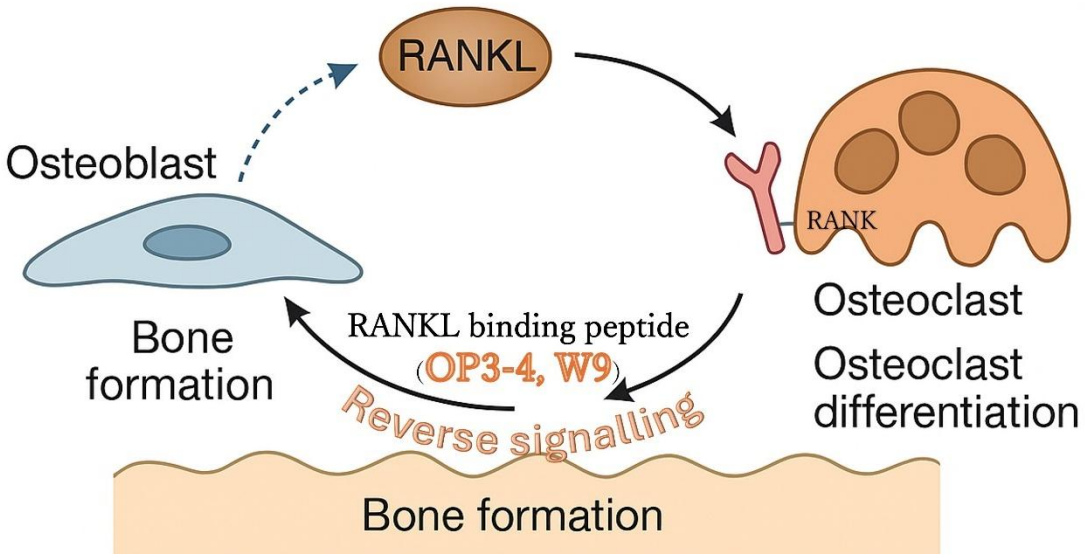

Figure 1. Schematic illustration of bidirectional roles of RANKL with RANKL reverse signaling in bone remodeling: RANKL normally stimulates osteoclast differentiation via RANK, but RANKL-binding peptides (OP3-4, W9) or osteoclast-derived extracellular vesicles activate reverse signaling in osteoblast-lineage cells, promoting bone formation.

Pharmacological exploitation of this pathway has centered on the development of RANKL-binding peptides. Mamun et al. [30] demonstrated that W9, when delivered locally with BMP-2, significantly enhanced bone regeneration in a murine calvarial defect model. Interestingly, the same calvarial defect model was employed by Ikebuchi et al. [14], who investigated extracellular vesicles (EVs) derived from mature osteoclasts. These EVs, containing RANK, were shown to activate reverse signaling and promote bone formation. Beyond local effects, systemic activity has also been documented. Kato et al. [31] reported that OP3-4, another RANKL-binding peptide, administration in a rheumatoid arthritis model not only inhibited bone destruction but also dose-dependently promoted bone formation, even under pathological conditions. Similarly, W9 was shown by Sato et al. [32] to suppress bone resorption in a murine bone resorption model. This evidence suggests that RANKL-binding peptides possess dual functionality, simultaneously reducing bone resorption and stimulating bone formation, broadening their therapeutic scope from local regenerative strategies to systemic disease management. RANKL reverse signaling engages the Src–PI3K–mTORC1–Runx2 pathway in osteoblast-lineage cells and initiates osteogenic transcription programs [14]. In practice, the magnitude and reliability of this response appear to be shaped by how peptides are presented and retained locally, which is governed by the delivery scaffold. The following sections examine scaffold features that enable such controlled, site-specific exposure.

3. Scaffold Materials for Local Delivery

An overview of representative scaffold–peptide–model combinations covered in this section is provided in Table 1; we begin with gelatin hydrogels, where release kinetics govern osteogenic outcomes (Figures 2–3(a)).

3.1. Gelatin Hydrogels: Sustained Release and Osteogenesis

Gelatin hydrogels, pioneered by Yasuhiko Tabata and colleagues at Kyoto University, have been widely applied as biodegradable carriers for growth factors and peptides. Mamun et al. [30] reported pivotal evidence that scaffold choice is critical when delivering RANKL-binding peptides. BMP-2 alone induced similar levels of bone regeneration regardless of whether collagen sponges or gelatin hydrogels were used as carriers. However, when W9 was co-delivered with BMP-2, gelatin hydrogels significantly outperformed collagen sponges, consistent with their sustained-release profile (Figure 2).

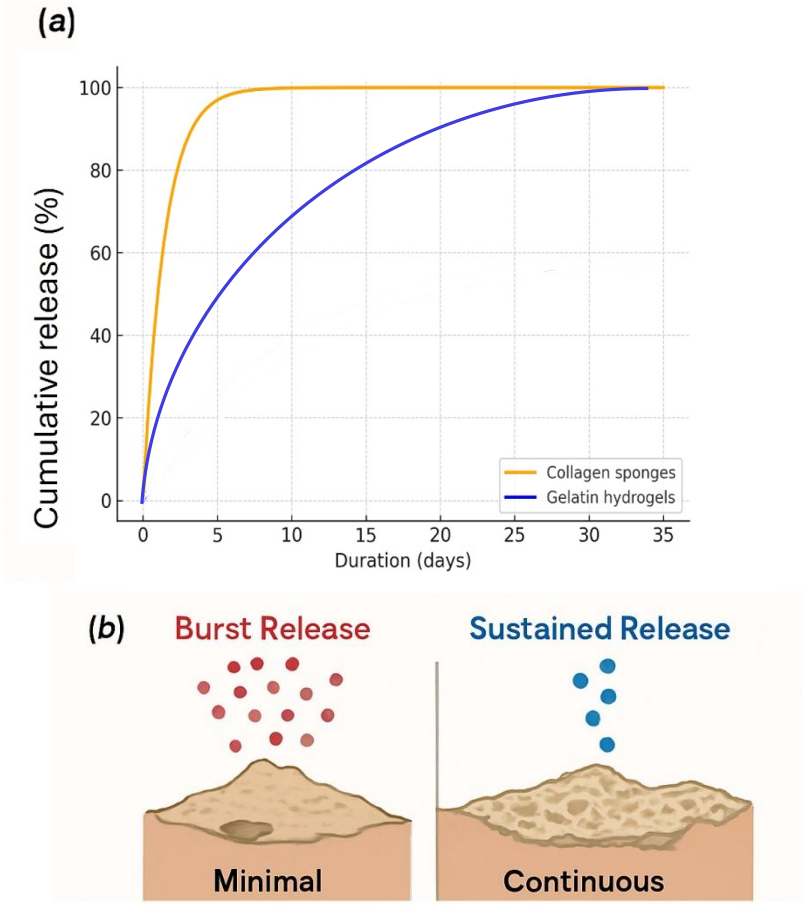

Figure 2. Release kinetics of W9 from different scaffolds: (a) Collagen sponges exhibit burst release within a few days, whereas gelatin hydrogels provide sustained release for at least four weeks, resulting in superior osteogenesis; (b) Comparison of release profiles of W9 from collagen sponges and gelatin hydrogels [30].

Consistent with the comparisons summarized in Table 1, collagen sponges exhibited a burst release that delivered most peptide within the first few days, whereas gelatin hydrogels provided sustained release, continuing to elute W9 beyond four weeks (Figure 2(a)). This prolonged bioavailability supports continuous activation of RANKL reverse signaling, thereby amplifying bone formation (Figure 3(a)) [30]. Interestingly, in the BMP-2–only condition, bone augmentation was comparable between collagen sponge and gelatin hydrogel. Together, these findings establish gelatin hydrogels as a preferred scaffold for W9 and underscore the broader principle that release kinetics can be as critical as molecular potency. By contrast, BMP-2–driven bone formation appears less dependent on prolonged release. At present, stable augmentation with a RANKL-binding peptide still requires co-delivery of BMP-2; however, peptide-only strategies may become feasible through systematic scaffold optimization—tuning chemistry and degradation/release kinetics, increasing local retention, engineering pore/perforation architecture for cell and vessel ingress, and enabling multivalent/clustered peptide presentation (including micro environmental buffering).

Zhang et al. [33] Developed a Gelatin–Sodium Alginate (Gel/SA) hydrogel system for sustained release of Simvastatin, a bone anabolic agent. They achieved controlled release over 7 days without burst behavior. It enhanced mechanical properties and compressive strength. It also promoted BMP-2 expression, cellular bioactivity, and osteoinductive performance in vitro.

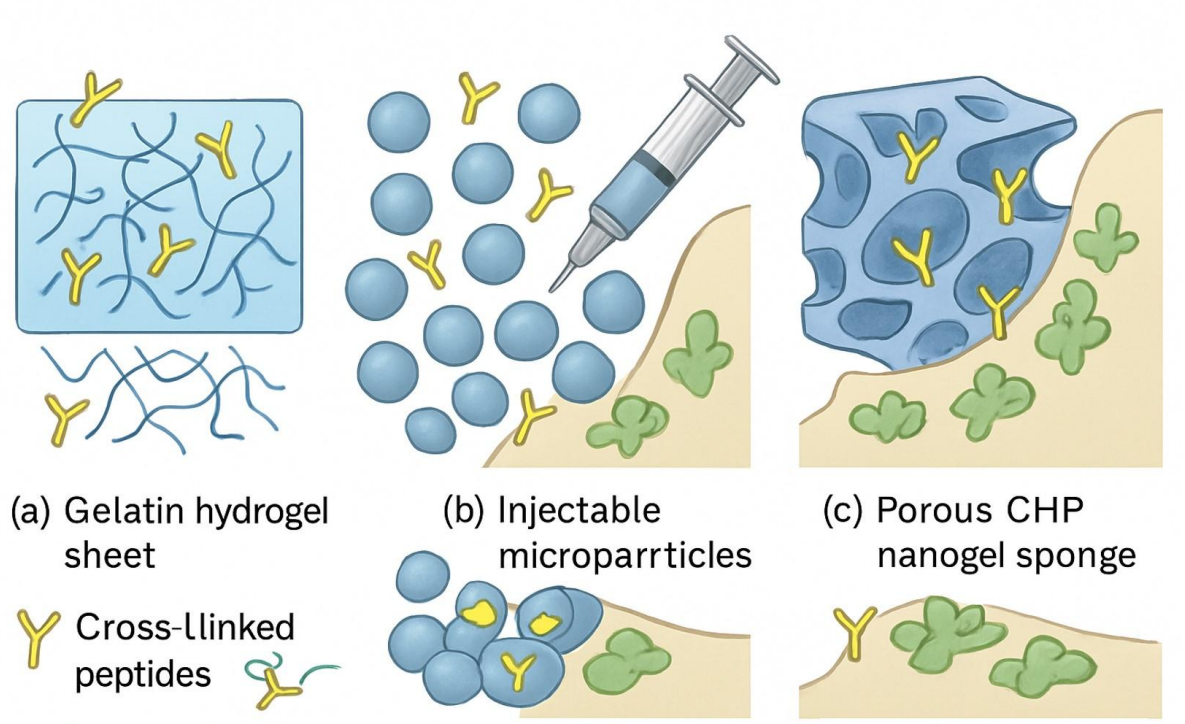

Figure 3. Scaffold platforms for local bone regeneration and representative biomaterial scaffolds engineered for the delivery of RANKL-binding peptides: (a) Gelatin hydrogel sheets provide a sustained-release matrix from a defined, implantable structure [14,30]; (b) Injectable gelatin microparticles offer a minimally invasive delivery method that conforms to a defect site [34,35]; (c) Freeze-dried porous nanogel sponges present a high surface area scaffold for peptide adsorption and cellular infiltration [36,37]. Despite their structural differences, each platform operates on the principle of sequestering the peptide (illustrated as yellow 'Y' shapes) to prolong its local bioavailability and enhance osteogenic signaling.

3.2. Injectable Gelatin Microparticles: Toward Non-Surgical Regeneration

Conventional orthopedic strategies for bone regeneration often employ so-called "injectable" biomaterials. However, in most cases these materials are not injected through intact tissue but rather placed into defects after surgical exposure or drilling. For example, injectable calcium phosphate cements and hydrogels are frequently delivered into surgically created bone cavities. While such methods reduce the need for large grafts or open fixation, they remain invasive and require operative access, often involving a longer procedure, slower recovery, and higher infection risk.

Non-surgical injection refers to the percutaneous administration of bioactive agents or scaffold-based formulations using a standard hypodermic needle (typically 18–27 gauge), without surgical incision, flap elevation, or implant exposure. Formulations should pass through a needle without clogging or phase separation, retain bioactivity after injection, and form a stable in situ depot or scaffold. This approach is minimally invasive, with short procedure time, rapid recovery, and lower risks of infection and bleeding (see Table 1 for representative doses and models).

Keo et al. [34] and Qi et al. [35] advanced gelatin-based systems by fabricating injectable microparticles (~20-30 μm) using a 26-gauge, 15° tip Hamilton needle attached to a 25-μL Hamilton syringe. These particles enabled submucosal injection without incision in a murine alveolar-bone model, a clear departure from conventional "injectable" methods that still require surgical exposure. In both studies, non-surgical injections of OP3-4 with BMP-2 through mucosa or subcutaneous tissue induced robust local bone formation without exposing the bone surface. Qi et al. [35] further showed that preventive injections preserved alveolar bone during outward tooth movement (Figure 3(b)). This distinction between "surgical-assisted injection" and truly "non-surgical injection" is critical for the future of regenerative medicine (Figure 4).

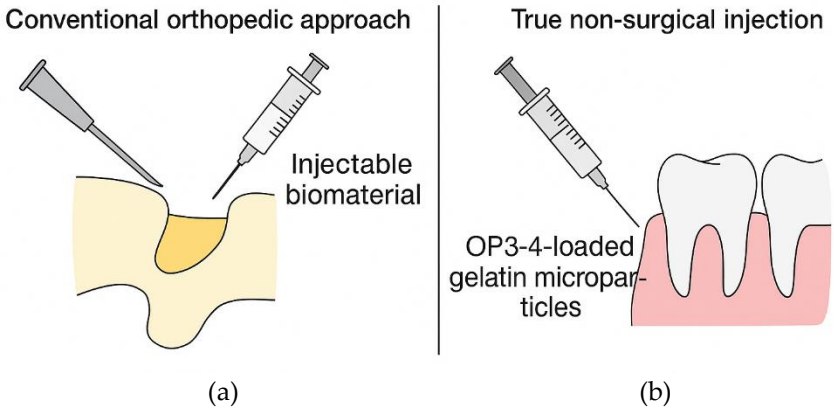

Figure 4. Surgical-assisted versus non-surgical injection paradigms and conceptual comparison of delivery strategies: (a) Conventional orthopedic approach where injectable biomaterials are placed into bone defects after surgical exposure; (b) non-surgical injection demonstrated in dental/orthodontic models, where OP3-4-loaded gelatin microparticles can be injected submucosally without the need for surgical incision, flap elevation, or implant exposure, to induce local bone formation.

A non-surgical injection refers to the delivery of therapeutic scaffolds or biomaterials entirely via needle-based methods, without any surgical incision, flap elevation, or operative exposure of the target site. By demonstrating that bone can be induced locally through simple injection, RANKL reverse signaling coupled with optimized scaffolds may establish a new category of therapies those that regenerate bone without the need for surgical intervention. Patrick et al. [38] developed a chitosan–gelatin-based microgels with hydroxyapatite to mimic bone matrix. It supported osteogenesis and vasculogenesis, promoting mesenchymal stromal cell differentiation and endothelial network formation. It achieved >95% defect closure in a murine model within 12 weeks.

This minimally invasive strategy allowed local bone induction in the alveolar bone, preventing buccal bone resorption during orthodontic tooth movement. Such an approach opens the possibility of expanding bone regeneration therapies to patient populations for whom surgery poses significant risks, such as elderly individuals or those with systemic comorbidities.

3.3. CHP Nanogels: Porosity and Chemical Modification

Kazunari Akiyoshi and colleagues developed cholesterol-bearing pullulan (CHP) nanogels as another scaffold platform. Unlike gelatin, these nanogels can be freeze-dried into porous matrices. Sato et al. [32] encapsulated W9 in CHP nanogels and demonstrated sustained systemic release that suppressed bone resorption. Xie et al. [37] extended this system to local regeneration in calvarial defect models. In one study, they showed that macropore size critically determined osteoconductivity [37], while in another, they demonstrated that chemical modifications of CHP nanogels altered osteogenic outcomes [38]. These results illustrate that not only release kinetics but also scaffold architecture and chemistry dictate the efficacy of RANKL-binding peptides (Figure 3(c)). Notably, perforated acryloyl-modified CHP nanogels (CHP-A) yielded greater bone formation only when OP3-4 was co-delivered with BMP-2, attributed to slower in vivo degradation—reinforced by freeze-drying–induced surface densification and a final fibronectin coating—which prolongs OP3-4 availability and supports late-stage osteoblast differentiation; concurrently, ~300–400 μm macropores promote cell ingress and angiogenesis, enhancing osteoconductivity [37]. In contrast, with BMP-2 alone, early-phase recruitment predominates and prolonged release offers little benefit; consistent with this, similar scaffold degradation at two weeks likely resulted in comparable early BMP-2 exposure, explaining the lack of difference between perforated and non-perforated groups [37]. In addition, CHPA exhibits pH-dependent degradation—slow under acidic conditions and faster at neutral to alkaline pH—implying that inflamed, acidic defects initially preserve the scaffold and that release accelerates as the microenvironment normalizes during healing.

3.4. Broader Hydrogel Platforms in Orthopedics

Beyond peptide-focused studies, a wide range of hydrogels have been investigated for orthopedic applications. Liu et al. [39] reviewed injectable hydrogels extensively, emphasizing their injectability, biocompatibility, and

potential for minimally invasive bone and cartilage repair. Recent developments include GelMA/κ-carrageenan/calcium phosphate composites [40], Zn-containing chitosan-based hydrogels with osteogenic activity, and enzyme-responsive PEG-based hydrogels for controlled degradation [41]. Bioinspired hydrogel designs that mimic extracellular matrix properties were recently highlighted. Together, these studies reinforce the notion that scaffold design—whether gelatin, CHP, or synthetic polymers must be tailored to the signal molecules they deliver.

4. Signal–Scaffold Matching: Design Principles

The efficacy of RANKL reverse signaling–based therapeutics is co-determined by the delivery scaffold. Here, signal–scaffold matching is defined as aligning release kinetics, spatial presentation, mechanical microenvironment, and biochemical affinity/retention of the carrier with the physicochemical and biological requirements of the signaling agent (Figure 5). When the pair is mismatched, rapid clearance, instability, or burst release blunt bioactivity; when matched, sustained exposure, protection from degradation, and appropriate spatial presentation maximize osteogenesis.

Beyond collagen sponges, platforms discussed in this review—gelatin hydrogels, CHP nanogels, mineral–collagen hybrids (e.g., HAp- or OCP-collagen [26–29]), and injectable microparticles—offer distinct mechanical and biological milieus. Osteoconductive mineral–collagen hybrids provide pre-structured templates for cell and vessel ingress and may buffer local pH; with RANKL-binding peptides, such scaffolds could reduce reliance on exogenous osteoinductive cues, moving toward peptide-only augmentation. This hypothesis warrants systematic evaluation of mineral phase, pore architecture, and modes of peptide presentation/retention.

For OP3-4, sustained release is essential—gelatin hydrogels provide gradual degradation and prolonged exposure. For W9, CHP nanogels afford nanoscale encapsulation and tunable porosity that preserve activity and modulate retention. Extracellular vesicles also benefit from hydrogel stabilization that prevents premature washout and confines action locally.

Design levers include stimuli-responsive hydrogels (enzymes, pH, temperature) [42], ECM-mimetic architectures for cell attachment/differentiation [43], and rational combinations with osteogenic pathways (ephrin–Eph [44], Wnt, PDGF), each delivered in carriers tailored to their kinetic and spatial needs.

In short, scaffolds should be treated not as passive supports but as active partners. Precisely matching scaffold properties to signaling requirements enables minimally invasive, patient-friendly bone-regeneration strategies that can outperform current standards.

5. Perspectives: Translational Outlook

Scaffold platforms such as gelatin hydrogels, CHP nanogels, and injectable microparticles illustrate diverse strategies for optimizing delivery. These systems support minimally invasive, non-surgical injection approaches that can reduce surgical burden and expand patient eligibility. Key translational considerations include:

1. **Safety:** Avoid ectopic bone formation and inflammatory responses.
2. **Dose control:** Achieve reproducible local exposure without systemic spillover.
3. **Imaging guidance:** Enable precise placement and longitudinal monitoring.
4. **Regulatory precedents:** Leverage approvals/standards for existing BMP-2 carriers to streamline development.

To fully realize the potential of signal-matched scaffold design, future work should integrate scaffold engineering with molecular biology, for example:

1. **EV stabilization:** Improve extracellular-vesicle bioactivity and shelf-life within protective matrices.
2. **Smart hydrogels**: Create stimuli-responsive materials that release cargo on demand (e.g., enzymatic, pH, temperature).

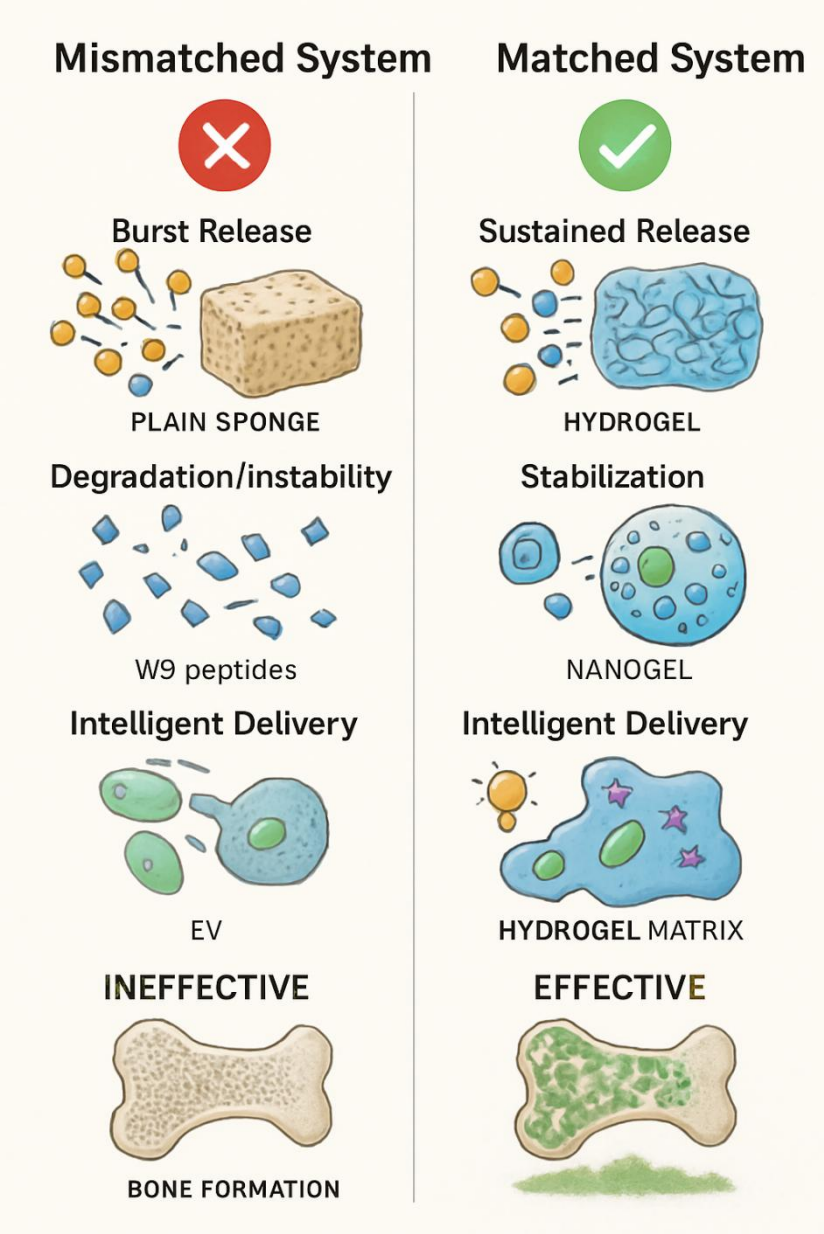

Figure 5. Concept of signal-matched scaffold design and framework illustrating the principle that scaffold choice must align with the signaling molecule: OP3-4 requires sustained release (gelatin hydrogel), W9 benefits from porous CHP nanogels, extracellular vesicles are stabilized by hydrogel matrices, and future osteogenic agents (e.g., Wnt, ephrin-Eph, PDGF) will require smart or bioinspired scaffolds.

3. **Combination pathways**: Explore synergistic signaling via Wnt, ephrin–Eph, PDGF, and other osteogenic axes.
4. **Mechanistic clarity**: Map downstream adapters (e.g., Src/PI3K/mTORC1) to scaffold-guided outcomes.

By aligning biomaterials precisely with signaling molecules, it may be possible to establish a new generation of regenerative therapies—ones that rebuild bone where needed without the risks and burdens of surgery.

Table 1. Comparative Summary: Bioactive Agents Across Scaffold Systems

Agent	Scaffold	Species; model/site	Dosing	Experimental Period	Primary Outcome	Ref.
W9 (WP9QY)	Type I collagen discs (ectopic implantation)	Mouse; subcutaneous implant	0.56 mg	12 days after implantation	↑ Ectopic bone volume; ↑ osteoblast surface and mineralized indices vs BMP-2 alone.	[45]
W9 (WP9QY)	Gelatin hydrogel (GH) vs Type I collagen (Col I)	Mouse; 3.5-mm calvarial defect	0.56 mg	28 days after implantation	Sustained W9 release from GH; GH+W9+BMP-2 > Col I+W9+BMP-2 for radio-opacity, BMC, histomorphometry	[30]
W9 (WP9QY)	(CHP) nanogels NanoClik nanoparticles	Mouse; once-daily subcutaneous injections of **microparticles**	8 mg/kg/day 24 mg/kg/day	4 days after implantation	24 mg/kg/day W9 + NanoClik nanoparticles prevented the low-calcium-diet–induced increase in bone resorption.	[46]
W9 and OP3-4	Gelatin hydrogel	Mouse; 3.5-mm calvarial defect	W9: 0.56 mg OP3-4: 0.66 mg	28 days after implantation	Peptides accelerate BMP-2-induced bone regeneration; peptide-induced ALP blocked by rapamycin; Akt/S6K1 ↑ (mTORC1)	[47]
LL37 (early) → W9 (sequential)	SIS hydrogel + PLGA microspheres	Rat; 8-mm calvarial defect	1 mg	1, 3 month after implantation	↑ bone regeneration and formation ↑ Antibacterial & immunomodulatory markers at 1 month; markers were Normalized at 3 months	[48]
OP3-4	Gelatin hydrogel placed into extraction socket	Mouse; mandibular incisor extraction	0.56 mg	21 days after implantation	De novo bone formation in socket; alveolar bone loss prevented; osteoclast number decreased	[49]

OP3-4	Injectable gelatin hydrogel (subperiosteal delivery) 20 μm diameter microparticles	Mouse; Single **microparticles** injection at maxillary right diastema	0.56 mg/5 μL	28 days after injection/ implantation	↑ BMC, ↑ BMD; mineralization from outside-in; ↑ early osteogenic markers.	[50]
OP3-4	Injectable gelatin hydrogel (subperiosteal delivery) 20-30 μm diameter microparticles	Mouse; Single **microparticles** injection at maxillary right diastema and miniscrew insertion after thickening	0.44 mg/4 μL	8 weeks after injection; screw placed at week 4	induced bone withstood screw insertion; formation activity maintained; adjacent basal bone BMD ↑.	[34]
OP3-4	Injectable gelatin hydrogel (subperiosteal delivery) 50 μm diameter microparticles	Mouse; Single **microparticle**s injection at maxillary right buccal side on an orthodontic-force–induced buccal dehiscence model	0.44 mg/4 μL	6 weeks after injection/ implantation	Prevention of buccal dehiscence; maintenance of buccal bone thickness and height.	[35]
OP3-4	Granular gelatin hydrogel (injection)	Rat: Single **microparticles** injection after surgical femoral defect	0.6 mg/mL	7, 14, 28 days after injection/ implantation	↑ bone remodelling indices. inhibiting osteoclast activation and promoting vascularization	[19]
OP3-4	CHP-A vs CHP-OA PEG-cross-linked nanogel hydrogels	Mouse; 3.5-mm calvarial defect	0.66 mg	4 weeks after implantation	With OP3-4 + BMP-2: CHP-A > CHP-OA for calcified area/formation; with BMP-2 alone: no significant difference.	[38]
OP3-4	Perforated vs non-perforated CHPA nanogel hydrogels	Mouse; 3.5-mm calvarial defect	0.66 mg	6 weeks after implantation	Perforated CHPA: slower scaffold degradation → sustained OP3-4 release; ~300–400 μm macropores facilitated cell migration/angiogenesis → greater bone formation (with OP3-4 + BMP-2).	[37]

SEVs (secreted extracellular vesicles: vesicular RANK)	Gelatin hydrogel sheet loaded with mature osteoclast-derived SEVs	Mouse; 3.5-mm calvarial defect	50 μg	4 weeks after implantation	Marked healing of calvarial injury; ↑ MAR and BFR/BS; reverse signalling via osteoblastic RANKL	[14]

Abbreviations: gelatin hydrogel (GH)； cholesteryl-bearing pullulan (CHP); acryloyl-modified CHP (CHPA); small intestinal submucosa (SIS); polylactic-co-glycolic acid (PLGA); bone mineral content (BMC); bone mineral density (BMD); mineral apposition rate (MAR); bone formation rate per bone surface (BFR/BS).

6. Conclusion

RANKL reverse signaling represents a paradigm shift in bone regeneration. Once regarded solely as a driver of osteoclastogenesis, RANKL is now recognized to transmit pro-osteogenic signals to osteoblast-lineage cells. Pharmacological activators such as OP3-4 and W9 harness this pathway, but their success depends critically on scaffold systems that provide sustained release, appropriate presentation/valency, structural support, and localized action.

Gelatin hydrogels, CHP nanogels, and injectable microparticles each showcase complementary delivery strategies. Pairing RANKL reverse signaling with tailored, signal-matched scaffolds offers a practical route to therapies that are effective, minimally invasive, and broadly applicable.

Looking ahead, integrating scaffold engineering with molecular biology will refine the signal-matched design principle. With biomaterials precisely tuned to their cargo, we may realize regenerative treatments that rebuild bone where and when it is needed—while minimizing operative risk and patient burden.

Author Contributions: K.A conceived and designed research; F.R drafted the manuscript and prepared figures; K.A revised the manuscript and approved the final version of the manuscript.

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