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REVIEW

A Procedural Overview of the Involvement of Small Molecules in the Nervous System in the Regulation of Bone Healing

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Abstract: Clinically, a multitude of factors can contribute to the development of bone defects. In the process of bone healing, the nervous system plays a vital role in bone regeneration. Small molecules from the nervous system, such as neurotrophic factors and neuropeptides, have been found to stimulate osteoblast proliferation and differentiation by activating signaling pathways associated with bone calcification and angiogenesis. These small molecules play a crucial regulatory role at various stages of bone healing. The systematic release mechanism of small molecules within the nervous system through diverse bone tissue engineering materials holds significant clinical implications for the controlled regulation of the bone healing process. This review provides an overview of the involvement of various nervous system small molecules at different stages of bone healing and discusses their regulatory mechanisms, aiming to establish a theoretical foundation for programmed regulation in bone regeneration and design of replacement materials in bone tissue engineering.

Keywords: nervous system, neurotrophin, neuropeptide, bone healing, procedural controlled release

Introduction

Bone tissue injury caused by various factors such as inflammation, trauma, fracture, tumor, surgery, etc. is a prevalent clinical issue[1] that can significantly impact bodily function and diminish quality of life in severe cases.[2–4] Deficiency in bone mass resulting from injury at specific sites may also impact the prognosis of associated treatments.[5] Various types of bone augmentation surgeries, such as autologous bone transplantation or allograft bone transplantation, are associated with certain drawbacks, including limited availability of autologous bone,[6] extensive tissue damage, high complication rates,[7,8] and challenges in patient acceptance. Additionally, allograft bone carries the potential risk of disease transmission or adverse immune reactions.[7,9,10]

The field of bone tissue engineering has emerged as a promising therapeutic approach, leveraging the principles of bionics to address the aforementioned limitations by incorporating diverse cell types, bioactive factors, and other components into materials for bone regeneration.[6,11] Although bone tissue engineering has achieved favorable outcomes in the treatment of conventional bone defects,[12,13] addressing large bone defects resulting from trauma and various bone diseases, specifically critical bone defects with a lesion length of at least 6 cm,[14–18] remains a challenging issue encountered by clinicians.[19] This could be attributed to the oversight of the crucial role played by the nervous system in bone repair.[20]

In recent years, the role of the nervous system in bone repair has garnered increasing attention and application from scientists.[21–25] Nerve tissue, which is primarily composed of neurons and glia, serves as the principal constituent of the nervous system. It has been widely observed to be distributed throughout the bone cortex[26] and bone trabeculae,[27] as well

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as in the bone marrow and periosteum.[28] Moreover, it plays a crucial role in facilitating bone tissue regeneration. Denervation experiments have directly demonstrated the influence of nerve tissue on bone resorption[29] and fracture healing.[30] Additionally, the presence of nerve fibers in the callus formed after bone injury[31] further emphasizes the significant role played by nerve tissue in the process of bone healing. Small molecules of the nervous system are a series of small molecules including neurotrophic factors and neuropeptides in the nervous system, which play an important role in promoting the growth and development of neurons and repairing after injury. In addition to their role in the nervous system, numerous studies have also demonstrated[32–38] that following bone injury, there is an elevation in the levels of these small molecules within the bone defects compared to normal tissues. This suggests their significant involvement in the process of bone repair and regeneration.

The ideal material for bone tissue engineering should possess the dual functionality of replacing missing bone fragments and promoting autologous bone formation. However, small molecules derived from the nervous system, despite being bioactive, are not suitable for direct clinical application due to their instantaneous cell signaling effects and short half-life.[39–43] The utilization of bone tissue engineering carriers for the treatment of these active small molecules not only enables better preservation of their biological activity, but also facilitates direct programmed controlled release within bone defects, thereby exerting a long-term and efficient role in biological regulation. This represents one of the future directions for application development in the field of bone tissue engineering. Therefore, this paper provides a comprehensive review of the roles played by various small molecules in the nervous system on bone tissue, elucidating their specific mechanisms of action in different processes of bone regeneration. This will serve as a valuable reference for the systematic and rational design of vectors for bone tissue engineering and the targeted utilization of small molecules from the nervous system.

Bone Tissue and Bone Healing Process

The bone tissue possesses a complex three-dimensional architecture, primarily categorized into two structural forms: cancellous bone and cortical bone. Cancellous bone exhibits a porous structure composed of anisotropic trabeculae acting as pillars, while cortical bone is comparatively dense to provide support and withstand pressure. Furthermore, an intricate neurovascular network traverses the bone tissue, regulating its biological behavior and supplying it with nutrition and sensory support.[44–47]

The process of bone healing is a complex and intricately coordinated physiological phenomenon that can be categorized into primary (direct) and secondary (indirect) bone healing.[48–50] Primary bone healing occurs under demanding circumstances, necessitating rigid fixation to prevent any movement of the bone fragments. This particular healing process seldom triggers inflammation but instead initiates a mechanism akin to normal bone remodeling. However, it should be noted that this recuperative process progresses at a sluggish pace, often spanning several months to years for complete recovery.

The secondary bone healing, which is a more commonly observed process, requires relatively simpler conditions for regeneration compared to primary bone healing. Therefore, it serves as the predominant form of bone tissue engineering materials' healing. This intricate process involves the regulation of multiple signaling pathways and bioactive factors. The process of normal bone healing involves the following sequential stages:[7,51,52] When bone defects occur, rupture of blood vessels leads to the formation of local hematoma. Inflammatory cells infiltrate into the hematoma tissue and secrete pro-inflammatory cytokines, recruiting macrophages for debris removal from damaged tissues. Subsequently, under the regulation of bioactive factors such as glial cell line-derived neurotrophic factor (GDNF), recruited mesenchymal bone progenitor cells proliferate and differentiate into chondrocytes, secreting cartilage matrix and forming soft callus. The callus then undergoes mineralization while proliferative cells release growth factors like vascular endothelial growth factor (VEGF) and bone morphogenetic protein (BMP), stimulating migration of osteogenic precursor cells and blood vessels to the site of bone defect. The soft callus is invaded by osteoclasts simultaneously, initiating the reabsorption of the mineralized cartilage matrix. The coordinated process of matrix absorption and bone tissue formation triggers the substitution of soft callus with hard bone callus, facilitating the subsequent formation and remodeling of woven bone. The process of bone healing can be described as a sequential progression through three partially overlapping stages:[49,53] inflammation, repair, and remodeling. Small molecules of the nervous system play diverse roles in each stage of bone

healing, facilitating the promotion of bone regeneration. Bone tissue engineering suggests that bone replacement materials should possess appropriate osteoconductivity, osteoinductivity, and osteogenesis.[44] Incorporating suitable bioactive factors can better fulfill these requirements. Therefore, exploring the integration of neurotrophic small molecules with bone substitute materials to enhance their role in promoting bone healing represents a promising future direction for bone tissue engineering. The Figure 1 below shows how various small molecules in the nervous system play a role in each of the three stages of bone healing and induce different differentiation fates between osteoblasts and osteoclasts.

Effects of Small Molecules of Nervous System on Bone Healing

Neurotrophins and Their Receptors

Neurotrophins (NTs) are a group of closely related proteins initially believed to serve as survival factors for sensory and sympathetic neurons, but subsequent research has revealed their crucial role in regulating the survival, development, and functionality of both peripheral and central nervous system neurons.[54] The first neurotrophic factor to be identified was Nerve Growth Factor (NGF), followed by Brain-derived neurotrophic factor (BDNF) during the purification of pig brains.[55] Four neurotrophic factors, including NGF, BDNF, NT-3 and NT-4/5, have been isolated and purified in mammals. Furthermore, through the implementation of serum-free neuronal culture techniques, novel specific protein molecules have been discovered in various tissue fluids and extracellular matrix. These include Glial cell line-derived neurotrophic factor (GDNF) and Platelet-derived growth factor (PDGF), as well as Fibroblast growth factor (FGF), among others. It has been demonstrated that these particular protein molecules also play a crucial role in promoting neuronal proliferation, differentiation, and survival.

The four types of neurotrophic factors (NTs) in mammals, also referred to as neurosurvival factors, play a crucial role in regulating various processes such as synaptic plasticity, neurodegenerative diseases, demyelinating diseases, and inflammation.[56] Apart from their pivotal function in the nervous system, these NTs have been demonstrated to exert

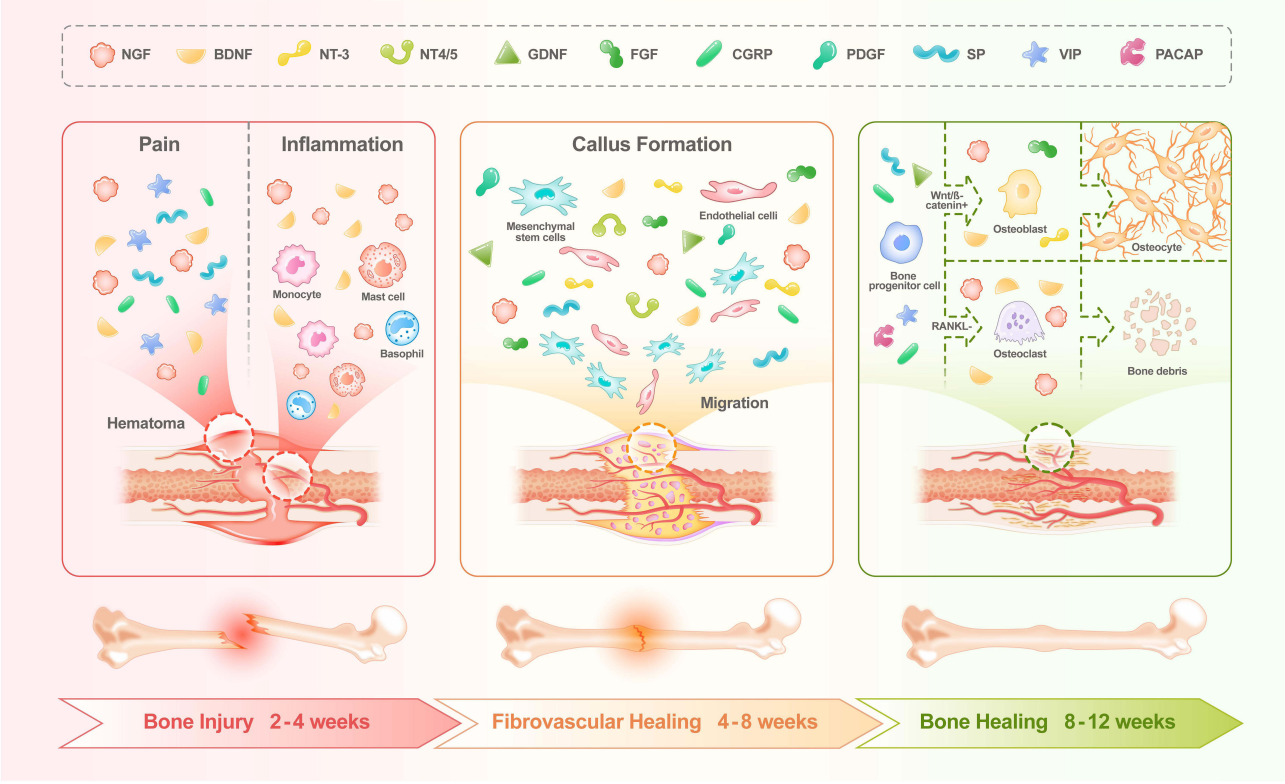

Figure 1 Schematic summary of the stages of bone healing and temporal patterns of small molecule expression in the nervous system.

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significant effects on bone tissue regeneration and skin wound healing,[57] as well as pregnancy and brain development,[58] inflammation, and allergies.[59]

There are two distinct types of neurotrophic factor receptors, each exhibiting varying affinities towards neurotrophic factors. The initial receptor identified, known as the p75 neurotrophic factor receptor ($p75^{NTR}$), functions as a low-affinity receptor for NTs. The $p75^{NTR}$ binds to all four NTs with equal affinity. Another group of receptors for NTs consists of three members belonging to the Trk receptor tyrosine kinase subfamily - TrkA, TrkB, and TrkC - which are recognized as high-affinity receptors for NTs. These receptors display specificity in their interactions with the four different types of NTs: NGF activates TrkA; BDNF and NT-4 activate TrkB; and NT-3 activates TrkC. Additionally, while NT-3 can still bind to the other two Trk receptors, its activation efficiency is relatively low.[54] In the context of bone healing, modulation of these distinct receptors' functions can potentially enhance bone regeneration. Therefore, this paper provides a comprehensive overview of small molecule-related receptors within the nervous system.

During the development of the central nervous system, $p75^{NTR}$ is extensively expressed in neurons and various glial cells, serving as a reliable marker for Schwann cells originating from either peripheral or central sources.[60] Apart from its distribution within the nervous system, $p75^{NTR}$ is also present in diverse mesenchymal cells.[61] Functionally, $p75^{NTR}$ engages in signal transduction by interacting with different proteins to mediate distinct biological processes.[60,62,63]

Xu et al[61] conducted cross-breeding experiments between $p75^{fl/fl}$ animals and $PDGFR^{\alpha mT/mG}$ lines to assess the necessity of $p75^{NTR}$ in skull defect repair. The experimental findings revealed a decrease in bone volume (BV), BV fraction, and bone fraction area in the CT scans of $p75^{PDGFR\alpha}$ mice, along with an increase in the mean diameter of the bone defect area. H&E staining in Figure 2[61] also showed significantly impaired healing between bone fronts in $p75^{NTR}$ mutant mice. To investigate the role of $p75^{NTR}$ in human skull osteoblasts, we observed $p75^{NTR}$ knockdown mediated by small interfering RNA (siRNA), which resulted in impaired cell migration and osteogenic differentiation.

Wang et al's study[64] also demonstrated that mice lacking thep75^{NTR} gene exhibited a reduction in alveolar bone mass compared to wild-type (WT) mice, and the mineralization induction process led to a decrease in osteogenic ability of Ecto Mesenchymal Stem cells (EMSCs). Furthermore, it was observed that the phosphatidylinositol 3-kinase/protein kinase B/β-catenin (PI3K/Akt/β-catenin) signaling pathway, which plays a positive role in regulating EMSCs' differentiated mineralization potential, was down-regulated as well.[64] Experimental findings indicated that $p75^{NTR}$ positively regulated the osteogenic differentiation of EMSCs by enhancing the PI3K/Akt/β-catenin pathway. Table 1 provides an overview of how neurotrophic factors affect bone through their respective receptors. In future research, if components responsible for regulating $p75^{NTR}$ activity can be incorporated into osteogenic materials, it may enhance the healing process of bone defects.

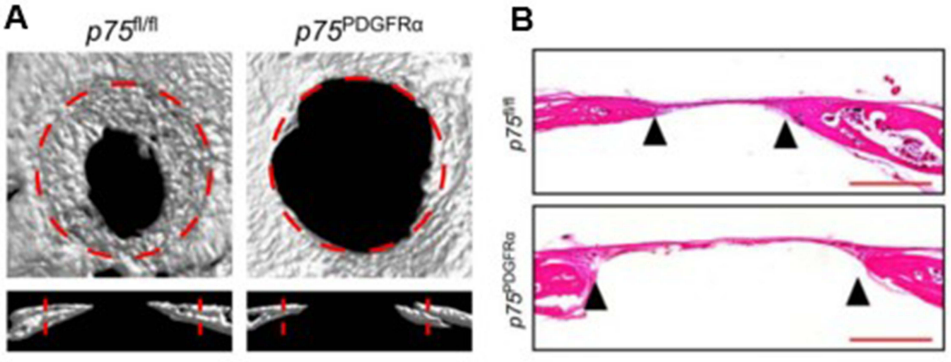

Figure 2 (**A**) Micro-CT reconstructions of the defect site in a top-down view (above) and coronal cross-sectional images (below) among $p75^{fl/fl}$ and $p75^{PDGFR\alpha}$ animals, 28 days after injury. Margins of original defect are indicated by red dashed lines. (**B**) Hematoxylin and eosin (H&E) staining of coronal cross section of the healing defect site from $p75^{fl/fl}$ and $p75^{PDGFR\alpha}$ mice at day 28 after injury. Black arrowheads indicate healing bone edges. Adapted from Xu J, Li Z, Tower RJ, et al. NGF-p75 signaling coordinates skeletal cell migration during bone repair. *Sci Adv.* 2022;8:eabl5716. Creative Commons.[61]

Table 1 The Receptors and Effect of Neurotrophins on Bone

Receptors	Neurotrophins	Effect	Ref.
p75	NGF, BDNF, NT-3, NT-4/5	Promotes bone marrow mesenchymal stem cell migration, Regulates the osteogenic differentiation of EMSC	[60–64]
TrkA	NGF	Regulates bone response to mechanical load, Regulates bone formation and bone resorption	[65–72]
TrkB	BDNF	Regulates new bone formation by inducing osteoblast proliferation Activates osteoclasts	[73–78]
	NT-4/5	Promotes blood vessel regeneration, Regulates the function of periodontal ligament cells	[79,80]
TrkC	NT-3	Improves fracture healing by improving osteoblast formation, Increases the formation and absorption of osteoclasts promotes heterotopic ossification formation	[81–85]
GFRα-1, GFRα-2	GDNF	Involved in the pathogenesis of bone pain; Regulates bone metabolism; Acts as a target-derived neurotrophic factor during dental innervation	[86–91]
Type A PDGF receptor, Type B PDGF receptor	PDGF	Improved reparative bone movement; Promotes angiogenesis	[92–100]
FGFR	FGF	Promotes fracture healing; Promotes bone resorption; Promotes angiogenesis	[101–107]

Nerve Growth Factor (NGF)

The role of NGF in bone pain conduction was initially identified within bone tissue. As the primary mediator of bone pain, NGF directly activates TrkA or indirectly transmits nociceptive signals to enhance the response of other pain pathways.[32,65,66]

In recent years, the role of NGF in bone regeneration has gradually become elucidated. Vincent Fitzpatrick et al[67] incorporated NGF, BMP2, and VEGF into the 3D printed ink to fabricate a scaffold material with appropriate pore size, which effectively promotes neurovascularization and bone tissue regeneration in cases of bone defects. This study confirms that NGF can synergistically interact with other growth factors. For instance, the upregulation of BMP-2 induces an increase in Runt-related transcription factor 2(RUNX2), osteopontin (OPN), and bone sialoprotein (BSP) expression in human mesenchymal stem cells (HMSCs), thereby facilitating osteogenic differentiation.[67] The addition of NGF to the titanium surface coating of the implant has also been demonstrated to enhance the differentiation of bone marrow mesenchymal stem cells into osteoblasts.[68] Moreover, NGF can upregulate osteoblast activity, inhibit osteoclast activity, and stimulate in vivo osteogenesis to facilitate fracture healing.[69] Local injection of β-NGF further improves bone healing function by increasing bone volume and promoting the formation of newly formed bone with a higher number of trabeculae, connective tissue density, and bone density.[70]

The diverse functions of NGF in bone regeneration are intricately linked to the cellular signaling pathways involved. NGF exerts its crucial role through two receptors, namely the high-affinity TrkA and the low-affinity $P75^{NTR}$. Studies have demonstrated that TrkA signaling plays a pivotal role in stress fracture repair.[32] In cases of bone defects, increased innervation occurs prior to vascularization, ossification, and mineralization of the fracture site. Inhibition of TrkA signaling results in reduced innervation, angiogenesis, and osteoblast activity within the callus at the site of stress fracture. Activation of TrkA receptors by NGF can induce reinnervation of skeletal muscle nerves, thereby promoting bone repair including revascularization and deposition of bone matrix.[32,71]

NGF may also play a role in bone repair through its low-affinity receptor $P75^{NTR}$. Studies have demonstrated that NGF-expressing macrophages stimulate the migration of bone cells and promote bone formation during the early stages

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of bone repair.[61,72] The combination of NGF and $P75^{NTR}$ can induce cell migration and initiate recruitment of various aptamers, including C-Jun N-terminal kinase signaling. KDM4B serves as a crucial epigenetic regulator in NGF-mediated osteogenesis.[72] Activated NGF-c-Jun binds to the promoter region of KDM4B and directly upregulates its expression. Subsequently, KDM4B actively demethylates the H3K9me3 marker on DLX5, which is a master osteogenic gene. Moreover, both KDM4B and c-Jun within the JNK signaling pathway collaborate to regulate NGF-mediated osteogenic differentiation by simultaneously recruiting the promoter region of DLX5.

Brain-Derived Neurotrophic Factor (BDNF)

BDNF is extensively distributed and extensively researched in the mammalian brain as a neurotrophic factor. BDNF signals are transmitted through the high-affinity TrkB receptor and the low-affinity $p75^{NTR}$ receptor. BDNF plays a crucial role in synaptic development and plasticity,[73] thereby highlighting its significant therapeutic potential in neurosystem-related diseases, depression, and neurodegenerative disorders such as Alzheimer's disease.[74]

BDNF is also expressed in human osteoblasts and mediates the process of fracture healing.[75] Nanoparticles loaded with BDNF have the potential to enhance the proliferation and differentiation of mesenchymal stem cells into osteoblasts, thereby promoting osteogenesis.[76] Consistent with NGF's role, BDNF regulates new bone production by up-regulating osteoblast activity, promoting their differentiation and mineralization, as well as facilitating fracture healing.[69,77] Furthermore, BDNF can improve trabecular microstructure, tibial biomechanical properties, and bone biochemical indices. It exhibits a certain therapeutic effect on postmenopausal osteoporosis.[75,78]

The osteogenic effect of BDNF is primarily mediated through TrKB receptors. FanXue et al[78] utilized 7, 8-dihydroxyflavone (7, 8-DHF), a plant-derived small molecule agonist for TrkB that mimics the functionality of BDNF, to demonstrate the activation of the Wnt/β-catenin signaling pathway by BDNF via TrkB receptors. This activation resulted in up-regulation of cyclin D1, phosphorylated glycogen synthetase kinase-3β (p-GSK3β), β-catenin, Runx2, Osterix and osteoprotegerin expression in MC3T3-E1 cells. The increased expression of osteoprotegerin promoted cell proliferation, osteogenic differentiation and mineralization processes. Furthermore, upon binding to the TrkB receptor, BDNF also exhibited inhibitory effects on osteoclast differentiation and bone resorption by suppressing transcription factor c-fos as well as key genes involved in osteoclast function such as tartrate-resistant acid phosphatase (TRAP), matrix metalloproteinase-9 (MMP-9) and Adamts5.

The BDNF/TrkB signaling pathway also activates Akt, leading to phosphorylation and inhibition of asparagine peptidase (AEP), which plays a regulatory role in the differentiation of human bone marrow stromal cells (hBMSC). 7, 8-DHF effectively inhibits the C/EBP-β/AEP pathway. Furthermore, the inhibition of Rank-L-induced RAW264.7 osteoclasts[75] provides additional evidence for the crucial involvement of BDNF in bone healing.

Neurotrophin-4/5 (NT-4/5)

NT-4/5, similar to BDNF, exhibits specific binding affinity for the TrkB receptor. When both BDNF and NT-4/5 coexist within the same environment, their effects vary depending on the sequence of exposure: BDNF does not influence the actions of NT-4/5; however, NT-4/5 inhibits the activity of BDNF.[79] During tooth development, prenatal oral and internal tooth epithelium have been found to express NT-4 mRNA,[80] suggesting a potential role for NT-4 in tooth morphogenesis. Furthermore, through activation of the ERK1/2 signaling pathway, NT-4/5 can regulate periodontal ligament cell function and induce expression of ALPase, OPN, and BMP-2 mRNA in HPL cells.[77] These findings may guide future research directions in oral studies.

Neurotrophin-3 (NT-3)

NT-3 is a neurotrophic protein that plays a crucial role in the survival and axonal regeneration of neurons in the spinal cord.[81] NT-3 specifically binds to the TrkC receptor, and under certain cellular conditions, it can also activate TrkA and TrkB with reduced efficiency.[54] Studies have demonstrated that NT-3 has the potential to mitigate brain damage,[82] as well as promote neuronal survival and differentiation. Additionally, the involvement of NT-3 in the process of fracture repair has garnered attention from relevant scholars. Su et al,[33] discovered that NT-3 and its receptor TrkC were significantly upregulated at the site of bone injury using a proximal tibial drilling injury repair model in young mice,

indicating their potential to promote bone repair. In vitro experiments revealed that NT-3 can enhance osteogenic differentiation and mineralization, stimulate BMP-2 secretion, and augment its activity during the osteogenic process.

The expression of NT-3 and TrkC receptors was observed in both normal bone and osteoclasts at the site of injury repair. Osteoblast-derived NT-3 can potentially function as a regulatory signal for osteoclast mineral uptake during bone injury.[83,84] Activation of the downstream kinase Erk1/2 of TrkC is involved in mediating the effect of NT-3 on osteoclast formation. Upon activation by TGF-β, NT-3 acts on TrkC to induce endothelial-mesenchymal transformation (EndMT) and enhance MSC production, leading to Heterotopic ossification (HO). Furthermore, NT-3 promotes the differentiation of MSCs into chondrocytes and osteoblasts by upregulating Sox9, OCN, RUNX2, and other bone markers.[84] Neovascularization promotion also contributes to HO formation mediated by NT-3.[84] Additionally, macrophage-derived NT-3 has been demonstrated to stimulate osteogenic differentiation of mesenchymal lineage tendon stem cells (TDSCs) through activating ERK1/2 and PI3K/Akt signaling pathways.[85]

Glial Cell Line-Derived Neurotrophic Factor (GDNF)

GDNF was identified in 1993 as a crucial neurotrophic factor for the survival of embryonic dopaminergic neurons in the midbrain.[86] It belongs to the TGF-β ligand superfamily.[87] Members of the GDNF family encompass GDNF, artemisinin, neurourea, and Persian peptide. All ligands within the GDNF family bind to various complexes through GFRα1-4. Among them, GDNF exhibits a strong affinity for GFRα-1 but typically utilizes the receptor tyrosine kinase RET as a signaling coreceptor.[87] Additionally, GDNF can also interact with other complexes via GFRα2.

Through the GDNF/GFRα1 (GDNF-GFRα1-RET-ERK) and neuroprotein/GFRα2 signaling pathways, GDNF exerts a significant influence on bone pathology or disease-related pain.[88,89] Additionally, GDNF is involved in the regulation of bone metabolism, with pre-osteoclast (poc)-derived GDNF promoting migration of bone marrow mesenchymal stem cells and enhancing osteogenic differentiation through GDNF-GFRα1-RET signaling.[87] Moreover, GDNF induces migration of Schwann cell precursors which accumulate in damaged periodontal tissue and play a crucial role in wound healing, particularly in alveolar bone tissue.[90] Furthermore, Schwann cells are vital for peripheral tissue regeneration including bone tissue regeneration; thus suggesting that GDNF indirectly influences the process of bone regeneration by regulating Schwann cell precursors. In the NRTN-GFRα2 neurotrophic axis, IL-6-induced skeletal sympathetic cholinergic nerve fibers sustain survival and function of bone cells during postnatal growth and development as well as physical activity during adolescence.[91]

Platelet-Derived Growth Factor (PDGF)

PDGF family comprises five dimeric ligands: PDGF-AA, -AB, -BB, -CC, and -DD.[9,92,93] Among its receptors are PDGFRα and PDGFRβ. Notably, PDGFRα is predominantly expressed in megakaryocytes while PDGFRβ is almost exclusively expressed in fibroblasts.[93] PDGF-BB, a chemotactic and mitotic factor within the PDGF family, facilitates the migration, proliferation and differentiation of various mesenchymal cell types such as endothelial progenitor cells and mesenchymal stem cells through signaling via PDGFRβ. This process promotes the establishment of a complete vascular network during bone healing,[94–96] thereby inducing vascularization into bone formation. It plays a pivotal role in promoting tissue repair and regeneration.[9] Following temporomandibular arthritis, the bone microenvironment exhibits an elevated presence of platelet-derived growth factor (PDGF). Furthermore, there is an upregulation of vascular endothelial growth factor (VEGF) and matrix metalloproteinase-9 (MMP-9). The formation of H-type blood vessels not only facilitates subchondral bone remodeling but also induces a bone pain response.[108] Compared to conventional bone repair methods that solely focus on new bone quantity, vascularization osteogenesis places greater emphasis on constructing a blood vessel network throughout the bone healing process. Studies have demonstrated that this mode of vascularization osteogenesis can generate new bone with superior quality; thus making it more suitable for healing large bone defects.

In a clinical study,[97] various biomaterials were introduced into the extraction wound after tooth removal to investigate alveolar ridge preservation and bone regeneration. The findings demonstrated that the addition of rhPDGF-BB enhanced the biological properties of newly formed bone and reduced bone resorption. This could be attributed to the differentiation of macrophage/non-absorbent osteoclast lineage cells into bone-resorbing osteoclasts during bone remodeling, as well as the secretion of PDGF-BB for recruiting endothelial cells and osteoblast progenitors, thereby forming a vaso-osteoblastic unit.[95] In one experiment, it was observed that older mice and mice on a high-fat diet (HFD) exhibited excessive secretion of PDGF-

BB from preosteoclasts in their bones/bone marrow compared to younger mice and those on a normal diet. Consequently, elevated serum levels of PDGF-BB led to both bone loss and arteriosclerosis.[98] This phenomenon further supports the association between PDGF-BB derived from osteoclasts and the process of bone formation. Also, Gao et al[99] also discovered that macrophage/monocyte-derived PDGF-BB could induce periosteal derived cells (PDC) to express periosteal bone proteins while attracting them for supporting osteogenesis along with H-type blood vessel formation (a vascular subtype characterized by high expression levels of CD31 and Emcn).

The PDGF-B derived from endothelial cells (EC) also serves as a crucial niche factor for BMSC, facilitating the osteogenic differentiation of bone marrow mesenchymal stem cells.[94] It plays a role in promoting osteogenesis and triggering the activation of bone stem and progenitor cells during bone repair through PDGFRβ signaling.[100]

Fibroblast Growth Factor (FGF)

FGF belongs to a superfamily of genes that exert pleiotropic effects on various biological processes by activating the FGF receptor tyrosine kinase (FGFR).[101] The FGF family comprises 22 members, out of which 18 are secreted and possess the ability to bind to one or more of the four FGF receptors 1–4 (FGFR1-4), thereby forming FGF signaling pathways.[102] These pathways regulate intricate cellular behaviors in vertebrates, including migration, proliferation, self-renewal, lineage commitment, aging, and survival.[103]

Many members of the FGF family are expressed in and around the membranous and endochondroskeletal elements, including FGF1, 2, 6–10, 17, 18, and 21–23.[103] FGF-2, FGF-9, and FGF-18 play a role in osteogenesis and may present a novel option for bone regeneration.[104] The bone-derived hormone FGF23 acts synergistically with parathyroid hormone (PTH) and the active vitamin D metabolite calcitriol to regulate calcium (Ca) and phosphate (P) homeostasis.[101,105] Deficiency of FGF23 results in hyperphosphatemia and ectopic calcification while excessive levels of FGF23 lead to hypophosphatemia and skeletal abnormalities.[106]

Another type, fibroblast growth factor 8 (FGF-8), also known as androgen-induced growth factor (AIGF), plays a crucial role in limb development. Within the bone microenvironment, FGF-8 produced or received by precursor chondrocyte cells binds to fibroblast growth factor receptor (FGFR), thereby inducing activation of downstream signaling pathways to varying extents. These signaling pathways include phospholipase Cγ (PLCγ)/Ca2+, RAS/mitogen-activated protein kinase-extracellular regulatory protein kinase (RAS/MAPK-MEK-ERK), and Wnt-β-catenin-Axin2, which ultimately govern chondrocyte proliferation, differentiation, cell survival, and migration.[107]

Neuropeptide

Neuropeptides, such as CGRP, SP, VIP, PACAP and NPY, are synthesized and secreted by neuronal cells to regulate various biological processes including bone homeostasis.[109] Table 2 summarizes the effects of different neuropeptides on bone healing.

Table 2 The Effect of Neuropeptides on Bone

Neuropeptides	Effect	Ref.
CGRP	Induced bone pain Promotes bone formation and inhibit bone resorption	[110–148]
SP	Promotes the production of vaso-related factors Increases the local blood flow and promote the development of callus Affects bone metabolism by directly affecting bone cells	[149–161]
VIP	Promotes the activity of osteoblast and inhibit the activity of osteoclast Improves bone density and mechanical properties	[162–170]
PACAP	Anti-inflammatory Protective cartilage	[171–178]
NPY	Directly involved in the regulation of osteoblast activity and helps bone homeostasis Indirectly involved in bone regulation by regulating blood vessels	[179–191]

Calcitonin Gene-Related Peptide (CGRP)

CGRP, a 37-amino acid neuropeptide belonging to the calcitonin family,[110] exhibits sensitivity to mechanical, chemical, and electrical stimulation,[111–115] and interacts with a receptor similar to the calcitonin receptor (CLR). In humans, CGRP is classified into two types: alpha-CGRP and β-CGRP. Alpha-CGRP is encoded by the Calca gene and predominantly expressed in both central and peripheral nervous systems. On the other hand, β-CGRP is encoded by the Calcb gene and primarily found in the enteric nervous system.[116,117] Apart from its distribution in neural tissues such as cardiovascular system, respiratory system, thyroid gland; CGRP also shows equal presence in bone marrow and periosteal tissue.[118] Furthermore, it can exert its effects on various non-neuronal tissues including bone tissue itself.[115,119–122]

CGRP and its receptor CLR, along with the receptor active Modification protein 1 (RAMP1) complex, have been demonstrated to play a crucial role in the pathogenesis of migraine.[123–128] Moreover, CGRP's involvement in bone turnover has also been confirmed.[129,130] Specifically, CGRP promotes bone formation and contributes significantly to α-CGRP's function.[129] Studies on mice lacking alpha-CGRP have revealed reduced bone formation and osteopenia, indicating that alpha-CGRP serves as a physiological activator of bone formation.

CGRP promotes osteoblast differentiation and function while inhibiting osteoclast development and activity.[131,132] Overexpression of CGRP in osteoblasts specifically upregulates insulin-like growth factor I (IGF-I) secretion and downregulates tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6) secretion. These cytokines, including IGF-I, TNF-α, and IL-6, are produced by various cell types including osteoblasts. IGF-I has been shown to promote positive bone balance while TNF-α and IL-6 have been implicated in the pathogenesis of osteoporosis. This finding suggests that CGRP protects bones through these pathways. Furthermore, CGRP receptors on the plasma membrane of periosteoblasts and osteoclasts bind to CGRP, inhibiting osteoclast formation and bone resorption by blocking RANKL-mediated NF-κB activation. As a result, this leads to positive bone balance and increased bone mineral density.[133]

The CGRP receptor has also been identified on the plasma membrane of bone marrow mesenchymal stem cells, and CGRP has the ability to stimulate the proliferation of these cells.[131] Additionally, it can induce osteoblast gene expression and synergize with Wnt signaling to enhance bone formation.[131,134–136]

The role of CGRP involves pain transmission and sensitization, with its pain intensity positively correlated to serum CGRP levels.[137,138] Following a fracture, there is an increase in CGRP content at the site of bone injury, indicating its potential involvement in the process of fracture pain transmission. Furthermore, CGRP plays a role in bone regeneration after a fracture. It reaches peak levels during the early post-fracture period and promotes bone formation by facilitating larger callus formation. Over time, CGRP gradually decreases to allow for normal or physiological bone remodeling.[139] In terms of bone healing, the expression and activity of NOS are closely associated with CGRP expression.[140] By inducing NOS-mediated vasodilation, local blood flow increases due to CGRP's promotion of fracture callus formation.[141] Additionally, nerve fibers containing sensory neuropeptide CGRP proliferate within and around the callus.[142] Apart from the NOS pathway, local administration of CGRP may also contribute to rapid recovery from bone defects by promoting positive bone balance. Moreover, it can assist in fracture healing through ERK phosphorylation stimulation.[143]

The bone tissue is innervated by a dense sensory neural network, with the CGRP-positive nerve being the most commonly distributed sensory nerve.[144] During fracture healing, CGRP-positive nerves exhibit regenerative properties.[22,23,145] Following neuronal depolarization at their endings, CGRP is released and exerts its biological functions in bone regeneration, including angiogenesis and osteogenesis.[146,147] Furthermore, researchers have discovered that loading CGRP into porous microspheres can provide protection for BMSCs against inflammatory environments and promote bone regeneration.[148] To some extent, this suggests the protective effect of CGRP on BMSCs in an inflammatory environment.

Substance P (SP)

SP is a tachykinin-like peptide composed of 11 amino acids that triggers a signal transduction cascade via the neurokinin-1 receptor (NK1-R).[149] Additionally, SP can be produced by non-neuronal cells and exert its function. SP+ nerves are widely distributed in periosteum, subchondral bone, bone marrow, ligaments, synovium, and other bone tissues.[150]

Delayed mobilization of mesenchymal stem cells may result in delayed bone healing following fracture.[151] Substance P can serve as a stimulating factor to facilitate the migration of CD29 mesenchymal stem cells towards the site of bone defect, thereby expediting the process of bone healing.[152–154] Furthermore, Substance P not only promotes mobilization and migration of mesenchymal cells but also enhances their proliferation and osteogenic differentiation.[155,156] Additionally, Substance P exerts an influence on bone metabolism and is considered an osteoneutronic mediator that regulates bone remodeling through increased neuro-bone crosstalk activity such as Ca2+ signaling.[157,158] Studies have reported that Substance P has been observed to augment the expression and mineralization of osteogenic markers in bone marrow stromal cells.[156,159] Moreover, by promoting the secretion of angiogenic cytokines to stimulate angiogenesis and enhance local blood flow, Substance P indirectly affects bone metabolism while participating in callus development and facilitating bone healing processes.[141]

The hydrogel experiment on skull regeneration revealed that chitin-PLGA-calcium sulfate hydrogel scaffolds, incorporating both SP and lactoferrin (LF) for dual regulation, exhibited superior osteogenic induction potential compared to single-component hydrogel scaffolds. This suggests that the synergistic combination of bone-inducing biomolecules and SP can effectively enhance bone regeneration.[160]

Jun-Kyu Lee et al[161] also discovered that the combined treatment of 4-hexylresorcinol (4HR) and substance P (SP) synergistically induces the phosphorylation of p38 MAPK and ERK in human umbilical vein endothelial cells (HUVECs), thereby upregulating the expression level of vascular endothelial growth factor (VEGF). This, in turn, promotes the migration and tubular ability of endothelial cells, ultimately accelerating bone formation and maturation. Furthermore, SP expression enhances HUVEC migration and angiogenesis while inhibiting osteoclast formation. Based on this synergistic effect, it is possible to achieve bone regeneration by inhibiting the RANKL pathway both in vitro and in vivo to counteract osteoporosis.

Vasoactive Intestinal Peptide (VIP)

VIP is a 28-amino acid prelysate product of VIP,[162] and it acts on three receptor subtypes: VPAC1, VPAC2, and PAC1.[163] Immunoreactive nerve fibers containing VIP were identified in the periosteum and bone of animals.[28] VIP plays a crucial role in bone metabolism and remodeling processes.[164] By activating specific VIP receptors in osteoclasts and osteoblasts, VIP can regulate bone resorption activity and promote osteoblast differentiation.[165–167] Specifically, VIP can enhance osteoblast activity to facilitate the production of bone tubercle alkaline phosphatase (ALP) and augment calcium accumulation,[168,169] while concurrently inhibiting osteoclast activation to reduce bone resorption.[166] Another study revealed that VIP can stimulate bone formation by activating the Wnt/beta-catenin signaling pathway in bone marrow stromal cells (BMSC).[170] In a mouse model of 6-hydroxydopamine-induced sympathectomy, local injection of VIP improved both the quality and mechanical properties of bones, leading to an upregulation in the expression of osteogenic markers.[164]

Pituitary Adenylate Cyclase Activating Polypeptide (PACAP)

PACAP is a neuropeptide[171] present in the hypothalamic-pituitary system with a significantly short half-life[172] in vivo. It exists in two bioactive forms, PACAP 1–38 and PACAP 1–27. PACAP exhibits three major G-protein-coupled receptors, namely PAC1, VPAC1, and VPAC2, among which PAC1 shows the highest affinity towards PACAP.[172,173] Through these receptors, PACAP can stimulate adenylate cyclase activation, elevate cellular cAMP concentration and initiate PKA phosphorylation activity. Consequently, it may activate the CREB transcription factor or Runx2 gene downstream of this kinase in osteoblasts. Moreover, PACAP demonstrates cross-talk effects with multiple pathways including Runx2-, BMP-, or WNT-associated pathways,[174,175] all of which have regulatory effects on osteogenesis. This suggests that PACAP plays an osteogenic role. Studies have shown that PACAP can modulate bone differentiation processes,[176] its absence can alter the microstructure of long bones,[177] and it has a protective effect against mechanical forces during cartilage differentiation as well.[175] In preclinical models of osteoarthritis (OA), it has been observed that PACAP possesses anti-inflammatory properties and protects cartilage integrity,[178] Therefore, PACAP exerts positive effects during both the inflammatory stage and bone remodeling stage of bone healing. Utilizing this property to enhance bone regeneration is one of the challenges we need to address.

Neuropeptide Y (NPY)

NPY is a highly conserved neurotransmitter peptide, predominantly released by sympathetic nerves in the central or peripheral nervous system.[179] Its immunoreactive fibers are primarily distributed in periosteal tissues, bone marrow, cortical bone, and blood vessels.[118,119,180–182] NPY receptors encompass Y1, Y2, Y4, Y5, and Y6 subtypes.[183]

NPY is involved in bone biology through various pathways. Initially, it was believed that NPY indirectly regulates bone by influencing blood vessels.[182,184] However, subsequent findings revealed the expression of NPY in MC3T3-E1 osteoblasts and bone marrow mesenchymal stem cells, as well as their role in osteogenic differentiation.[185] Additionally, the presence of NPY receptors in bone cells has been identified,[150,186] indicating a direct involvement of NPY in regulating osteoblast activity. Studies have demonstrated that cultured bone marrow stromal cells (BMSC) and osteoblasts express only the Y1 receptor while lacking expression of Y2, Y4, Y5 or Y6 receptors.[187] Nevertheless, further investigation confirmed the expression of Y2 receptor in MC3T3-E1 preosteoblasts derived from mouse skulls,[188] with its mRNA expression occurring under conditions promoting osteoblast differentiation.[189] In studies involving Y2 $^{-/-}$ knockout mice, an increase in both the number of bone progenitor cells and osteoblastic activity was observed. This led to elevated trabecular bone volume and thickness along with enhanced bone formation within these mice lacking functional Y2 $^{-/-}$ receptors.[190] The anatomical structure of the NPY/NPY receptor is illustrated in Figure 3 below.

NPY functions as a negative regulator of Y1 receptor expression.[189] Stimulation of bone progenitor cells with NPY leads to significant downregulation of the Y1 receptor, thereby enhancing osteoblast phenotypic markers and demonstrating the osteogenic promoting effect of NPY. Furthermore, during the early stages of osteoblast differentiation, NPY inhibits the transcriptional activity of RANKL promoter in osteoblasts, resulting in increased expression of OPG.[189,191] When directly acting on osteoblasts, the NPY pathway plays a crucial role in maintaining bone homeostasis.

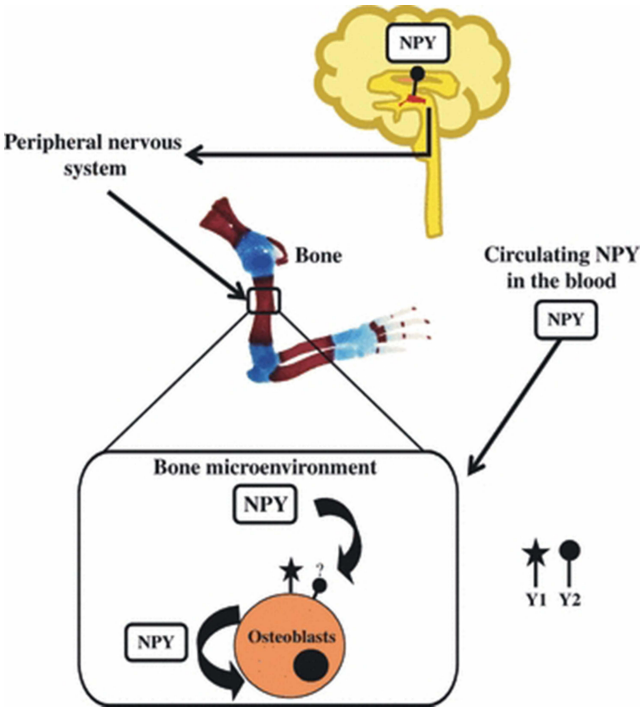

Figure 3 Anatomical structures with NPY/NPY receptors. Peripheral nerve fibers derived from basal, dorsal root and sympathetic ganglia innervate the bone and release NPY in the sites of innervation. Besides peripheral innervation, bone biology is also centrally regulated by NPY (highly expressed in the hypothalamus) and probably also by autocrine mechanisms, as osteoblasts (expressing Y1 and possibly Y2) are themselves capable of producing and secreting NPY. Reprinted from Franquinho F, Liz MA, Nunes AF, et al. Neuropeptide Y and osteoblast differentiation – the balance between the neuro-osteogenic network and local control. *FEBS J.* 2010;277(18):3664–3674. © 2010 The Authors Journal compilation © 2010 FEBS.[192]

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Programmed Regulatory Role of Nervous System Small Molecules in Bone Regeneration

Stage of Bone Injury

In the initial stage following bone defect occurrence, blood vessels rupture, leading to local hematoma formation, inflammation formation, and pain sensation. NGF,[193] BDNF,[65] CGRP,[137] SP,[138,194,195] VIP[196] and PACAP are believed to be involved in the initiation, maintenance and sensitization of peripheral pain. This process is indirectly regulated by recruiting sensory nerves during bone regeneration. In the early inflammatory response phase, there may be a variety of small molecules acting together. Functional NGF receptors have been identified in monocytes,[197] B cell or T cell clones.[198] In stressful situations, NGF functions as a broad "alarm" molecule that can recruit and activate local and systemic defense systems. It also causes monocyte toxicity, basophil differentiation, mast cell formation and degranulation.[199] Apart from the immune response triggered by NGF, microglia - the main innate immune cells of the central nervous system - participate in early immune responses by releasing BDNF. The effects of microglia may also be regulated by BDNF.[200] Additionally GDNF and NT-3 are involved in immune responses but their effects are not prominent.[201] FGF, CGRP and SP act as anti-inflammatory factors during the inflammatory stage of bone repair to prevent excessive inflammation. Specifically,FGR-21 inhibits IL-1 expression in lipopolysaccharide-induced THP-1 cells in vivo.Additionally,FGR-21 is associated with an increase in IL-10 through ERK1/2 and NF-kB pathways.[202] CGRP participates in validation processes by inducing overexpression of IL-10 in innate immune cells while inhibiting NF-kB activity.[203] As a superior anti-inflammatory agent. SP can inhibit expression of inflammatory cytokines around injury sites.[161]

Stage of Fibrovascular Healing

During the fibrovascular healing phase of bone regeneration, blood vessels and mesenchymal stem cells/progenitor cells (MSCS) are recruited into the temporary callus to re-establish the vascular network at the bone defect. Small molecules in the nervous system promote vascular regeneration and subsequently osteogenesis through various mechanisms, forming a complex neuro-vascular-bone regulatory network. NGF acts as a chemical attractant for ECs, facilitating their migration in human and pig aorta[204,205] and faster recruitment to bone defect sites. Additionally, NGF promotes VEGF production and induces angiogenesis by binding to TrkA receptors.[206,207] In a study[208] examining patients with both clavicular fractures and TBI, elevated levels of NGF, CD31 (an endothelial cell marker), and VEGF were also observed to enhance VEGF-mediated angiogenesis for accelerated fracture healing. BDNF can bind to TrkB receptors to stimulate VEGF production;[209] however, unlike NGF, its pro-angiogenic effect is not inhibited by VEGF neutralization. NT-4/5 and NT-3 also possess angiogenic properties.[209] In human bone marrow mesenchymal stem cells, NT-3 not only significantly increases the release of vasoactive substances such as VEGF and NGF in vitro[210] but also enhances blood circulation recovery after ischemia or ischemia-induced injury.[211] GDNFs promote BMSC migration while controlling microvascular networks independently of VEGFs activity,[212] thereby promoting vascular network growth.[213] PDGF acts as a chemoattractant and mitogen for mesenchymal cells promoting angiogenesis along with recruiting bone progenitor cells.[214] FGFs and VEGFs influence survival, promotion, migration and differentiation of endothelial cells.[215] CGRP itself exhibits potent vasodilation and angiogenesis-inducing effects,[216] while CGRP-positive nerves with both vascular and non-vascular terminations have been identified in rat long bones[150] thereby highlighting the vasogenic role of CGRP in peribone tissues. Nitric oxide produced by SP can act as an autocrine regulator of microvascular events essential for neovascularization.[217]

Stage of Bone Healing

After the completion of vascular network construction, bone progenitor cells recruited to the bone defect site initiate osteoblast and chondrocyte development, thereby promoting bone formation through intrachondral and intramembranous ossification. This is followed by cartilage mineralization, woven bone formation, and remodeling. As a crucial participant in bone healing,[218] NGF regulates the survival and differentiation of osteoblast cell lines by activating the BMP2/Runx2 signaling pathway.[219] Additionally, it can induce osteoclast generation independently of RANKL,[220] thus regulating both osteogenesis and bone resorption processes. NGF plays a direct role in bone repair and remodeling during bone

regeneration. When stimulated by mechanical load, osteoblasts produce NGF which activates TrkA receptors in the periosteum to enhance Wnt/β-catenin signaling in both osteoblasts and osteocytes through NGF-TRKA signaling.[221] BDNF shares similar effects on osteogenesis as NGF does; it positively regulates new bone formation by inducing proliferation, differentiation, and mineralization of osteoblasts[222] while also contributing to RANKL secretion from human BMSC for enhanced osteoclast generation,[223] ultimately achieving effective bone remodeling. However, BDNF and its receptor TrkB are localized to the fracture tissue during the inflammatory and early stages of bone formation, where BDNF is exclusively present in the granulation tissue along the periphery of woven bone, while being absent in chondrocytes and mature bone. NT-3 promotes osteoblast differentiation and contributes to bone regeneration. By regulating endothelium-interstitial transition (EndMT), NT-3 may induce ectopic ossification. Proosteoclast (poc)-derived GDNF also enhances osteogenic differentiation of BMSC through GDNF-GFRα1-RET signaling pathway. In developing rats, moderate bFGF promotes endochondral osteogenesis but inhibits endoperiosteal osteogenesis.[224] CGRP and SP can also induce osteoblast differentiation of BMSC via Wnt/β-catenin signaling pathway. Additionally, CGRP blocks RANKL-mediated NF-κB activation in bone marrow-derived macrophages (BMM), thereby suppressing osteoclast generation and bone resorption.[131,132] In BMSCs, SP appears to stimulate BMP-2 expression and migration, thus influencing bone formation.[155] VIP and PACAP attenuate the stimulatory effect of vitamin D3 on RANKL and RANK expression by reversing vitamin D3's inhibitory effect on osteophorin expression—a RANK antagonist—thereby inhibiting bone resorption.[225]

Conclusions

Numerous studies have confirmed that nerve tissue within bone tissue regulates the behavior of bone tissue through signaling pathways, and there is also an interaction between bone repair and nerve growth.[226,227] Currently, when constructing certain bone tissue engineering scaffolds, consideration has been given to the role of nerve tissue, leading to the development of relevant scaffolds based on bionics principles.[20] Through specific signaling pathways, various small molecules from the nervous system can promote osteogenic differentiation of bone progenitor cells, inhibit osteoclastic differentiation, and coordinate migration of bone cells. This direct promotion facilitates bone healing. Additionally, these molecules indirectly contribute to promoting bone regeneration by facilitating migration of vascular endothelial cells, reconstruction of vascular networks, and establishment of a neuro-vascular-bone regulatory network. Figure 4 below shows how neuro-bone tissue engineering is used.

Due to the short half-life of small molecules in the nervous system, their bioavailability is limited or their direct application is ineffective. When developing bone replacement materials, one approach involves encapsulating small molecules from the nervous system within a carrier to achieve controlled release. For instance, Liang et al[229] loaded naringenin (NG) and CGRP into a hydroxyapatite-sodium alginate composite hydrogel scaffold, enabling gradual release of CGRP during bone regeneration to sustain its role in promoting bone growth. However, finding suitable carrier materials as an in vivo platform for these small molecules remains a key challenge in future bone tissue engineering endeavors. Currently, certain biocomposite scaffolds have demonstrated the ability to enhance local bone healing[230,231] and exhibit stem cell-promoting effects,[232] indicating the potential for these scaffolds to be modified with neurogenic factors to facilitate bone regeneration. This approach, however, introduces additional design challenges, including the efficient loading of these small molecules and the creation of an optimal environment for their controlled release, thereby ensuring the effective functioning of all stages of bone healing.[228]

Additionally, exploring appropriate substitutes for nervous system small molecules that can exert similar osteogenic effects is also a promising direction for bone tissue engineering research. Indeed, utilizing Trk receptor agonists like 7, 8-DHF to activate osteogenic signaling pathways associated with neurotrophins has been shown to be feasible; Considering the role of various small molecules at various stages, the synthesis of endogenous bone peptides with osteogenic activity[233] can regulate bone metabolism and promote bone homeostasis, which is a further application of biomaterials.

While this paper has summarized the role of numerous neuropeptide small molecules in bone regeneration, many members of the extensive neuropeptide family do not directly engage in bone tissue healing. Instead, they exert an indirect regulatory influence via the endocrine system, a field referred to as bone neuroendocrinology.[234] Moreover, sensory neurons within the nervous system collaborate synergistically with these small molecules to enhance their

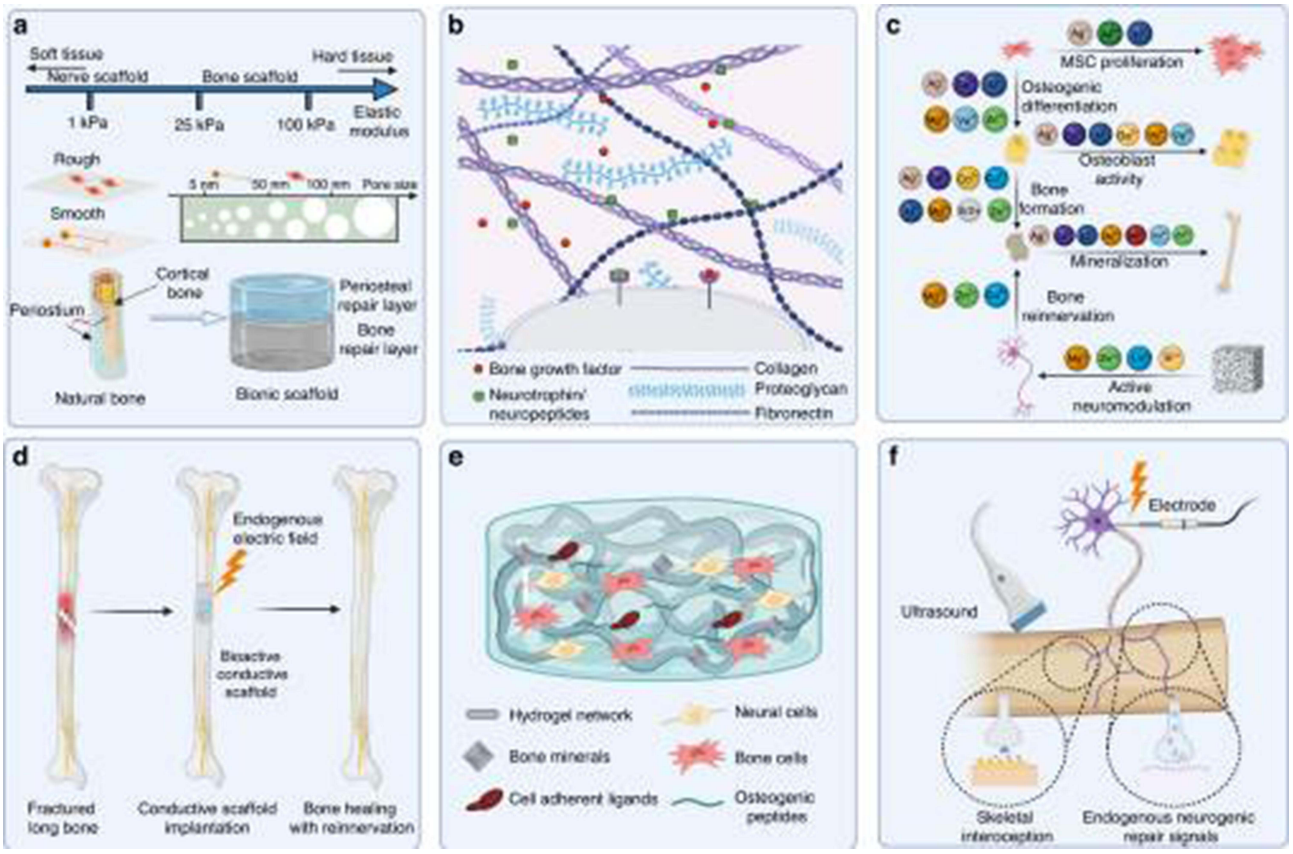

Figure 4 Schematic representation of neuro-bone tissue engineering strategies. (**a**) Regulate cell differentiation by controlling the surface morphology and structures of scaffolds; (**b**) regulate cellular behavior by modifying scaffolds with neural growth factor/neuropeptides. (**c**) Incorporate bioactive ions that can influence cellular behavior into scaffolds. (**d**) Scaffolds containing electroactive nanoparticles. (**e**) Coculture systems mimic the crosstalk between different cells during bone regeneration. (**f**) External field stimulation activates endogenous neurogenic repair signals, including skeletal interoception (left) and neuropeptide secretion (right).Reprinted from Sun W, Ye B, Chen S, et al. Neuro-bone tissue engineering: emerging mechanisms, potential strategies, and current challenges. *Bone Res*. 2023;11(1):65. Creative Commons.[228]

effects.[235] The human body maintains a complex and intricately regulated internal environment; for instance, menopausal women may experience decreased bone mineral density due to estrogen deficiency, resulting in osteoporosis.[236] It is evident that each physiological system interacts and influences one another. Further in-depth research is required to fully understand the various regulatory substances involved in bone healing.

Programmed regulation of bone regeneration has emerged as a prominent research area in recent years, with various small molecules within the nervous system playing crucial roles throughout the entire process. However, an urgent challenge that remains is how to design materials capable of controlled release and optimal functionality at specific time points. Therefore, this paper aims to provide a comprehensive review on the involvement of small molecules from the nervous system in bone regeneration, integrating their biological behaviors across different stages. The ultimate goal is to offer theoretical support for future advancements in designing bone replacement materials.

Data Sharing Statement

No data was used for the research described in the article.

Ethics Approval and Consent to Participate

This is a review article. Therefore, no patients or animals were involved in the preparation of this manuscript.

Consent for Publication

All authors have agreed on the journal to which the article has been submitted.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

No potential conflict of interest was reported by the authors.

References

1. Amini AR, Laurencin CT, Nukavarapu SP. Bone tissue engineering: recent advances and challenges. *Critical Rev Biomed Eng*. 2012;40:363–408. doi:10.1615/CritRevBiomedEng.v40.i5.10
2. Hao Q, Wu Y, Wu Y, et al. Tumor-derived exosomes in tumor-induced immune suppression. *Int J Mol Sci*. 2022;23(3):1461. doi:10.3390/ijms23031461
3. Murphy MP, Koepke LS, Lopez MT, et al. Articular cartilage regeneration by activated skeletal stem cells. *Nature Med*. 2020;26(10):1583–1592. doi:10.1038/s41591-020-1013-2
4. Shin CS, Cabrera FJ, Lee R, et al. 3D-bioprinted inflammation modulating polymer Scaffolds for soft tissue repair. *Adv Mater*. 2021;33(4):e2003778. doi:10.1002/adma.202003778
5. Rocchietta I, Fontana F, Simion M. Clinical outcomes of vertical bone augmentation to enable dental implant placement: a systematic review. *J Clin Periodontol*. 2008;35(s8):203–215. doi:10.1111/j.1600-051X.2008.01271.x
6. Safari B, Davaran S, Aghanejad A. Osteogenic potential of the growth factors and bioactive molecules in bone regeneration. *Int J Biol Macromol*. 2021;175:544–557. doi:10.1016/j.ijbiomac.2021.02.052
7. Mehta M, Schmidt-Bleek K, Duda GN, Mooney DJ. Biomaterial delivery of morphogens to mimic the natural healing cascade in bone. *Adv Drug Delivery Rev*. 2012;64(12):1257–1276. doi:10.1016/j.addr.2012.05.006
8. Golubovsky JL, Ejikeme T, Winkelman R, Steinmetz MP. Osteobiologics. *Operat Neurosurg*. 2021;21(Supplement_1):S2–s9. doi:10.1093/ons/opaa383
9. Gillman CE, Jayasuriya AC. FDA-approved bone grafts and bone graft substitute devices in bone regeneration. *Mater Sci Eng C Mater Biol Appl*. 2021;130:112466. doi:10.1016/j.msec.2021.112466
10. Sohn HS, Oh JK. Review of bone graft and bone substitutes with an emphasis on fracture surgeries. *Biomater Res*. 2019;23(9). doi:10.1186/s40824-019-0157-y
11. Perez JR, Kouroupis D, Li DJ, et al. Tissue engineering and cell-based therapies for fractures and bone defects. *Front Bioeng Biotechnol*. 2018;6:105. doi:10.3389/fbioe.2018.00105
12. Fang H, Zhu D, Yang Q, et al. Emerging zero-dimensional to four-dimensional biomaterials for bone regeneration. *J Nanobiotechnol*. 2022;20(1):26.
13. Wan Z, Zhang P, Liu Y, Lv L, Zhou Y. Four-dimensional bioprinting: current developments and applications in bone tissue engineering. *Acta Biomater*. 2020;101:26–42. doi:10.1016/j.actbio.2019.10.038
14. Wang Z, Zou C, Zhan X, et al. Application of double plate fixation combined with Masquelet technique for large segmental bone defects of distal tibia: a retrospective study and literature review. *BMC Surg*. 2024;24(1):103. doi:10.1186/s12893-024-02396-1
15. Meselhy MA, Singer MS, Halawa AM, et al. Gradual fibular transfer by Ilizarov external fixator in post-traumatic and post-infection large tibial bone defects. *Arch Orthop Trauma Surg*. 2018;138(5):653–660. doi:10.1007/s00402-018-2895-z
16. Hamdy RC, Bernstein M, Fragomen AT, Rozbruch SR. What's new in limb lengthening and deformity correction. *J Bone Joint Surg*. 2017;99(16):1408–1414. doi:10.2106/JBJS.17.00464
17. Houdek MT, Bayne CO, Bishop AT, Shin AY. The outcome and complications of vascularised fibular grafts. *Bone Joint J*. 2017;99-b:134–138.
18. Tarchala M, Harvey EJ, Barralet J. Biomaterial-stabilized soft tissue healing for healing of critical-sized bone defects: the Masquelet technique. *Adv Healthcare Mater*. 2016;5(6):630–640. doi:10.1002/adhm.201500793
19. Lopes D, Martins-Cruz C, Oliveira MB, Mano JF. Bone physiology as inspiration for tissue regenerative therapies. *Biomaterials*. 2018;185:240–275. doi:10.1016/j.biomaterials.2018.09.028
20. Xu Y, Xu C, He L, et al. Stratified-structural hydrogel incorporated with magnesium-ion-modified black phosphorus nanosheets for promoting neuro-vascularized bone regeneration. *Bioact Mater*. 2022;16:271–284. doi:10.1016/j.bioactmat.2022.02.024
21. Wan Q-Q, Qin W-P, Ma Y-X, et al. Crosstalk between bone and nerves within bone. *Adv Sci*. 2021;8(7):2003390. doi:10.1002/advs.202003390
22. Brazill JM, Beeve AT, Craft CS, Ivanusic JJ, Scheller EL. Nerves in bone: evolving concepts in pain and anabolism. *J Bone Miner Res*. 2019;34(8):1393–1406. doi:10.1002/jbmr.3822
23. Chen H, Hu B, Lv X, et al. Prostaglandin E2 mediates sensory nerve regulation of bone homeostasis. *Nat Commun*. 2019;10(1):181. doi:10.1038/s41467-018-08097-7
24. Assefa F, Lim J, Kim J-A, et al. Secretoneurin, a neuropeptide, enhances bone regeneration in a mouse calvarial bone defect model. *Tissue Eng and Regener Med*. 2021;18(2):315–324. doi:10.1007/s13770-020-00304-1

25. Assefa F. The role of sensory and sympathetic nerves in craniofacial bone regeneration. *Neuropeptides*. 2023;99:102328. doi:10.1016/j.npep.2023.102328
26. Cooper RR. Nerves in cortical bone. *Science*. 1968;160(3825):327–328. doi:10.1126/science.160.3825.327
27. Sayilekshmy M, Hansen RB, Delaissé J-M, et al. Innervation is higher above bone remodeling surfaces and in cortical pores in human bone: lessons from patients with primary hyperparathyroidism. *Sci Rep*. 2019;9(1):5361. doi:10.1038/s41598-019-41779-w
28. Hohmann EL, Elde RP, Rysavy JA, Einzig S, Gebhard RL. Innervation of periosteum and bone by sympathetic vasoactive intestinal peptide-containing nerve fibers. *Science*. 1986;232(4752):868–871. doi:10.1126/science.3518059
29. Adam C, Llorens A, Baroukh B, Cherruau M, Saffar JL. Effects of capsaicin-induced sensory denervation on osteoclastic resorption in adult rats. *Exp Physiol*. 2000;85(1):62–66. doi:10.1017/s0958067000019308
30. Apel PJ, Crane D, Northam CN, et al. Effect of selective sensory denervation on fracture-healing: an experimental study of rats. *J Bone Joint Surg*. 2009;91(12):2886–2895. doi:10.2106/JBJS.H.01878
31. Siddiqui JA, Partridge NC. Physiological bone remodeling: systemic regulation and growth factor involvement. *Physiology*. 2016;31(3):233–245. doi:10.1152/physiol.00061.2014
32. Li Z, Meyers CA, Chang L, et al. Fracture repair requires TrkA signaling by skeletal sensory nerves. *J Clin Invest*. 2019;129(12):5137–5150. doi:10.1172/JCI128428
33. Su Y-W, Chung R, Ruan C-S, et al. Neurotrophin-3 induces BMP-2 and VEGF activities and promotes the bony repair of injured growth plate cartilage and bone in rats. *J Bone Miner Res*. 2016;31(6):1258–1274. doi:10.1002/jbmr.2786
34. Tao R, Mi B, Hu Y, et al. Hallmarks of peripheral nerve function in bone regeneration. *Bone Res*. 2023;11(1):6. doi:10.1038/s41413-022-00240-x
35. Villar MJ, Wiesenfeld-Hallin Z, Xu X-J, et al. Further studies on galanin-, substance P-, and CGRP-like immunoreactivities in primary sensory neurons and spinal cord: effects of dorsal rhizotomies and sciatic nerve lesions. *Exp Neurol*. 1991;112(1):29–39. doi:10.1016/0014-4886(91)90111-O
36. Noguchi K, Senba E, Morita Y, Sato M, Tohyama M. α-CGRP and β-CGRP mRNAs are differentially regulated in the rat spinal cord and dorsal root ganglion. *Brain Res Mol Brain Res*. 1990;7(4):299–304. doi:10.1016/0169-328X(90)90080-W
37. Hökfelt T, Zhang X, Wiesenfeld-Hallin Z. Messenger plasticity in primary sensory neurons following axotomy and its functional implications. *Trends Neurosci*. 1994;17(1):22–30. doi:10.1016/0166-2236(94)90031-0
38. Li X-Q, Verge VMK, Johnston JM, Zochodne DW. CGRP peptide and regenerating sensory axons. *J Neuropathol Exp Neurol*. 2004;63(10):1092–1103. doi:10.1093/jnen/63.10.1092
39. Liu X, Obianyo O, Chan CB, et al. Biochemical and biophysical investigation of the brain-derived neurotrophic factor mimetic 7, 8--dihydroxyflavone in the binding and activation of the TrkB receptor. *J Biol Chem*. 2014;289(40):27571–27584. doi:10.1074/jbc.M114.562561
40. Liu C, Chan CB, Ye K. 7,8-dihydroxyflavone, a small molecular TrkB agonist, is useful for treating various BDNF-implicated human disorders. *Transl Neurodegener*. 2016;5(1). doi:10.1186/s40035-015-0048-7
41. Felts PA, Smith KJ, Gregson NA, Hughes RAC. Brain-derived neurotrophic factor in experimental autoimmune neuritis. *J neuroimmunol*. 2002;124(1–2):62–69. doi:10.1016/S0165-5728(02)00017-6
42. Ochs G, Penn RD, York M, et al. A Phase I/II trial of recombinant methionyl human brain derived neurotrophic factor administered by intrathecal infusion to patients with amyotrophic lateral sclerosis. *Amyotrophic Lateral Sclerosis Other Motor Neuron Disorder*. 2000;1(3):201–206. doi:10.1080/14660820050515197
43. Thoenen H, Sendtner M. Neurotrophins: from enthusiastic expectations through sobering experiences to rational therapeutic approaches. *Nat Neurosci*. 2002;5(S11):1046–1050. doi:10.1038/nn938
44. Stahl A, Yang YP. Regenerative approaches for the treatment of large bone defects. *Tissue Eng Part B*. 2021;27(6):539–547. doi:10.1089/ten.teb.2020.0281
45. Mercado-Pagán Á E, Stahl AM, Shanjani Y, Yang Y. Vascularization in bone tissue engineering constructs. *Ann Biomed Eng*. 2015;43(3):718–729. doi:10.1007/s10439-015-1253-3
46. van der Linden JC, Weinans H. Effects of microarchitecture on bone strength. *Curr Osteoporosis Rep*. 2007;5(2):56–61. doi:10.1007/s11914-007-0003-3
47. Ott SM. Cortical or trabecular bone: what's the difference? *Am J Nephrol*. 2018;47(6):373–375. doi:10.1159/000489672
48. Einhorn TA, Gerstenfeld LC. Fracture healing: mechanisms and interventions. *Nat Rev Rheumatol*. 2015;11(1):45–54. doi:10.1038/nrrheum.2014.164
49. Claes L, Recknagel S, Ignatius A. Fracture healing under healthy and inflammatory conditions. *Nat Rev Rheumatol*. 2012;8(3):133–143. doi:10.1038/nrrheum.2012.1
50. Marsell R, Einhorn TA. The biology of fracture healing. *Injury*. 2011;42(6):551–555. doi:10.1016/j.injury.2011.03.031
51. Ai-Aql ZS, Alagl AS, Graves DT, Gerstenfeld LC, Einhorn TA. Molecular mechanisms controlling bone formation during fracture healing and distraction osteogenesis. *J Dent Res*. 2008;87(2):107–118. doi:10.1177/154405910808700215
52. Hankenson KD, Gagne K, Shaughnessy M. Extracellular signaling molecules to promote fracture healing and bone regeneration. *Adv Drug Delivery Rev*. 2015;94:3–12. doi:10.1016/j.addr.2015.09.008
53. Maruyama M, Rhee C, Utsunomiya T, et al. Modulation of the inflammatory response and bone healing. *Front Endocrinol*. 2020;11:386. doi:10.3389/fendo.2020.00386
54. Reichardt LF. Neurotrophin-regulated signalling pathways. *Philos Trans R Soc London Ser B*. 2006;361:1545–1564. doi:10.1098/rstb.2006.1894
55. Barde YA, Edgar D, Thoenen H. Purification of a new neurotrophic factor from mammalian brain. *EMBO J*. 1982;1:549–553. doi:10.1002/j.1460-2075.1982.tb01207.x
56. Al-Qudah MA, Al-Dwairi A. Mechanisms and regulation of neurotrophin synthesis and secretion. *Neurosciences*. 2016;21:306–313. doi:10.17712/nsj.2016.4.20160080
57. Chéret J, Lebonvallet N, Carré JL, Misery L, Le gall-ianotto C. Role of neuropeptides, neurotrophins, and neurohormones in skin wound healing. *Wound Repair Regen*. 2013;21:772–788. doi:10.1111/wrr.12101
58. Sahay A, Kale A, Joshi S. Role of neurotrophins in pregnancy and offspring brain development. *Neuropeptides*. 2020;83:102075. doi:10.1016/j.npep.2020.102075

59. Manti S, Brown P, Perez MK, Piedimonte G. The role of neurotrophins in inflammation and allergy. *Vitamin Hormones*. 2017;104:313–341.
60. Becker K, Cana A, Baumgärtner W, Spitzbarth I. p75 neurotrophin receptor: a double-edged sword in pathology and regeneration of the central nervous system. *Vet Pathol*. 2018;55:786–801. doi:10.1177/0300985818781930
61. Xu J, Li Z, Tower RJ, et al. NGF-p75 signaling coordinates skeletal cell migration during bone repair. *Sci Adv*. 2022;8:eabl5716. doi:10.1126/sciadv.abl5716
62. Hempstead BL. The many faces of p75NTR. *Curr Opin Neurobiol*. 2002;12(3):260–267. doi:10.1016/S0959-4388(02)00321-5
63. Roux PP, Barker PA. Neurotrophin signaling through the p75 neurotrophin receptor. *Progress Neurobiol*. 2002;67:203–233. doi:10.1016/S0301-0082(02)00016-3
64. Wang Y, Yang K, Li G, et al. p75NTR −/− mice exhibit an alveolar bone loss phenotype and inhibited PI3K/Akt/β-catenin pathway. *Cell Proliferation*. 2020;53:e12800. doi:10.1111/cpr.12800
65. Pezet S, McMahon SB. Neurotrophins: mediators and modulators of pain. *Ann Rev Neurosci*. 2006;29(1):507–538. doi:10.1146/annurev.neuro.29.051605.112929
66. Mantyh PW. The neurobiology of skeletal pain. *Eur J Neurosci*. 2014;39(3):508–519. doi:10.1111/ejn.12462
67. Fitzpatrick V, Martín-Moldes Z, Deck A, et al. Functionalized 3D-printed silk-hydroxyapatite scaffolds for enhanced bone regeneration with innervation and vascularization. *Biomaterials*. 2021;276:120995. doi:10.1016/j.biomaterials.2021.120995
68. Ye J, Gong P. NGF-CS/HA-coating composite titanium facilitates the differentiation of bone marrow mesenchymal stem cells into osteoblast and neural cells. *Biochem Biophys Res Commun*. 2020;531(3):290–296. doi:10.1016/j.bbrc.2020.06.158
69. Sun S, Diggins NH, Gunderson ZJ, et al. No pain, no gain? The effects of pain-promoting neuropeptides and neurotrophins on fracture healing. *Bone*. 2020;131:115109. doi:10.1016/j.bone.2019.115109
70. Rivera KO, Russo F, Boileau RM, et al. Local injections of β-NGF accelerates endochondral fracture repair by promoting cartilage to bone conversion. *Sci Rep*. 2020;10(1):22241. doi:10.1038/s41598-020-78983-y
71. Meyers CA, Lee S, Sono T, et al. A neurotrophic mechanism directs sensory nerve transit in cranial bone. *Cell Rep*. 2020;31(8):107696. doi:10.1016/j.celrep.2020.107696
72. Liu Z, Suh JS, Deng P, et al. Epigenetic regulation of NGF-mediated osteogenic differentiation in human dental mesenchymal stem cells. *Stem Cells*. 2022;40(9):818–830. doi:10.1093/stmcls/sxac042
73. Wang CS, Kavalali ET, Monteggia LM. BDNF signaling in context: from synaptic regulation to psychiatric disorders. *Cell*. 2022;185(1):62–76. doi:10.1016/j.cell.2021.12.003
74. Colucci-D'Amato L, Speranza L, Volpicelli F. Neurotrophic factor BDNF, physiological functions and therapeutic potential in depression, neurodegeneration and brain cancer. *Int J mol Sci*. 2020;21(20):7777. doi:10.3390/ijms21207777
75. Xiong J, Liao J, Liu X, et al. A TrkB agonist prodrug prevents bone loss via inhibiting asparagine endopeptidase and increasing osteoprotegerin. *Nat Commun*. 2022;13(1):4820. doi:10.1038/s41467-022-32435-5
76. Loy TL, Vehlow D, Kauschke V, et al. Effects of BDNF and PEC Nanoparticles on Osteocytes. *Molecules*. 2020;26(1):25. doi:10.3390/molecules26010025
77. Sun R, Bai L, Yang Y, et al. Nervous system-driven osseointegration. *Int J Mol Sci*. 2022;23:8893.
78. Xue F, Zhao Z, Gu Y, et al. 7,8-Dihydroxyflavone modulates bone formation and resorption and ameliorates ovariectomy-induced osteoporosis. *eLife*. 2021;10. doi:10.7554/eLife.64872
79. Torres-Cruz FM, César Vivar-Cortés I, Moran I, et al. NT-4/5 antagonizes the BDNF modulation of corticostriatal transmission: role of the TrkB.T1 receptor. *CNS Neurosci Ther*. 2019;25(5):621–631. doi:10.1111/cns.13091
80. Nosrat CA, Fried K, Lindskog S, Olson L. Cellular expression of neurotrophin mRNAs during tooth development. *Cell Tissue Res*. 1997;290(3):569–580. doi:10.1007/s004410050962
81. Su Y-W, Zhou X-F, Foster BK, et al. Roles of neurotrophins in skeletal tissue formation and healing. *J Cell Physiol*. 2018;233(3):2133–2145. doi:10.1002/jcp.25936
82. Maisonpierre PC, Belluscio L, Squinto S, et al. Neurotrophin-3: a neurotrophic factor related to NGF and BDNF. *Science*. 1990;247(4949):1446–1451. doi:10.1126/science.247.4949.1446
83. Su Y-W, Chim SM, Zhou L, et al. Osteoblast derived-neurotrophin-3 induces cartilage removal proteases and osteoclast-mediated function at injured growth plate in rats. *Bone*. 2018;116:232–247. doi:10.1016/j.bone.2018.08.010
84. Zhang J, Wang L, Cao H, et al. Neurotrophin-3 acts on the endothelial-mesenchymal transition of heterotopic ossification in rats. *J Cell Mol Med*. 2019;23(4):2595–2609. doi:10.1111/jcmm.14150
85. Zhang J, Wang L, Chu J, et al. Macrophage-derived neurotrophin-3 promotes heterotopic ossification in rats. *Lab Investigat*. 2020;100(5):762–776. doi:10.1038/s41374-019-0367-x
86. Pezet S. Neurotrophins and pain. *Biologie aujourd'hui*. 2014;208:21–29. doi:10.1051/jbio/2014002
87. Yi S, Kim J, Lee SY. GDNF secreted by pre-osteoclasts induces migration of bone marrow mesenchymal stem cells and stimulates osteogenesis. *BMB Rep*. 2020;53(12):646–651. doi:10.5483/BMBRep.2020.53.12.199
88. Nencini S, Ringuet M, Kim DH, Greenhill C, Ivanusic JJ. GDNF, neurturin, and artemin activate and sensitize bone afferent neurons and contribute to inflammatory bone pain. *J Neurosci*. 2018;38:4899–4911. doi:10.1523/JNEUROSCI.0421-18.2018
89. Yuan Z-L, Liu X-D, Zhang Z-X, et al. Activation of GDNF-ERK-Runx1 signaling contributes to P2X3R gene transcription and bone cancer pain. *iScience*. 2022;25(9):104936. doi:10.1016/j.isci.2022.104936
90. Itoyama T, Yoshida S, Tomokiyo A, et al. Possible function of GDNF and Schwann cells in wound healing of periodontal tissue. *J Periodontal Res*. 2020;55(6):830–839. doi:10.1111/jre.12774
91. Gadomski S, Fielding C, García-García A, et al. A cholinergic neuroskeletal interface promotes bone formation during postnatal growth and exercise. *Cell Stem Cell*. 2022;29:528–544.e529. doi:10.1016/j.stem.2022.02.008
92. Fredriksson L, Li H, Eriksson U. The PDGF family: four gene products form five dimeric isoforms. *Cytokine Growth Factor Rev*. 2004;15(4):197–204. doi:10.1016/j.cytogfr.2004.03.007
93. Kramer F, Dernedde J, Mezheyeuski A, et al. Platelet-derived growth factor receptor β activation and regulation in murine myelofibrosis. *Haematologica*. 2020;105(8):2083–2094. doi:10.3324/haematol.2019.226332

94. Sivaraj KK, Jeong H-W, Dharmalingam B, et al. Regional specialization and fate specification of bone stromal cells in skeletal development. *Cell Rep*. 2021;36(2):109352. doi:10.1016/j.celrep.2021.109352
95. Peng Y, Wu S, Li Y, Crane JL. Type H blood vessels in bone modeling and remodeling. *Theranostics*. 2020;10(1):426–436. doi:10.7150/thno.34126
96. Zhang M, Yu W, Niibe K, et al. The effects of platelet-derived growth Factor-BB on bone marrow stromal cell-mediated vascularized bone regeneration. *Stem Cells Inter*. 2018;2018:3272098. doi:10.1155/2018/3272098
97. Mendoza-Azpur G, Cornejo H, Olaechea A, et al. Anorganic Bovine bone plus recombinant human platelet-derived growth Factor-BB in ridge preservation: a pilot study. *Int J Oral Maxillofacial Implant*. 2022;37(2):356–364. doi:10.11607/jomi.9022
98. Santhanam L, Liu G, Jandu S, et al. Skeleton-secreted PDGF-BB mediates arterial stiffening. *J Clin Invest*. 2021;131(20). doi:10.1172/JCI147116
99. Gao B, Deng R, Chai Y, et al. Macrophage-lineage TRAP+ cells recruit periosteum-derived cells for periosteal osteogenesis and regeneration. *J Clin Invest*. 2019;129(6):2578–2594. doi:10.1172/JCI98857
100. Böhm A-M, Dirckx N, Tower RJ, et al. Activation of skeletal stem and progenitor cells for bone regeneration is driven by PDGFRβ signaling. *Dev Cell*. 2019;51(2):236–254.e212. doi:10.1016/j.devcel.2019.08.013
101. Mace ML, Olgaard K, Lewin E. New aspects of the kidney in the regulation of Fibroblast Growth Factor 23 (FGF23) and mineral homeostasis. *Int J Mol Sci*. 2020;21(22):8810. doi:10.3390/ijms21228810
102. Schliermann A, Nickel J. Unraveling the connection between fibroblast growth factor and bone morphogenetic protein signaling. *Int J Mol Sci*. 2018;19(10):3220. doi:10.3390/ijms19103220
103. Tuzon CT, Rigueur D, Merrill AE. Nuclear fibroblast growth factor receptor signaling in skeletal development and disease. *Curr Osteoporosis Rep*. 2019;17(3):138–146. doi:10.1007/s11914-019-00512-2
104. Charoenlarp P, Rajendran AK, Iseki S. Role of fibroblast growth factors in bone regeneration. *Inflamm Regen*. 2017;37(1):10. doi:10.1186/s41232-017-0043-8
105. Czaya B, Faul C. The role of fibroblast growth Factor 23 in inflammation and anemia. *Int J Mol Sci*. 2019;20(17):4195. doi:10.3390/ijms20174195
106. Simic P, Babitt JL. Regulation of FGF23: beyond bone. *Curr Osteoporosis Rep*. 2021;19(6):563–573. doi:10.1007/s11914-021-00703-w
107. Chen H, Cui Y, Zhang D, Xie J, Zhou X. The role of fibroblast growth factor 8 in cartilage development and disease. *J Cell Mol Med*. 2022;26(4):990–999. doi:10.1111/jcmm.17174
108. Qin W, Gao J, Yan J, et al. Microarray analysis of signalling interactions between inflammation and angiogenesis in subchondral bone in temporomandibular joint osteoarthritis. *Biomater Transl*. 2024;5(2):175–184. doi:10.12336/biomatertransl.2024.02.007
109. Niedźwiedzki T, Filipowska J. Bone remodeling in the context of cellular and systemic regulation: the role of osteocytes and the nervous system. *J Mol Endocrinol*. 2015;55(2):R23–36. doi:10.1530/JME-15-0067
110. Hendrikse ER, Bower RL, Hay DL, Walker CS. Molecular studies of CGRP and the CGRP family of peptides in the central nervous system. *Cephalalgia*. 2019;39(3):403–419. doi:10.1177/0333102418765787
111. Lau Y-C, Qian X, Po K-T, Li L-M, Guo X. Electrical stimulation at the dorsal root ganglion preserves trabecular bone mass and micro-architecture of the tibia in hindlimb-unloaded rats. *Osteop Inter*. 2015;26(2):481–488. doi:10.1007/s00198-014-2866-3
112. Lau Y-C, Lai Y-M, Po K-T, et al. Dorsal root ganglion electrical stimulation promoted intertransverse process spinal fusion without decortications and bone grafting: a proof-of-concept study. *Spine J*. 2014;14(10):2472–2478. doi:10.1016/j.spinee.2014.04.001
113. Yuen-chi Lau R, Qian X, Po KT, Li LM, Guo X. Response of Rat Tibia to prolonged unloading under the influence of electrical stimulation at the dorsal root Ganglion. *Neuromodulation*. 2017;20:284–289. doi:10.1111/ner.12488
114. Naot D, Musson DS, Cornish J. The activity of peptides of the Calcitonin family in bone. *Physiol Rev*. 2019;99(1):781–805. doi:10.1152/physrev.00066.2017
115. Russell FA, King R, Smillie SJ, Kodji X, Brain SD. Calcitonin gene-related peptide: physiology and pathophysiology. *Physiol Rev*. 2014;94:1099–1142. doi:10.1152/physrev.00034.2013
116. Mulderry PK, Ghatki MA, Spokks RA, et al. Differential expression of alpha-CGRP and beta-CGRP by primary sensory neurons and enteric autonomic neurons of the rat. *Neuroscience*. 1988;25:195–205. doi:10.1016/0306-4522(88)90018-8
117. Sternini C. Enteric and visceral afferent CGRP neurons. Targets of innervation and differential expression patterns. *Ann NY Acad Sci*. 1992;657(1):170–186. doi:10.1111/j.1749-6632.1992.tb22766.x
118. Ahmed M, Srinivasan GR, Theodorsson E, et al. Extraction and quantitation of neuropeptides in bone by radioimmunoassay. *Regul Pept*. 1994;51(3):179–188. doi:10.1016/0167-0115(94)90063-9
119. Hill EL, Elde R. Distribution of CGRP-, VIP-, D beta H-, SP-, and NPY-immunoreactive nerves in the periosteum of the rat. *Cell Tissue Res*. 1991;264:469–480. doi:10.1007/BF00319037
120. Irie K, Hara-Irie F, Ozawa H, Yajima T. Calcitonin gene-related peptide (CGRP)-containing nerve fibers in bone tissue and their involvement in bone remodeling. *Microsc Res Tech*. 2002;58:85–90. doi:10.1002/jemt.10122
121. Barwell J, Gingell JJ, Watkins HA, et al. Calcitonin and calcitonin receptor-like receptors: common themes with family B GPCRs? *Br J Pharmacol*. 2012;166(1):51–65. doi:10.1111/j.1476-5381.2011.01525.x
122. Hay DL, Garelja ML, Poyner DR, Walker CS. Update on the pharmacology of calcitonin/CGRP family of peptides: IUPHAR Review 25. *Br J Pharmacol*. 2018;175(1):3–17. doi:10.1111/bph.14075
123. Gallai V, Sarchielli P, Floridi A, et al. Vasoactive peptide levels in the plasma of young migraine patients with and without aura assessed both interictally and ictally. *Cephalalgia*. 1995;15(5):384–390. doi:10.1046/j.1468-29821995.1505384.x
124. Goadsby PJ, Edvinsson L, Ekman R. Release of vasoactive peptides in the extracerebral circulation of humans and the cat during activation of the trigeminovascular system. *Ann Neurol*. 1988;23(2):193–196. doi:10.1002/ana.410230214
125. Goadsby PJ, Edvinsson L, Ekman R. Vasoactive peptide release in the extracerebral circulation of humans during migraine headache. *Ann Neurol*. 1990;28(2):183–187. doi:10.1002/ana.410280213
126. Buzzi MG, Carter WB, Shimizu T, Heath H, Moskowitz MA. Dihydroergotamine and sumatriptan attenuate levels of CGRP in plasma in rat superior sagittal sinus during electrical stimulation of the trigeminal ganglion. *Neuropharmacology*. 1991;30:1193–1200. doi:10.1016/0028-3908(91)90165-8

127. MacDonald NJ, Butters L, O'Shaughnessy DJ, Riddell AJ, Rubin PC. A comparison of the effects of human alpha calcitonin gene-related peptide and glyceryl trinitrate on regional blood velocity in man. *Br J Clin Pharmacol*. 1989;28:257–261. doi:10.1111/j.1365-2125.1989.tb05424.x
128. Escott KJ, Beattie DT, Connor HE, Brain SD. Trigeminal ganglion stimulation increases facial skin blood flow in the rat: a major role for calcitonin gene-related peptide. *Brain Res* 1995;669:93–99. doi:10.1016/0006-8993(94)01247-F
129. Köhli P, Appelt J, Otto E, et al. Effects of CGRP receptor antagonism on glucose and bone metabolism in mice with diet-induced obesity. *Bone*. 2021;143:115646. doi:10.1016/j.bone.2020.115646
130. Schinke T, Liese S, Priemel M, et al. Decreased bone formation and osteopenia in mice lacking α-Calcitonin gene-related peptide. *J Bone Miner Res*. 2004;19(12):2049–2056. doi:10.1359/jbmr.040915
131. Wang L, Shi X, Zhao R, et al. Calcitonin-gene-related peptide stimulates stromal cell osteogenic differentiation and inhibits RANKL induced NF-κB activation, osteoclastogenesis and bone resorption. *Bone*. 2010;46(5):1369–1379. doi:10.1016/j.bone.2009.11.029
132. Boyce BF, Xing L. Functions of RANKL/RANK/OPG in bone modeling and remodeling. *Arch Biochem Biophys* 2008;473(2):139–146. doi:10.1016/j.abb.2008.03.018
133. Ballica R, Valentijn K, Khachatryan A, et al. Targeted expression of calcitonin gene-related peptide to osteoblasts increases bone density in mice. *J Bone Miner Res*. 1999;14(7):1067–1074. doi:10.1359/jbmr.1999.14.7.1067
134. Mrak E, Guidobono F, Moro G, et al. Calcitonin gene-related peptide (CGRP) inhibits apoptosis in human osteoblasts by β-catenin stabilization. *J Cell Physiol*. 2010;225(3):701–708. doi:10.1002/jcp.22266
135. Tian G, Zhang G, Tan YH. Calcitonin gene-related peptide stimulates BMP-2 expression and the differentiation of human osteoblast-like cells in vitro. *Acta Pharmacol Sin*. 2013;34:1467–1474. doi:10.1038/aps.2013.41
136. Zhou R, Yuan Z, Liu J, Liu J. Calcitonin gene-related peptide promotes the expression of osteoblastic genes and activates the WNT signal transduction pathway in bone marrow stromal stem cells. *Mol Med Rep*. 2016;13:4689–4696. doi:10.3892/mmr.2016.5117
137. Schou WS, Ashina S, Amin FM, Goadsby PJ, Ashina M. Calcitonin gene-related peptide and pain: a systematic review. *J Headache Pain*. 2017;18(34). doi:10.1186/s10194-017-0741-2
138. Saito T, Koshino T. Distribution of neuropeptides in synovium of the knee with osteoarthritis. *Clin Orthop Related Res*. 2000;376:172–182. doi:10.1097/00003086-200007000-00024
139. Xu J, Wang J, Chen X, et al. The effects of calcitonin gene-related peptide on bone homeostasis and regeneration. *Curr Osteoporosis Rep*. 2020;18(6):621–632. doi:10.1007/s11914-020-00624-0
140. Li Y, Tan Y, Zhang G, Yang B, Zhang J. Effects of calcitonin gene-related peptide on the expression and activity of nitric oxide synthase during mandibular bone healing in rabbits: an experimental study. *J Oral Maxillofacial Surg*. 2009;67(2):273–279. doi:10.1016/j.joms.2008.06.077
141. Aoki M, Tamai K, Saotome K. Substance P- and calcitonin gene-related peptide-immunofluorescent nerves in the repair of experimental bone defects. *Int Orthop*. 1994;18(5):317–324. doi:10.1007/BF00180235
142. Li J, Ahmad T, Spetea M, Ahmed M, Kreicbergs A. Bone reinnervation after fracture: a study in the rat. *J Bone Miner Res*. 2001;16(8):1505–1510. doi:10.1359/jbmr.2001.16.8.1505
143. Tang P, Duan C, Wang Z, et al. NPY and CGRP inhibitor influence on ERK pathway and macrophage aggregation during fracture healing. *Cell Physiol Biochem*. 2017;41(4):1457–1467. doi:10.1159/000468405
144. Mi J, Xu J-K, Yao Z, et al. Implantable electrical stimulation at dorsal root Ganglions Accelerates osteoporotic fracture healing via calcitonin gene-related peptide. *Adv Sci*. 2022;9(1):e2103005. doi:10.1002/advs.202103005
145. Ni S, Ling Z, Wang X, et al. Sensory innervation in porous endplates by Netrin-1 from osteoclasts mediates PGE2-induced spinal hypersensitivity in mice. *Nat Commun*. 2019;10(1):5643. doi:10.1038/s41467-019-13476-9
146. Li J, Kreicbergs A, Bergström J, Stark A, Ahmed M. Site-specific CGRP innervation coincides with bone formation during fracture healing and modeling: a study in rat angulated tibia. *J Orthop Res*. 2007;25(9):1204–1212. doi:10.1002/jor.20406
147. Rusanen M, Korkala O, Grönblad M, Partanen S, Nederström A. Evolution of substance P immunofluorescent nerves in callus tissue during fracture healing. *J Trauma*. 1987;27:1340–1343. doi:10.1097/00005373-198712000-00004
148. Luo J, Chen H, Wang G, et al. CGRP-loaded porous microspheres protect BMSCs for alveolar bone regeneration in the periodontitis microenvironment. *Adv Healthcare Mater*. 2023;12(28):e2301366. doi:10.1002/adhm.202301366
149. Liu D, Jiang LS, Dai LY. Substance P and its receptors in bone metabolism. *Neuropeptides*. 2007;41:271–283. doi:10.1016/j.npep.2007.05.003
150. Bjurholm A. Neuroendocrine peptides in bone. *Int Orthop*. 1991;15(4):325–329. doi:10.1007/BF00186871
151. Pang J, Guo H-L, Ding D-F, et al. Changes of mesenchymal stromal cells mobilization and bone turnover in an experimental bone fracture model in ovariectomized mice. *Int J Clin Exp Pathol*. 2015;8(9):10228–10238.
152. Kim SH, Hur W, Kim JE, et al. Self-assembling peptide nanofibers coupled with neuropeptide substance P for bone tissue engineering. *Tissue Eng Part A*. 2015;21(7–8):1237–1246. doi:10.1089/ten.tea.2014.0472
153. Hong HS, Lee J, Lee E, et al. A new role of substance P as an injury-inducible messenger for mobilization of CD29+ stromal-like cells. *Nature Med*. 2009;15(4):425–435. doi:10.1038/nm.1909
154. Al-Baadani MA, Xu L, Cai K, et al. Preparation of co-electrospinning membrane loaded with simvastatin and substance P to accelerate bone regeneration by promoting cell homing, angiogenesis and osteogenesis. *Mater Today*. 2023;21:100692. doi:10.1016/j.mtbio.2023.100692
155. Fu S, Mei G, Wang Z, et al. Neuropeptide Substance P improves osteoblastic and angiogenic differentiation capacity of bone marrow stem cells in vitro. *Biomed Res Int*. 2014;2014:596023. doi:10.1155/2014/596023
156. Chen D, Liu P, Li M, et al. Nacre-mimetic hydroxyapatite/chitosan/gelatin layered scaffolds modifying substance P for subchondral bone regeneration. *Carbohydr Polym*. 2022;291:119575. doi:10.1016/j.carbpol.2022.119575
157. Imai S, Matsusue Y. Neuronal regulation of bone metabolism and anabolism: calcitonin gene-related peptide-, substance P-, and tyrosine hydroxylase-containing nerves and the bone. *Microsc Res Tech*. 2002;58(2):61–69. doi:10.1002/jemt.10119
158. Mori T, Ogata T, Okumura H, et al. Substance P regulates the function of rabbit cultured osteoclast; increase of intracellular free calcium concentration and enhancement of bone resorption. *Biochem Biophys Res Commun*. 1999;262(2):418–422. doi:10.1006/bbrc.1999.1220
159. Wang L, Zhao R, Shi X, et al. Substance P stimulates bone marrow stromal cell osteogenic activity, osteoclast differentiation, and resorption activity in vitro. *Bone*. 2009;45(2):309–320. doi:10.1016/j.bone.2009.04.203

160. Amirthalingam S, Lee SS, Rajendran AK, et al. Addition of lactoferrin and substance P in a chitin/PLGA-CaSO4 hydrogel for regeneration of calvarial bone defects. *Mater Sci Eng C*. 2021;126:112172. doi:10.1016/j.msec.2021.112172
161. Lee J-K, Kim D-S, Park S-Y, et al. Osteoporotic bone regeneration via Plenished Biomimetic PLGA Scaffold with sequential release system. *Small*. 2024;20(23):e2310734. doi:10.1002/smll.202310734
162. Konttinen Y, Imai S, Suda A. Neuropeptides and the puzzle of bone remodeling: state of the art. *Acta Orthop Scand*. 1996;67(6):632–639. doi:10.3109/17453679608997772
163. Schulz S, Rocken C, Mawrin C, et al. Immunocytochemical identification of VPAC1, VPAC2, and PAC1 receptors in normal and neoplastic human tissues with subtype-specific antibodies. *Clin Cancer Res*. 2004;10(24):8235–8242. doi:10.1158/1078-0432.CCR-04-0939
164. Shi L, Liu Y, Yang Z, et al. Vasoactive intestinal peptide promotes fracture healing in sympathectomized mice. *Calcified Tissue Inter*. 2021;109(1):55–65. doi:10.1007/s00223-021-00820-9
165. Lundberg P, Lundgren I, Mukohyama H, et al. Vasoactive intestinal peptide (VIP)/pituitary adenylate cyclase-activating peptide receptor subtypes in mouse calvarial osteoblasts: presence of VIP-2 receptors and differentiation-induced expression of VIP-1 receptors. *Endocrinology*. 2001;142:339–347. doi:10.1210/endo.142.1.7912
166. Ransjö M, Lie A, Mukohyama H, Lundberg P, Lerner UH. Microisolated mouse osteoclasts express VIP-1 and PACAP receptors. *Biochem Biophys Res Commun*. 2000;274(2):400–404. doi:10.1006/bbrc.2000.3151
167. Lundberg P, Lie A, Bjurholm A, et al. Vasoactive intestinal peptide regulates osteoclast activity via specific binding sites on both osteoclasts and osteoblasts. *Bone*. 2000;27(6):803–810. doi:10.1016/S8756-3282(00)00394-X
168. Hohmann EL, Levine L, Tashjian AH Jr. Vasoactive intestinal peptide stimulates bone resorption via a cyclic adenosine 3′,5′-monophosphate-dependent mechanism. *Endocrinology*. 1983;112:1233–1239. doi:10.1210/endo-112-4-1233
169. Lerner UH, Lundberg P, Ransjö M, Persson P, Håkanson R. Helodermin, helospectin, and PACAP stimulate cyclic AMP formation in intact bone, isolated osteoblasts, and osteoblastic cell lines. *Calcified Tissue Inter*. 1994;54:284–289. doi:10.1007/BF00295952
170. Shi L, Feng L, Zhu M-L, et al. Vasoactive intestinal peptide stimulates bone marrow-mesenchymal stem cells osteogenesis differentiation by activating Wnt/β-Catenin signaling pathway and promotes rat skull defect repair. *Stem Cells Dev*. 2020;29(10):655–666. doi:10.1089/scd.2019.0148
171. Miyata A, Arimura A, Dahl RR, et al. Isolation of a novel 38 residue-hypothalamic polypeptide which stimulates adenylate cyclase in pituitary cells. *Biochem Biophys Res Commun*. 1989;164:567–574. doi:10.1016/0006-291X(89)91757-9
172. Vaudry D, Falluel-Morel A, Bourgault S, et al. Pituitary adenylate cyclase-activating polypeptide and its receptors: 20 years after the discovery. *Pharmacol Rev*. 2009;61(3):283–357. doi:10.1124/pr.109.001370
173. Gourlet P, Vandermeers A, Vertongen P, et al. Development of high affinity selective VIP1 receptor agonists. *Peptides*. 1997;18(10):1539–1545. doi:10.1016/S0196-9781(97)00228-3
174. Juhász T, Helgadottir SL, Tamás A, Reglődi D, Zákány R. PACAP and VIP signaling in chondrogenesis and osteogenesis. *Peptides*. 2015;66:51–57. doi:10.1016/j.peptides.2015.02.001
175. Juhász T, Szentléleky E, Somogyi C, et al. Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) pathway is induced by mechanical load and reduces the activity of Hedgehog signaling in chondrogenic micromass cell cultures. *Int J Mol Sci*. 2015;16(8):17344–17367. doi:10.3390/ijms160817344
176. Juhász T, Matta C, Katona É, et al. Pituitary adenylate cyclase-activating polypeptide (PACAP) signalling enhances osteogenesis in UMR-106 cell line. *J Mol Neurosci*. 2014;54(3):555–573. doi:10.1007/s12031-014-0389-1
177. Józsa G, Szegeczki V, Pálfi A, et al. Signalling alterations in bones of Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) gene deficient mice. *Int J Mol Sci*. 2018;19(9):2538. doi:10.3390/ijms19092538
178. Giunta S, Castorina A, Marzagalli R, et al. Ameliorative effects of PACAP against cartilage degeneration. Morphological, immunohistochemical and biochemical evidence from in vivo and in vitro models of rat osteoarthritis. *Int J Mol Sci*. 2015;16(3):5922–5944. doi:10.3390/ijms16035922
179. Zukowska-Grojec Z, Neuropeptide Y. A novel sympathetic stress hormone and more. *Ann NY Acad Sci*. 1995;771:219–233. doi:10.1111/j.1749-6632.1995.tb44683.x
180. Bjurholm A, Kreicbergs A, Terenius L, et al. Neuropeptide Y-, tyrosine hydroxylase- and vasoactive intestinal polypeptide-immunoreactive nerves in bone and surrounding tissues. *J Auton Nerv Syst*. 1988;25(2–3):119–125. doi:10.1016/0165-1838(88)90016-1
181. Tabarowski Z, Gibson-Berry K, Felten SY. Noradrenergic and peptidergic innervation of the mouse femur bone marrow. *Acta Histochem*. 1996;98(4):453–457. doi:10.1016/S0065-1281(96)80013-4
182. Ahmad T, Ugarph-Morawski A, Li J, et al. Bone and joint neuropathy in rats with type-2 diabetes. *Regul Pept*. 2004;119(1–2):61–67. doi:10.1016/j.regpep.2003.12.008
183. Lee NJ, Herzog H. NPY regulation of bone remodelling. *Neuropeptides*. 2009;43(6):457–463. doi:10.1016/j.npep.2009.08.006
184. Ericsson A, Schalling M, McIntyre KR, et al. Detection of neuropeptide Y and its mRNA in megakaryocytes: enhanced levels in certain autoimmune mice. *Proc Natl Acad Sci USA*. 1987;84(16):5585–5589. doi:10.1073/pnas.84.16.5585
185. Nunes AF, Liz MA, Franquinho F, et al. Neuropeptide Y expression and function during osteoblast differentiation--insights from transthyretin knockout mice. *FEBS J*. 2010;277:263–275. doi:10.1111/j.1742-4658.2009.07482.x
186. Bjurholm A, Kreicbergs A, Schultzberg M, Lerner UH. Neuroendocrine regulation of cyclic AMP formation in osteoblastic cell lines (UMR-106-01, ROS 17/2.8, MC3T3-E1, and Saos-2) and primary bone cells. *J Bone Miner Res*. 1992;7:1011–1019. doi:10.1002/jbmr.5650070903
187. Lundberg P, Allison SJ, Lee NJ, et al. Greater bone formation of Y2 knockout mice is associated with increased osteoprogenitor numbers and altered Y1 receptor expression. *J Biol Chem*. 2007;282(26):19082–19091. doi:10.1074/jbc.M609629200
188. Hosaka H, Nagata A, Yoshida T, et al. Pancreatic polypeptide is secreted from and controls differentiation through its specific receptors in osteoblastic MC3T3-E1 cells. *Peptides*. 2008;29(8):1390–1395. doi:10.1016/j.peptides.2008.03.015
189. Teixeira L, Sousa DM, Nunes AF, et al. NPY revealed as a critical modulator of osteoblast function in vitro: new insights into the role of Y1 and Y2 receptors. *J Cell Biochem*. 2009;107(5):908–916. doi:10.1002/jcb.22194
190. Baldock PA, Sainsbury A, Couzens M, et al. Hypothalamic Y2 receptors regulate bone formation. *J Clin Invest*. 2002;109(7):915–921. doi:10.1172/JCI0214588

191. Amano S, Arai M, Goto S, Togari A. Inhibitory effect of NPY on isoprenaline-induced osteoclastogenesis in mouse bone marrow cells. *Biochimica Et Biophysica Acta*. 2007;1770(6):966–973. doi:10.1016/j.bbagen.2007.02.009
192. Franquinho F, Liz MA, Nunes AF, et al. Neuropeptide Y and osteoblast differentiation – the balance between the neuro-osteogenic network and local control. *FEBS J*. 2010;277(18):3664–3674. doi:10.1111/j.1742-4658.2010.07774.x
193. Yasui M, Shiraishi Y, Ozaki N, et al. Nerve growth factor and associated nerve sprouting contribute to local mechanical hyperalgesia in a rat model of bone injury. *Eur J Pain*. 2012;16(7):953–965. doi:10.1002/j.1532-2149.2011.00094.x
194. Witoński D, Wagrowska-Danielewicz M. Distribution of substance-P nerve fibers in the knee joint in patients with anterior knee pain syndrome. A preliminary report. *Knee Surg Sports Traumatol Arthrosc*. 1999;7(3):177–183. doi:10.1007/s001670050144
195. Saxler G, Löer F, Skumavc M, Pförtner J, Hanesch U. Localization of SP- and CGRP-immunopositive nerve fibers in the Hip joint of patients with painful osteoarthritis and of patients with painless failed total hip arthroplasties. *Eur J Pain*. 2007;11(1):67–74. doi:10.1016/j.ejpain.2005.12.011
196. Jiang W, Wang H, Li Y-S, Luo W. Role of vasoactive intestinal peptide in osteoarthritis. *J Biomed Sci*. 2016;23(1):63. doi:10.1186/s12929-016-0280-1
197. Ehrhard PB, Ganter U, Stalder A, Bauer J, Otten U. Expression of functional trk protooncogene in human monocytes. *Proc Natl Acad Sci USA*. 1993;90(12):5423–5427. doi:10.1073/pnas.90.12.5423
198. Ehrhard PB, Erb P, Graumann U, Otten U. Expression of nerve growth factor and nerve growth factor receptor tyrosine kinase Trk in activated CD4-positive T-cell clones. *Proc Natl Acad Sci USA*. 1993;90(23):10984–10988. doi:10.1073/pnas.90.23.10984
199. Asaumi K, Nakanishi T, Asahara H, Inoue H, Takigawa M. Expression of neurotrophins and their receptors (TRK) during fracture healing. *Bone*. 2000;26(6):625–633. doi:10.1016/S8756-3282(00)00281-7
200. Rossetti AC, Paladini MS, Trepci A, et al. Differential neuroinflammatory response in male and female mice: a role for BDNF. *Front Mol Neurosci*. 2019;12:166. doi:10.3389/fnmol.2019.00166
201. Shinoda M, Hoffer BJ, Olson L. Interactions of neurotrophic factors GDNF and NT-3, but not BDNF, with the immune system following fetal spinal cord transplantation. *Brain Res* 1996;722(1–2):153–167. doi:10.1016/0006-8993(96)00208-9
202. Li J-Y, Wang N, Khoso MH, et al. FGF-21 elevated IL-10 production to correct LPS-induced inflammation. *Inflammation*. 2018;41(3):751–759. doi:10.1007/s10753-018-0729-3
203. Holzmann B. Antiinflammatory activities of CGRP modulating innate immune responses in health and disease. *Curr Protein Peptide Sci*. 2013;14(4):268–274. doi:10.2174/13892037113149990046
204. Dollé J-P, Rezvan A, Allen FD, Lazarovici P, Lelkes I. Nerve growth factor-induced migration of endothelial cells. *J Pharmacol Exp Ther*. 2005;315(3):1220–1227. doi:10.1124/jpet.105.093252
205. Rahbek UL, Dissing S, Thomassen C, Hansen AJ, Tritsaris K. Nerve growth factor activates aorta endothelial cells causing PI3K/Akt- and ERK-dependent migration. *Pflügers Archiv*. 2005;450(5):355–361. doi:10.1007/s00424-005-1436-0
206. Cantarella G, Lempereur L, Presta M, et al. Nerve growth factor–endothelial cell interaction leads to angiogenesis in vitro and in vivo. *FASEB J*. 2002;16(10):1307–1309. doi:10.1096/fj.01-1000fje
207. Lazarovici P, Gazit A, Staniszewska I, Marcinkiewicz C, Lelkes PI. Nerve growth factor (NGF) promotes angiogenesis in the quail chorioallantoic membrane. *Endothelium*. 2006;13(1):51–59. doi:10.1080/10623320600669053
208. Zhang R, Liang Y, Wei S. The expressions of NGF and VEGF in the fracture tissues are closely associated with accelerated clavicle fracture healing in patients with traumatic brain injury. *Ther Clin Risk Manage*. 2018;14:2315–2322. doi:10.2147/TCRM.S182325
209. Kermani P, Rafii D, Jin DK, et al. Neurotrophins promote revascularization by local recruitment of TrkB+ endothelial cells and systemic mobilization of hematopoietic progenitors. *J Clin Invest*. 2005;115(3):653–663. doi:10.1172/JCI200522655
210. Shen L, Zeng W, Wu Y-X, et al. Neurotrophin-3 accelerates wound healing in diabetic mice by promoting a paracrine response in mesenchymal stem cells. *Cell Transplant*. 2013;22(6):1011–1021. doi:10.3727/096368912X657495
211. Cristofaro B, Stone OA, Caporali A, et al. Neurotrophin-3 is a novel angiogenic factor capable of therapeutic neovascularization in a mouse model of limb ischemia. *Arteriosclerosis Thromb Vasc Biol*. 2010;30(6):1143–1150. doi:10.1161/ATVBAHA.109.205468
212. Blais M, Lévesque P, Bellenfant S, Berthod F. Nerve growth factor, brain-derived neurotrophic factor, Neurotrophin-3 and Glial-derived neurotrophic factor enhance angiogenesis in a tissue-engineered in vitro model. *Tissue Eng Part A*. 2013;19(15–16):1655–1664. doi:10.1089/ten.tea.2012.0745
213. Zhong Z, Gu H, Peng J, et al. GDNF secreted from adipose-derived stem cells stimulates VEGF-independent angiogenesis. *Oncotarget*. 2016;7(24):36829–36841. doi:10.18632/oncotarget.9208
214. Hollinger JO, Hart CE, Hirsch SN, Lynch S, Friedlaender GE. Recombinant human platelet-derived growth factor: biology and clinical applications. *J Bone Joint Surg*. 2008;90:48–54. doi:10.2106/JBJS.G.01231
215. Cross MJ, Claesson-Welsh L. FGF and VEGF function in angiogenesis: signalling pathways, biological responses and therapeutic inhibition. *Trends Pharmacol Sci*. 2001;22:201–207. doi:10.1016/S0165-6147(00)01676-X
216. Mapp PI, McWilliams DF, Turley MJ, Hargin E, Walsh DA. A role for the sensory neuropeptide calcitonin gene-related peptide in endothelial cell proliferation in vivo. *Br J Pharmacol*. 2012;166:1261–1271. doi:10.1111/j.1476-5381.2012.01848.x
217. Ziche M, Morbidelli L, Masini E, et al. Nitric oxide mediates angiogenesis in vivo and endothelial cell growth and migration in vitro promoted by substance P. *J Clin Invest*. 1994;94(5):2036–2044. doi:10.1172/JCI117557
218. El Baassiri MG, Dosh L, Haidar H, et al. Nerve growth factor and burn wound healing: update of molecular interactions with skin cells. *Burns*. 2023;49(5):989–1002. doi:10.1016/j.burns.2022.11.001
219. Jin P, Yin F, Huang L, et al. Retracted article: Guangxi cobra venom-derived NGF promotes the osteogenic and therapeutic effects of porous BCP ceramic. *Exp Mol Med*. 2017;49(4):e312. doi:10.1038/emm.2016.173
220. Hemingway F, Taylor R, Knowles HJ, Athanasou NA. RANKL-independent human osteoclast formation with April, BAFF, NGF, IGF I and IGF II. *Bone*. 2011;48(4):938–944. doi:10.1016/j.bone.2010.12.023
221. Tomlinson RE, Li Z, Li Z, et al. NGF-TrkA signaling in sensory nerves is required for skeletal adaptation to mechanical loads in mice. *Proc Natl Acad Sci USA*. 2017;114(18):E3632–e3641. doi:10.1073/pnas.1701054114
222. Cai -X-X, Luo E, Yuan Q. Interaction between Schwann cells and osteoblasts in vitro. *Int J Oral Sci*. 2010;2(2):74–81. doi:10.4248/IJOS10039

223. Ai L-S, Sun C-Y, Zhang L, et al. Inhibition of BDNF in multiple myeloma blocks osteoclastogenesis via down-regulated stroma-derived RANKL expression both in vitro and in vivo. *PLoS One*. 2012;7(10):e46287. doi:10.1371/journal.pone.0046287
224. Nagai H, Tsukuda R, Mayahara H. Effects of basic fibroblast growth factor (bFGF) on bone formation in growing rats. *Bone*. 1995;16 (3):367–373. doi:10.1016/8756-3282(94)00049-2
225. Mukohyama H, Ransjö M, Taniguchi H, Ohyama T, Lerner UH. The inhibitory effects of vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide on osteoclast formation are associated with upregulation of osteoprotegerin and downregulation of RANKL and RANK. *Biochem Biophys Res Commun*. 2000;271(1):158–163. doi:10.1006/bbrc.2000.2599
226. Dolan CP, Yan M, Zimmel K, et al. Axonal regrowth is impaired during digit tip regeneration in mice. *Dev Biol*. 2019;445(2):237–244. doi:10.1016/j.ydbio.2018.11.010
227. Mohammad KS, Neufeld DA. Denervation retards but does not prevent toetip regeneration. *Wound Repair Regen*. 2000;8(4):277–281. doi:10.1046/j.1524-475x.2000.00277.x
228. Sun W, Ye B, Chen S, et al. Neuro-bone tissue engineering: emerging mechanisms, potential strategies, and current challenges. *Bone Res*. 2023;11(1):65. doi:10.1038/s41413-023-00302-8
229. Liang T, Wu J, Li F, et al. Drug-loading three-dimensional scaffolds based on hydroxyapatite-sodium alginate for bone regeneration. *J Biomed Mat Res Part A*. 2021;109(2):219–231. doi:10.1002/jbm.a.37018
230. Mahmoud EM, Sayed M, El-Kady AM, Elsayed H, Naga SM. In vitro and in vivo study of naturally derived alginate/hydroxyapatite bio composite scaffolds. *Int J Biol Macromol*. 2020;165:1346–1360. doi:10.1016/j.ijbiomac.2020.10.014
231. Yang J, Fatima K, Zhou X, He C. Meticulously engineered three-dimensional-printed scaffold with microarchitecture and controlled peptide release for enhanced bone regeneration. *Biomater transl*. 2024;5(1):69–83. doi:10.12336/biomatertransl.2024.01.007
232. Sayed M, Mahmoud EM, Saber SM, et al. Effect of the injectable alginate/ nano-hydroxyapatite and the silica/ nano-hydroxyapatite composites on the stem cells: a comparative study. *J Non-Crystalline Solids*. 2023;610:122327.
233. Lewis JW, Frost K, Neag G, et al. Therapeutic avenues in bone repair: harnessing an anabolic osteopeptide, PEPITEM, to boost bone growth and prevent bone loss. *Cell Rep Med*. 2024;5(5):101574. doi:10.1016/j.xcrm.2024.101574
234. Kim S-M, Sultana F, Korkmaz F, et al. Neuroendocrinology of bone. *Pituitary*. 2024;27(6):761–777. doi:10.1007/s11102-024-01437-5
235. Shu Y, Tan Z, Pan Z, et al. Inhibition of inflammatory osteoclasts accelerates callus remodeling in osteoporotic fractures by enhancing CGRP +TrkA+ signaling. *Cell Death Differ*. 2024;31(12):1695–1706. doi:10.1038/s41418-024-01368-5
236. Liu H, Zhang H, Wang S, et al. Bone-targeted bioengineered bacterial extracellular vesicles delivering siRNA to ameliorate osteoporosis. *Compos B Eng*. 2023;225:110610.