Journal of Tissue Engineering
Volume 15: 1–38

DOI: 10.1177/20417314241303818
journals.sagepub.com/home/tej
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Bioinspired synthetic peptide-based biomaterials regenerate bone through biomimicking of extracellular matrix

Sareh Azadi[1], Mohammad Ali Yazdanpanah[2], Ali Afshari[2], Niloofar Alahdad[2], Solmaz Chegeni[3], Abdolhamid Angaji[1], Seyed Mahdi Rezayat[4,5] and Shima Tavakol[3,6]

Abstract

There have been remarkable advancements in regenerative medicine for bone regeneration, tackling the worldwide health concern of tissue loss. Tissue engineering uses the body's natural capabilities and applies biomaterials and bioactive molecules to replace damaged or lost tissues and restore their functionality. While synthetic ceramics have overcome some challenges associated with allografts and xenografts, they still need essential growth factors and biomolecules. Combining ceramics and bioactive molecules, such as peptides derived from biological motifs of vital proteins, is the most effective approach to achieve optimal bone regeneration. These bioactive peptides induce various cellular processes and modify scaffold properties by mimicking the function of natural osteogenic, angiogenic and antibacterial biomolecules. The present review aims to consolidate the latest and most pertinent information on the advancements in bioactive peptides, including angiogenic, osteogenic, antimicrobial, and self-assembling peptide nanofibers for bone tissue regeneration, elucidating their biological effects and potential clinical implications.

Keywords

Bone regeneration, biological motifs, bioactive peptide, angiogenesis, antimicrobial peptides

Date received: 18 August 2024; accepted: 15 November 2024

Introduction

Bone defects resulting from trauma, infection, inflammation, tumors, or chronic diseases can have severe physical and psychological consequences for patients. Reconstructing and repairing critical bone defects poses significant challenges, mainly when they are located deep within the body and have irregular shapes.[1] Bone fractures can result in work absenteeism, decreased productivity, disability, impaired quality of life, health loss, and high healthcare costs. They also significantly impact individuals, families, societies, and healthcare systems. According to a meta-analysis of 113 studies, the average cost of hospital treatment for a hip fracture was estimated at US$10,075, and the total cost of health and social care for one hip fracture after 12 months was a global average of

[1]Department of Medical Biotechnology, Iran University of Medical Sciences, Tehran, Iran
[2]Department of Cell and Molecular Biology, Faculty of Biological Science, Kharazmi University, Tehran, Iran
[3]Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran
[4]Department of Medical Nanotechnology, Tehran University of Medical Sciences, Tehran, Iran
[5]Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[6]Department of Research and Development, Tavakol Biomimetic Technologies Company, Tehran, Iran

Corresponding author:
Shima Tavakol, Department of Medical Nanotechnology, Cellular and Molecular Research Center, Iran University of Medical Sciences, Next to Milad Tower, Tehran 1449614535, Iran.
Email: shima.tavakol@yahoo.com

$43,669.[2] Bone grafts and artificial materials (Alloplastic) are substitutes for reconstructing such fractures. While autografts are considered the most effective treatment, allografts, and xenografts are also employed for bone regeneration. However, these methods have certain limitations, including donor site morbidity, graft rejection, and insufficient bone formation, all of which are influenced by the patient's general health status.[3] Researchers in the medical field are currently emphasizing the development of treatment strategies to improve the process of bone healing. These approaches involve integrating specific molecules that enhance bones' responsiveness to healing, thereby addressing the limitations and concerns associated with other grafting materials.[4] In recent decades, there has been significant expansion in research related to bone tissue engineering and biomolecules such as peptides. *Bioactive peptides* are short sequences of amino acids commonly extracted from the active regions of proteins. The analogs of biologically active proteins can influence crucial biological and physiological processes, regulate cell activities, and modulate intercellular communication. These bioactive molecules are readily producible, processable, and modifiable and can be prepared under well-defined and controlled conditions. The primary origins of peptides comprise extracellular matrix proteins (e.g. collagen, fibronectin, bone sialoprotein, and laminin), soluble growth factors (GFs), and engineered and natural peptides.[5] Most peptides function similarly to their original proteins, triggering signaling pathways through direct engagement with cell receptors. Other peptides are used to modify biomaterials, enhancing their regenerative attributes.[6] It is worth mentioning that different bioactive peptides show considerable potential *for in vitro* and *in vivo* bone formation; some have even found clinical applications in tissue engineering.[7] Many peptides have been developed and investigated as potential candidates for the upregulation of bone healing response. Among all the peptides that have been extensively researched significant focus has been directed toward angiogenesis, osteogenesis, antimicrobial peptides (AMPs) and self-assembling peptide nanofibers. They are considered promising targets for bone regeneration due to their crucial role in cell proliferation, differentiation, extracellular matrix synthesis, biomineralization, and angiogenesis. Besides, these peptides have demonstrated their ability to support and stimulate bone healing, making them highly suitable for clinical use as therapeutic options.[8]

The focus of this analysis is to explore the recent advancements in the development of bioactive peptides for the purpose of enhancing bone regeneration. Additionally, this review aims to gather the most significant data on bioactive peptides concerning bone regeneration, angiogenesis, antimicrobial, and self-assembling peptide nanofiber properties, as well as their biological effects and clinical implications.

Cell signaling cascade in normal osteogenesis

The BMP/TGF-β signaling pathway

Peptides serve various functions in cell communication pathways and are vital for regulating cellular functions and promoting tissue regeneration. Consequently, having a deep understanding of the intricate molecular signaling pathways at play is imperative for the advancement of bone implants, alternatives, and scaffolds using cells for bone regeneration. Key molecular signaling pathways in bone regeneration include transforming growth factor β (TGF-β) and bone morphogenetic proteins (BMP) that are, both members of the multifunctional TGF-β superfamily. These proteins influence cell behavior by binding to their receptors in a particular manner. The TGF-β pathway begins with the release of ligands near the bone extracellular matrix (ECM). The binding of TGF-β to the tetrameric receptor, consisting of two subunits of TGF-β types I receptors (TβRI) and two subunits of TGF-β types II receptors (TβRII), or binding of BMP2 to type BMP receptors leads to transphosphorylation of type I receptor leading to transphosphorylation of the receptor components (Figure 1).[9]

This activation can occur through two pathways: Smad-dependent signaling and non-Smad-dependent signaling.[9–11] In the Smad-dependent pathway, regulatory Smads (R-Smad) such as Smad1/5/8 (BMP signaling pathway) or Smad 2/3 (TGF-β signaling pathway) are phosphorylated and combine with Smad4 to create a complex known as Co-Smad Then, the complex is transported to the nucleus, and regulates the process of transcribing target genes such as Runx2, Osx, and Dlx5 (Figure 2).[9] In this pathway, Smad 6 and Smad 7 act as inhibitory biomolecules by ubiquitinating R-Smads and inhibiting BMP/Smad and TGF-β signaling pathways, respectively.[12] However, histone deacetylases (HDACs) such as HDAC9, HDRP, HDAC10, HDAC11, HDAC7, and HDAC6 inhibit Runx2's activity.[13,14] In the non-Smad-dependent pathway, phosphorylated TGF-β activation kinase1 (TAK1), with the help of TAK1 binding protein 1 (TAB1), initiates MKK-P38. This pathway then activates either P38 or ERK1/2, which enhances the transcriptional ability of Runx2 through phosphorylation. The non-Smad-dependent pathway promotes the proliferation of mesenchymal stem cells and advances the early stages of differentiation into osteoblasts. Moreover, TAK1 influences the activation of Smad1/3/5, as well.[11]

Role of angiogenesis in bone regeneration

Bone is a highly vascularized connective tissue, and the vasculature within the skeletal system plays a crucial role in ossification, regeneration, and remodeling processes.[15] Approximately 10%–15% of the total cardiac output is

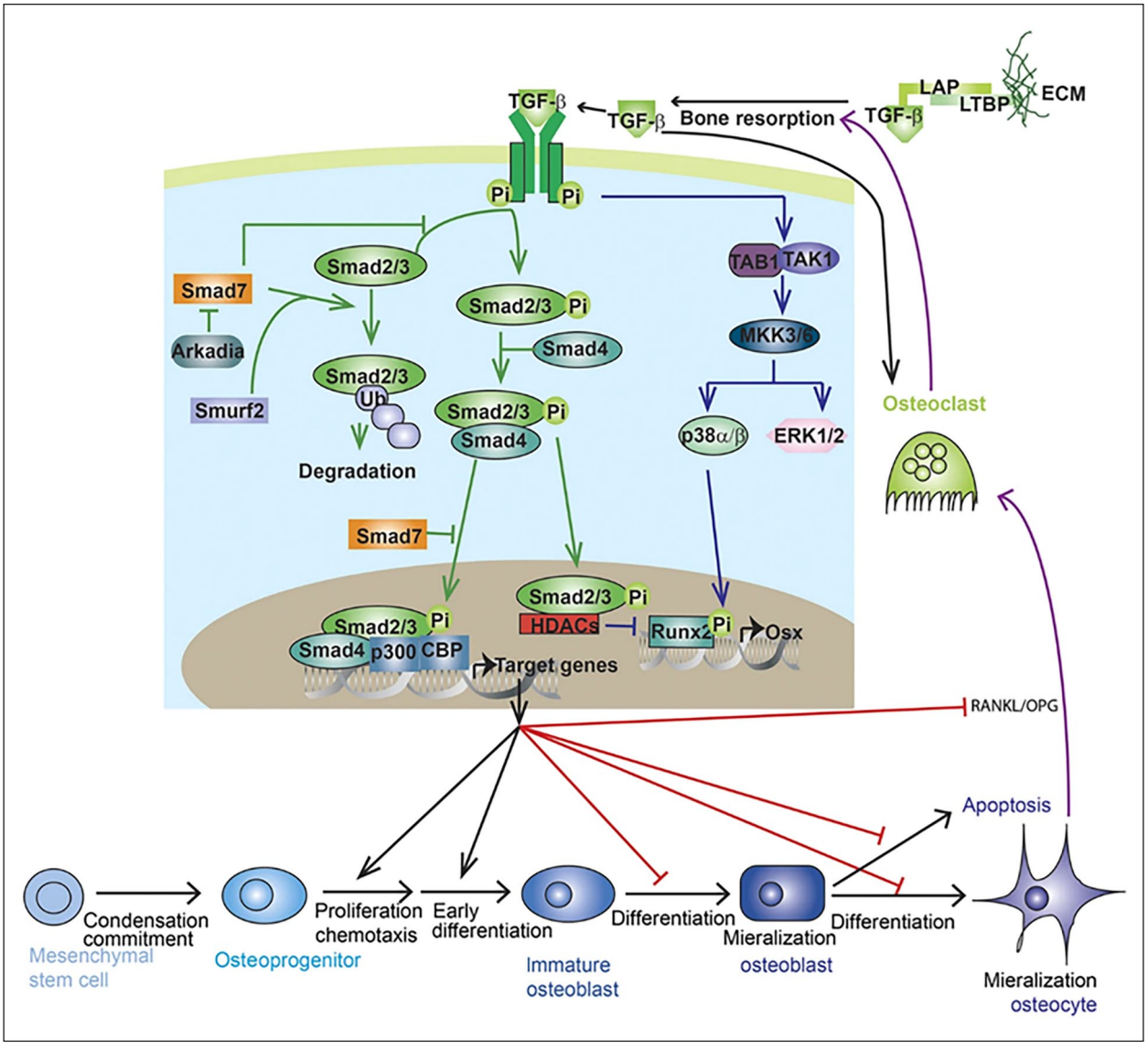

Figure 1. TGF-β signaling in bone. TGF-β binds to a tetrameric receptor complex consisting of two TGF-β type I receptors (TβRI) and two type II receptors (TβRII). The binding of TGF-β to TβRII leads to the transphosphorylation of TβRI, resulting in the activation of downstream signaling pathways. There are two main signaling pathways activated by TGF-β: the Smad-dependent pathway and the non-Smad-dependent pathway. In the Smad-dependent pathway, the phosphorylated R-Smad (Smad2 or Smad3) forms a complex with Smad4 and translocates into the nuclei. The non-Smad-dependent pathway involves the phosphorylation of TAK1, which then recruits TAB1. This initiates a signaling cascade involving the activation of MKK-p38 MAPK or MKK-ERK1/2, leading to the regulation of gene expression. Figure was reused from Wu et al.[9]

directed toward the human skeletal system.[16] In addition to supplying oxygen and nutrients and removing metabolites from bones, blood vessels also significantly function in delivering specific hormones, GFs, and neurotransmitters secreted by other tissues, such as brain-derived serotonin, to support the survival and activity of bone cells.[17] Osteoblasts, which differentiate into osteocytes, develop from precursor mesenchymal stem cells capable of osteogenesis through two distinct mechanisms: endochondral ossification or intramembranous ossification.[18] In intramembranous ossification, mesenchymal condensate cells directly differentiate into osteoblasts, forming flat bones like the skull and facial

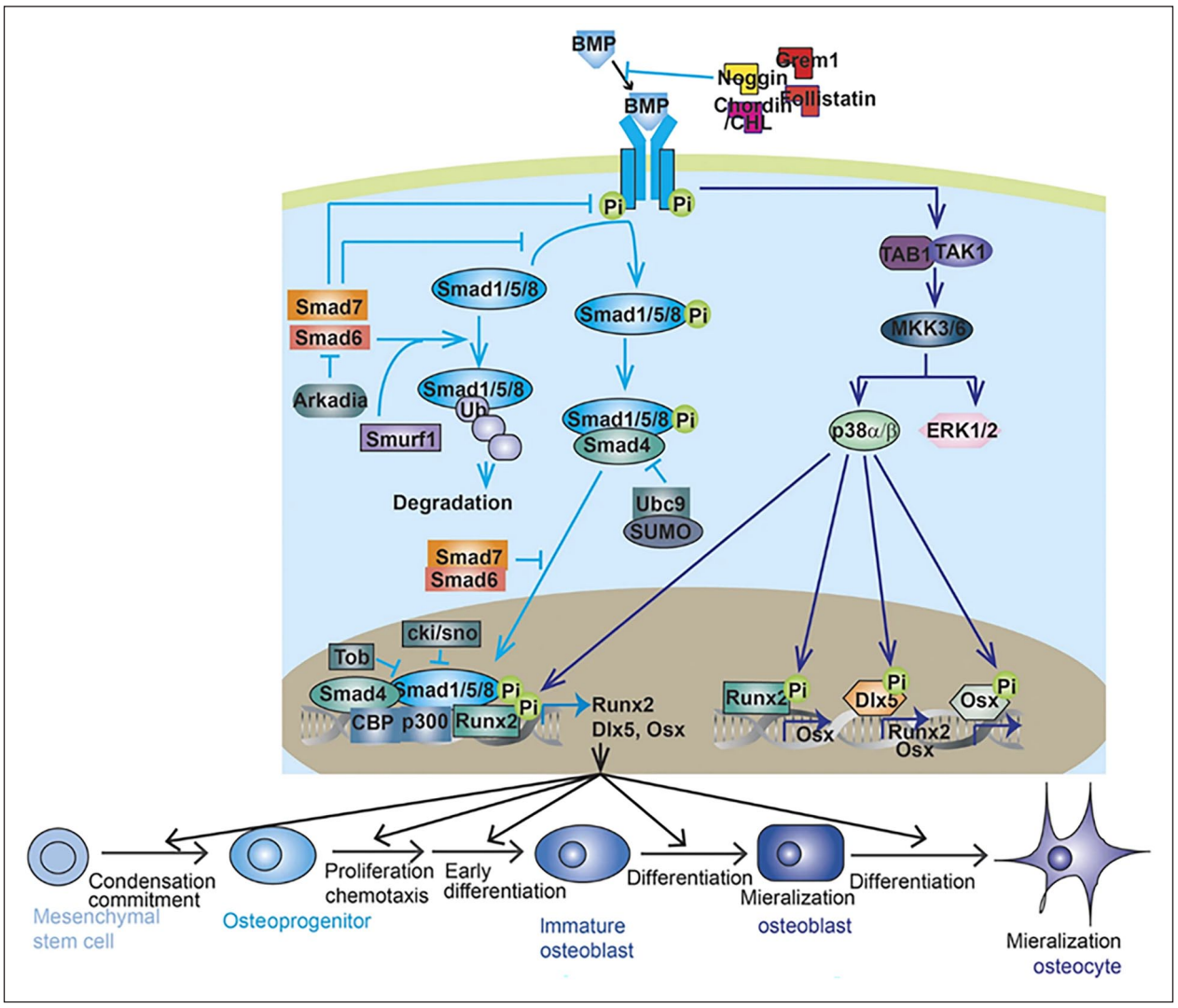

Figure 2. BMP signaling pathway in bone regeneration. BMP signaling is regulated by various binding proteins, such as Noggin, Grem1, Chordin, CHL, and Fellistatin, which act as antagonists to BMP activity. BMPs bind to type II receptors, leading to the transphosphorylation of type I receptors. This transphosphorylation event triggers both Smad-dependent and non-Smad-dependent signaling pathways. In the Smad-dependent pathway, phosphorylated R-Smad proteins (Smad1, 5, or 8) form complexes with Smad4 and translocate into the nuclei. Once in the nuclei, these complexes recruit co-factors and Runx2, a transcription factor that regulates osteogenic gene expression. Figure was reused from Wu et al.[9]

bones. These flat bones consist of compact bone layers with bone marrow in between. The differentiating mesenchymal cells release proangiogenic factors including Vascular Endothelial Growth Factor-A (VEGF-A) and osteogenic factors that support the growth of osteoprogenitors and osteoblasts, ultimately forming ossification centers. Also, the blood vessels are drawn to ossification centers, which in turn promotes the process of osteogenesis.[19] On the other hand, endochondral ossification is responsible for forming long bones through an intermediate stage of chondrocyte differentiation and avascular cartilage formation. This process is involved in the development of most bones in the body, including the femur and tibia long bones, as well as vertebral bodies. Mesenchymal condensates undergo differentiation into avascular cartilage, which is finally get replaced by bone tissue.[20]

Crosstalk between osteogenesis and vasculogenesis

The development of vascular networks in organs and tissues involves two main processes: vasculogenesis and angiogenesis. Vasculogenesis refers to the formation of

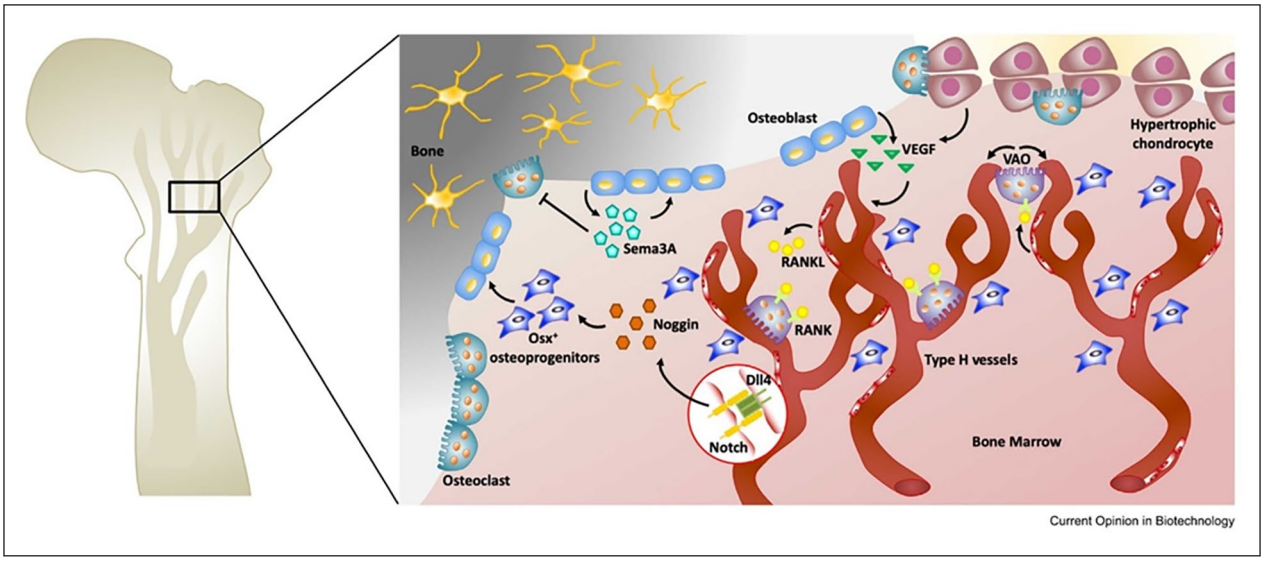

Figure 3. Cross-talk of osteogenesis and angiogenesis. During the process of osteogenesis, there is a significant cross-talk between vascular and bone cells. Type H vessels, which are specialized vascular structures, have a crucial function in the regulation of bone formation. Within the microenvironment that promotes bone formation, various cell types must synchronize their activities, and this coordination is achieved through a complex network of molecular signals. Several well-studied interactions are particularly noteworthy in this context, including VEGF, RANK/RANKL, Sema3A, Dll4/Notch, and BMP signaling (Noggin). Figure was reused from Di Maggio and Banfi.[25]

blood vessels from progenitor cells, while angiogenesis involves the creation of new blood vessels from existing ones through sprouting or splitting.[21] During embryonic development, this process can also happen in adults under certain conditions, such as wound healing, tissue regeneration, or tumor growth. However, mature endothelial cells (ECs) and smooth muscle cells (SMCs) remain inactive in adults unless these conditions arise.[22] Blood vessel formation during embryonic development depends on interactions between developing tissue and the vasculature. It starts with mesodermal stem cells differentiating into hemangioblasts, which then cluster to form blood islands. These blood islands give rise to hematopoietic stem cells and angioblasts, also known as endothelial progenitor cells (EPCs).[23] Angiogenesis is a complex process involving multiple steps heavily influenced by cell interactions and ECM. These interactions are mediated by the expression of VEGF and the activation of various signaling pathways. New blood vessels can form through the division of existing vessels (splitting angiogenesis or intussusception) or through the sprouting of ECs from existing vessels after the degradation of the basement membrane. These mechanisms are followed by the formation of lumens and the maturation of blood vessels.[24] In the initial phases of sprouting angiogenesis, blood plasma proteins and extracellular matrix (ECM) components like fibronectin, vitronectin, fibrinogen, and collagen form a provisional ECM. This environment encourages the growth and movement of endothelial cells (ECs), which help develop lumens and vascular tubes (Figure 3).[25] The final step is blood vessel stabilization, achieved by recruiting pericytes and other stromal cells and forming the basement membrane.[24,26] It is important to note that timely and abundant blood vessel growth (angiogenesis) is essential for healing common bone injuries and effectively using engineered tissues to repair bone defects. Two types of vascular ECs have been identified in developing bone structures, each with distinct physical, molecular, and functional characteristics. These types are known as type H vascular ECs, which have high levels of CD31 and EMCN (types of EC proteins) expression, and type L vascular ECs, which have lower levels of CD31 and EMCN.[27] Type H vessels tend to differentiate into arteries, unlike type L vessels. This suggests a strong connection between type H vessels and the formation of new vessels, particularly arteries. Located mainly in the metaphyseal region of bone, type H vessels are surrounded by osteoprogenitors that express Runx2+ and Osterix+. Additionally, factors like hypoxia-inducible factor-1α (HIF-1α), Notch, platelet-derived growth factor type BB (PDGF-BB), and slit guidance ligand 3 (SLIT3) all play essential roles in coordinating the link between type H vessel development and bone formation.[28]

Peptides

Peptides involved in angiogenesis

Efficient regulation of angiogenesis is crucial for successful bone repair and restoration of mature and functional bone. As a result, a viable strategy for bone regenerative medicine could be targeting the angiogenic regulation of lineage cells can that form bone tissues.[29] Incorporating pro-angiogenic molecules into biomaterials and scaffolds holds excellent promise in bone engineering. In addition to well-known angiogenic GFs like VEGF, fibroblast growth factor 2 (FGF-2), and PDGF, several peptides have been discovered in recent years that can induce endothelial cells to promote angiogenesis. These peptides can originate from GFs mentioned earlier, fragments of ECM proteins unveiled during the healing process, or innovative designs. It is important to note that angiogenic motifs, specific sequences found in various angiogenic factors, are critical in initiating specific events in the angiogenesis pathway. These motifs contribute to the process of angiogenesis by facilitating specific events that lead to the formation of new blood vessels[30] (Figure 4(b)).

Biological motifs in angiogenesis

RDD

AGGF1, a protein that stimulates the formation of new blood vessels, is a key player in various cellular processes. Its interaction with integrin $\alpha5\beta1$ through a specific region, the angiogenic domain, located between the 604th and 613th amino acids of AGGF1, with the sequence FQRDDAPAS, is a fascinating aspect. This region is not only crucial for endothelial cell attachment, movement, and the formation of tube-like structures lining blood vessels but also activates AKT, a protein involved in cell survival and growth, adding to the intricate web of cellular interactions. In an attempt to delve deeper into the significance of the angiogenic domain, a 15-amino acid peptide was engineered with a single amino acid mutation from -RDD- to -RGD- (a well-known integrin-binding motif). However, this mutated peptide proved to be ineffective in blocking the function of AGGF1, highlighting the intricate and complex nature of the RDD motif in the angiogenic domain and its crucial role in the activity of AGGF1. Furthermore, research using a mouse model for peripheral artery disease (PAD) called hindlimb ischemia C57BL/6N mice, a widely used model for studying PAD due to its similarity to human PAD in terms of disease progression and response to treatment, has demonstrated that the functional angiogenic domain of AGGF1 is essential for AGGF1-mediated therapeutic angiogenesis. This suggests that the angiogenic domain of AGGF1 is crucial for its potential use in promoting the growth of new blood vessels as a treatment for PAD.[31]

TRAP

Tyrosine-rich amelogenin peptide (TRAP) is a unique short fragment of 45 amino acids. It is derived from the low molecular weight Fraction C of enamel matrix derivative (EMD), a complex mixture of proteins and peptides involved in tooth development. TRAP, the primary component responsible for the vasculogenic activity of EMD, has been found to have distinct properties. In laboratory studies using human periodontal ligament cells (HPC) in vitro, TRAP has been found to stimulate the expression of endothelial markers and induce the differentiation of HPC cells into angiogenic cells. Additionally, synthetic TRAP peptide has demonstrated the unique ability to promote cell migration, tube formation, and blood vessel formation in both in vitro experiments using human umbilical vein endothelial cells (HUVEC) and ex vivo studies using a chick embryo chorioallantoic membrane (CAM) model. Nevertheless, it has been shown that TRAP enhances the expression of specific genes in HPC cells. These genes include VEGFR2, a tyrosine kinase receptor for VEGF ligand, and Tie-1 and Tie-2, which are tyrosine kinase receptors for angiopoietin and are exclusively expressed by endothelial cells. TRAP also increases VE-cadherin expression, an adhesion molecule specific to endothelial cells. However, the early endothelial gene VEGFR1 remains unchanged by the TRAP peptide. According to the findings presented, TRAP and its modified derivatives can stimulate the formation of new blood vessels and facilitate wound healing in vivo, suggesting their potential application as an angiogenic biomolecule. However, it is important to note that (potential risks or limitations associated with the use of TRAP), which should be taken into consideration when exploring its applications.[32]

C-C

C-C motif ligand 2 (CCL2) is a type of chemokine that participates in various biological processes, including inflammation and immune response. Research has demonstrated that CCL2 can stimulate angiogenesis *in* vitro. In the presence of CCL2, specific genes associated with angiogenesis, such as VEGF A, VEGFR-2, and matrix metalloproteinases 9 (MMP9), are overexpressed. These genes are crucial for neovascularization. CCL2 induces the formation of tube-like structures by HUVECs when cultured with ECM. The increased expression of VEGF, as triggered by CCL2, likely contributes to the enhanced formation of these tubes and the promotion of angiogenesis. The study suggests that CCL2 exhibits a more significant potential for angiogenesis at 50 ng/ml concentration in a co-culture system of human adipose-derived stem cells (hADSCs) and HUVECs. This finding implies that the paracrine regulation of hADSCs, which produce VEGF, may play a role in this process. These results could have

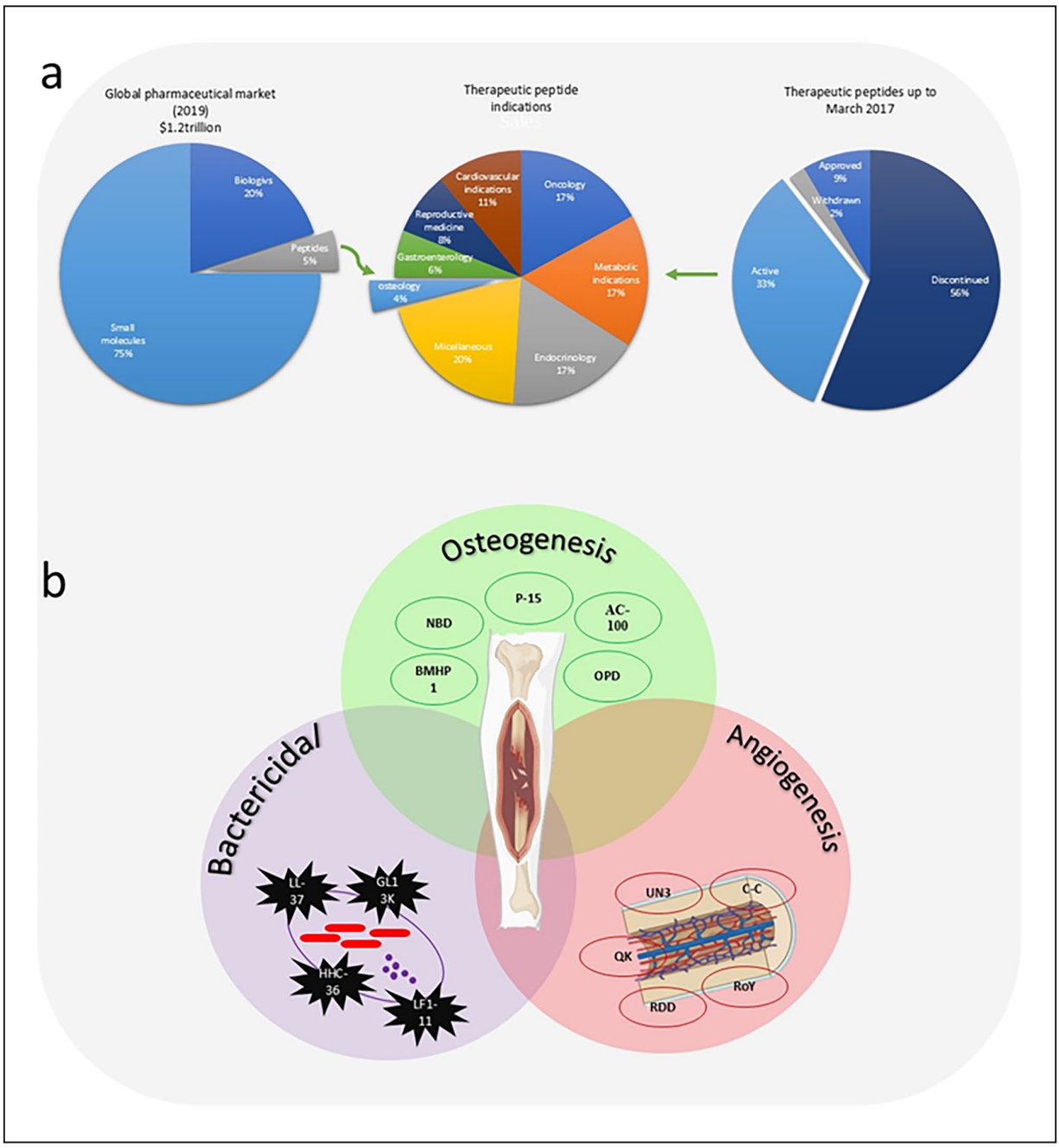

Figure 4. Market analysis of therapeutic peptides and their applications in bone regeneration (a) Market overview and therapeutic peptide indications. The overall figure highlights the market relevance of therapeutic peptides, and their diverse applications and status in market (withdraw, approved, discountined, and active). (b) Peptides for bone regeneration: This section depicts the application of peptides in bone regeneration, illustrating the overlap between osteogenesis, angiogenesis, and bactericidal activities. Specific peptides are listed within each category Osteogenesis such as NBD, BMHP-1, P-15, AC-100, and OPD. These peptides are associated with bone formation. Angiogenesis such as UN3, QK, RDD, C-C, and ROY. These peptides are associated with blood vessel formation, crucial for bone healing. Bactericidal/Antimicrobial such as LL-37, GL1-3k, HHC-36, and LF1-11. These peptides exhibit antimicrobial properties, important for preventing infection at the bone regeneration site. The figure shows the overlap between the three activities, indicating that some peptides may contribute to multiple aspects of bone regeneration.

significant implications for the development of therapeutic interventions targeting angiogenesis-related conditions.[33]

Qk

QK peptide, known to have similar properties as VEGF, promotes the growth of new blood vessels in vitro. It induces the formation and organization of capillaries. Notably, previous studies on rats have consistently shown that QK has similar effects on blood vessel dilation as VEGF. It also consistently promotes the growth of new blood vessels in different models of angiogenesis, including ischemic hindlimb, wound healing, and Matrigel plugs. In the ischemic hindlimb model, treatment enhances blood flow and increases capillary density to a greater extent than treatment with VEGF15, providing reassurance about its potential effectiveness. The practical implications of QK are evident in the wound healing model, where it accelerates the closure of full-thickness punch biopsy wounds, hinting at its potential in clinical wound healing applications. In the Matrigel plug model, QK treatment outperforms VEGF15, leading to a more significant infiltration of peripheral capillaries. These compelling findings underscore the proangiogenic properties of QK, comparable to the complete VEGF protein. Consequently, QK shows promise for clinical applications in conditions such as chronic ischemia.[34]

Comb1 and UN3

Comb1 and UN3 peptides have been demonstrated to be effective in improving wound healing in mice with impaired healing abilities and in a pre-clinical swine model. They enhance angiogenesis, granulation tissue formation, and re-epithelialization in these models. In diabetic porcine wound tissues, Comb1 and UN3 peptides stimulate the same processes after injury. They interact with proteins found in human keratinocytes, dermal microvascular endothelial cells, and fibroblasts. The specific binding patterns of these interactions depend on how the cells respond to injury. In diabetic pig tissues, these peptides significantly increase the production of mRNA for pro-angiogenic GFs and their receptors, as well as factors that attract immune and stem cells and re-epithelialization. Furthermore, Comb1 and UN3 enhance mRNA expression for GFs and cytokines involved in various healing processes, such as angiogenesis, mobilization of progenitor cells, epithelialization, activation of fibroblasts, and remodeling of ECM in diabetic porcine wounds. They also promote a balanced expression of MMPs and tissue inhibitors of metalloproteinases (TIMP), which play a crucial role in the turnover of ECM and wound resolution. Lastly, in granulation tissue, peptides promote mRNA expression of key angiogenic mediators, including VEGFA, FGF2, and VEGFR1/2. This suggests that Comb1 and UN3 have a therapeutic potential for improving wound healing, particularly in diabetic conditions.[35]

KRX-725

KRX-725, a short peptide of 9 amino acids, is derived from the second intracellular loop of S1P3 (sphingosine 1-phosphate). It activates a Gi-dependent MEK-ERK signaling pathway, triggering a pro-angiogenic response. The angiogenic effects of this peptide were examined using an ex vivo aortic ring model, where it stimulated the growth of sprouts with multiple layers and a lumen, mirroring the effects of the natural ligand S1P. KRX-725 promotes the formation of dense and extensive vascular sprouts, including the organization of endothelial and smooth muscle layers and the development of a lumen. It also has synergistic effects when combined with pro-angiogenic factors like VEGF, SCF (stem cell factor), and bFGF, highlighting its capacity to boost neovascularization. In vivo studies using a mouse corneal pocket assay revealed that KRX-725 initiates neovascularization and works synergistically with bFGF. Given its ability to stimulate extensive angiogenesis and synergize with pro-angiogenic factors, KRX-725, despite being a short peptide, holds the potential for treating pathological conditions like peripheral vascular disease, cardiac ischemia, or tissue grafts.[36]

PRG and KLT

PRG (PRGDSGYRGDS) and KLT (KLTWQELY QLKYKGI) peptides significantly improve cell adhesion and proliferation. PRG contains two RGD binding sequences, PRGDS and YRGDS, while KLT acts as an agonist for VEGF, activating the VEGF-dependent signaling pathway. Both PRG and KLT play essential roles in promoting the migration of endothelial cells, forming angiogenic sprouts, and developing capillary lumens. These peptides are incorporated into self-assembling peptide scaffolds called RAD/PRG and RAD/KLT, which act as functional motifs. These scaffolds have been shown to enhance endothelial cell migration, sprouting wildly, and the formation of capillary vessels both in vitro and in vivo. The functional motifs PRG and KLT augment the responsiveness of endothelial cells to VEGF, thereby leading to enhanced sprouting. Notably, the RAD/KLT scaffold exhibits superior angiogenic activity, promoting tissue invasion and the formation of new capillary vessels in the chorioallantoic membrane, even in the absence of VEGF. These findings suggest that RAD/PRG and RAD/KLT scaffolds could have broad potential applications in tissue regeneration, including bone regeneration, where angiogenesis is critical.[37]

SDKP

SDKP, a tetrapeptide, acts as a negative physiological regulator of hematopoiesis. It is found in plasma at nanomolar concentrations and widely distributed in tissues, with a higher level in hematopoietic organs like the thymus, spleen, and bone marrow. In vitro studies have

shown that SDKP stimulates the migration and differentiation of endothelial cells, forming capillary-like structures. This suggests its potential role in modulating endothelial cell function. It also enhances the secretion of MMP-1, an enzyme crucial for ECM remodeling and promoting angiogenesis. In vivo experiments have provided concrete evidence of SDKP's effects, showing that it promotes angiogenesis in various models. These include CAM, rat abdominal muscle, and subcutaneously implanted Matrigel plugs in rats. These findings indicate that SDKP directly affects endothelial cells, triggering an angiogenic response both in vitro and in vivo. The potential of SDKP role in regulating hematopoiesis and the engraftment of bone marrow, possibly through interactions with angiogenic and hematopoietic pathways, is also highlighted. The data strongly suggests that SDKP could be a potential therapeutic agent for diseases associated with impaired angiogenesis.[38]

YR or RoY

RoY, a synthetic peptide consisting of 12 amino acids (YPHIDSLGHWRR), binds specifically to the 78 kDa glucose-regulated protein (GRP78) receptor. This receptor is excessively expressed on the surface of vascular endothelial cells during hypoxic conditions. In vitro studies have shown that the RoY peptide boosts angiogenic activity by improving the proliferation, migration, and tube formation of vascular endothelial cells under hypoxia. Combined with chitosan chloride (CSCl) hydrogel, the RoY peptide further enhances angiogenesis under hypoxia following a myocardial infarction (MI). As compared whit the CSCl hydrogel alone, the CSCl-RoY hydrogel performs better in promoting the survival, proliferation, migration, and tube formation of HUVECs under hypoxic conditions. The CSCl-RoY hydrogel also influences the expression of the GRP78 receptor on the membrane surface of HUVECs under hypoxia. It activates the Akt and ERK1/2 signaling pathways involved in cell survival and proliferation, thereby enhancing the angiogenic activity of HUVECs under hypoxia. In vivo studies using a rat model of MI have demonstrated that the CSCl-RoY hydrogel improves angiogenesis in the MI region, reduces the size of the infarct, increases the thickness of the heart wall, and enhances cardiac function. These findings suggest that the CSCl-RoY hydrogel has potential applications in injectable cardiac tissue engineering.[39]

GHK

The GHK peptide, derived from collagen breakdown, plays a significant role in angiogenesis, forming new blood vessels. This peptide and its derivatives are used in biomaterials like cryogels to boost angiogenic properties and facilitate the transformation of endothelial cells into specialized phenotypes with altered morphology. When combined with the RGD peptide, the GHK peptide stimulates the production of proangiogenic factors, including FGF-2, VEGF, IL-6, IL-8, and MCP-1, in endothelial cells. This combination results in a synergistic effect. The GHK peptide and its copper complex, GHK-Cu, have the ability to promote cell adhesion, viability, and proliferation in human skin fibroblasts (HSF) and HUVEC. Furthermore, GHK-Cu has been shown to regulate the balance of redox reactions and increase the content of glutathione (GSH) in ECs, which may contribute to their regenerative effects. The angiogenic activity of the GHK peptide is enhanced when it is complexed with copper ions (GHK-Cu), promoting the formation of tubular structures by endothelial cells. The incorporation of GHK peptide and its derivatives into biomaterials has yielded promising results in promoting angiogenesis, indicating their potential for use in tissue regeneration and vascular tissue engineering.[40]

Exendin-4

Exendin-4 (Ex-4) is a GLP-1 receptor agonist with promise for type 2 diabetes mellitus treatment. Its extended half-life and unique anti-oxidative and anti-inflammatory properties make it a potential game-changer in wound healing. Beyond these benefits, Ex-4 has demonstrated cardioprotective effects in a murine MI model and angiogenic effects, as confirmed by both in vitro and in vivo studies. By enhancing the proliferation and differentiation of endothelial progenitor cells through increased VEGF generation, Ex-4 has shown potential in promoting angiogenesis. A study on hindlimb ischemic injury revealed the protective role of Ex-4 by stimulating angiogenesis. The topical application of Ex-4 or the local injection of ADSCs has been proven to be effective in treating skin wounds in diabetic mice.[41]

OPD or SV

The SV peptide has shown comparable angiogenic activity to VEGF, making it beneficial for promoting angiogenesis. It has been observed to stimulate the migration and motility of oral fibroblasts and keratinocytes, possibly by activating the TGF-b1 receptor. The SV peptide also enhances the proliferation of fibroblasts and myofibroblasts, contributing to the healing of oral wounds. It supports the production of ECM by fibroblasts, a crucial component for maintaining tissue integrity and organ structure during wound healing. By boosting angiogenesis, increasing the proliferation of fibroblasts and myofibroblasts, and activating the TGF-b1 receptor, the SV peptide may accelerate the healing of mucosal wounds. Thus, it holds the potential for treating persistent mucosal wounds caused by trauma or surgery, including oral cancer.[42] Table 1 represents summary of the most relevant bioactive peptides used in angiogenesis.

Table 1. The bioctive peptides involved in angiogenesis, major function, source, and their interactions.

Peptide name	Sequence	Major Function	Source	Interactions	Type Of Study	References
RDD	FQRDDAPASV	Necessary for EC adhesion and migration, capillary tube formation, and AKT activation	AGGF1	integrin $\alpha5\beta1$	HUVECs and MVECs in vitro, hindlimb ischemia model for PAD in C57BL/6N mice	Wang et al.[31]
TRAP	MPLPPHPGSPGYINLSYEVLPL LSLNLSYEVLTPLSLQGPTQ SPEDLHYPYSYGEP	Promotes cell migration, tubule formation, blood vessel formation, and wound healing	Fraction C of enamel matrix derivative (EMD)	Unknown	HUVECs and HPCs in vitro, The chick embryo chorioallantoic membrane (CAM) ex vivo	Amin et al.[32]
C-C (Part of CCL2)	CC	Increases the expression of angiogenesis-related genes like VEGF A, VEGFR, MMP9, Tubule formation	Cell types present in the co-culture system like hADSCs and HUVECs	C-C chemokine receptors (CCR)	hADSCs and HUVECs in vitro, Matrigel tube formation assay	Zhu et al.[33]
QK	KLTWQELYQLKYKGI	Vessel density promotion in ischemic hind limbs and increases the rate of cutaneous wound healing	VEGF	Flt-1 and Kdr receptors	Matrigel *in vitro*, hindlimb ischemia rat model *in vivo*	Santulli et al.[34]
Comb1	DINECEIGAPAGEETEVTV EGLEPG	Improves cell migration, proliferation, and tube creation *in vitro*, Increases vessel density in damaged cutaneous wounds, promotes the mRNA expression of key angiogenic mediators, including VEGFA, FGF2, and VEGFR1/2	Extracellular matrix	Dermal and epidermal cell populations, keratinocytes, fibroblasts, and vascular endothelial cells	Diabetic Yorkshire swine model *in vivo*, HMVECs *in vitro*, RT-qPCR	Sheets et al.[35]
UN3	NH_2-ELLESYIDGRPTATSEY QTFFNPR-Amide		Platelet-Rich Plasma			
KRX-725	MRPYDANKR	Increases aortic ring development, and promotes vascularization by co-treatment with KRX-725 and VEGF or bFGF	C-terminal end of the i2 loop of $S1P_3$ (sphingosine 1-phosphate)	ERK (extracellular signal-regulated kinase)	HUVECs and HA-VSMCs *in vitro*, Aortic ring assay used C57BL/6 and BALB/c mice, or Sprague-Dawley rats *in vivo*	Licht et al.[36]
PRG	PRGDSGYRGDS	Enhances endothelial cell migration, sprouting, and capillary vessel formation.	Custom-synthesized through solid phase, 2-unit RGD binding peptide	Integrin avb3	*In vivo* chick embryo CAM assay, 3D migration/sprouting bead assay using HUVECs *in vitro*	Liu et al.[37]
KLT	KLTWQELYQLKYKGI		Custom-synthesized VEGF-mimicking peptide	The VEGF-dependent signaling pathway as a VEGF agonist		
SDKP	SDKP	Stimulates endothelial cell migration and differentiation into capillary-like structures, enhances the secretion of MMP-1 (crucial for angiogenesis)	Bone marrow	Endothelial cells	Cell culture using EA.hy926 endothelial and A549 lung carcinoma cells, *In vivo* chick embryo CAM assay	Liu et al.[38]
YR or RoY	YPHIDSLGHWRR	Increased cell proliferation and migration *in vitro*, improves angiogenesis in the MI region, reduces infarct size, increases wall thickness, and enhances cardiac function	Synthetic	Glucose-regulated protein (GRP78) receptor	Cell culture using HUVECs *in vitro*, myocardial infarction SD rat model *in vivo*	Shu et al.[39]
GHK	GHK	Promotes cell adhesion, viability, and proliferation in both human skin fibroblasts (HSF) and endothelial cells (HUVEC)	ECM-derived	Integrin $\beta1$	HSF and HUVECs *in vitro*	Zoughaib et al.[40]
Exendin 4	HGEGTFTSDLSKQMEEEA VRLFIEWLKNGGPSSGAPPPS	Increases the migration, sprouting, and tube formation *in vitro*. induces wound healing in diabetic model and protection against an ischemic injury *in vivo*	Glucagon-like peptide 1 (GLP-1)	PKA- and PI3K/Akt-dependent pathways	HUVECs *in vitro*, Diabetic db/db mice *in vivo*, mice hindlimb ischemia model *in vivo*	Seo et al.[41]
OPD or SV (Osteopontin-derived peptide)	SVVGLR	Stimulates the migration and motility of oral fibroblasts and keratinocytes, induces wound healing	Osteopontin	Integrin signaling	Normal human gingival fibroblasts (NHGF) *in vitro*, wound healing assay *in vitro*	Tanaka et al.[42]

In summary, the pursuit of effective therapies for tissue regeneration and the treatment of diseases caused by insufficient blood vessel formation motivates research into angiogenic peptides. These peptides, derived from various sources, have significant potential in regenerative medicine due to their ease of development, cost-effective synthesis, and favorable safety profiles. Their role in promoting angiogenesis is crucial, as inadequate blood supply can hinder tissue regeneration, particularly in bone where a lack of vascularization can lead to cell deficiency and insufficient GFs. Angiogenic peptides, such as RDD, TRAP, QK, Comb1, and UN3, act by binding to endothelial cell receptors, triggering processes that lead to the formation of new blood vessels. While they offer potential benefits like improved wound healing and bone regeneration, challenges remain in addressing potential off-target effects, immunogenicity, and limited stability. Current trends in angiogenic peptide research involve developing more stable and targeted peptides, exploring their synergistic effects with other GFs, and incorporating them into biomaterials for localized delivery.

Biological motifs in osteogenesis

While GFs, serving as molecular signals, play a vital role in regulating cell behavior, their use in tissue engineering comes with certain limitations, including high production expenses, high required dosage, intricate manufacturing processes, immunogenicity, as well as a short half-life. In addition, many of molecular signals are pleiotropic, and a single GF may have various active domains, making their effects challenging to control.[5] An exciting alternative recently emerged is using biomimetic peptides in soluble form or as self-assembling peptide nanofibers.[43–45] These new osteogenic peptides allow for a higher density of active sites to be exposed to target cells, as more molecules can be grafted per unit area on a biomaterial. Furthermore, peptides have the practical advantage of enhancing cellular processes such as adhesion, proliferation, differentiation, and angiogenesis.[46] To date, several peptides have been developed to regulate the osteogenic response and bone healing (Figure 2).

Various osteogenic ECM-derived peptides are used for bone tissue repair such as Arg-Gly-Asp (RGD) and Lys-Arg-Ser-Arg (KSRS) from fibronectin, vitronectin, bone sialoprotein (BSP), Asp-Gly-Glu-Ala (DGEA) from collagen, and Tyr-Ile -Gly -Ser -Arg (YIGSR) and Ile-Lys-Val-Ala-Val (IKVAV) from laminin and also, osteogenic GFs, such as BMPs, FGF, PDGF, TGF-β, VEGF, and insulin-like growth factors (IGFs). These factors are applied to scaffolding materials to enhance their ability to regenerate bone.[47,48] The ECM-derived peptides, GFs-derived peptides have been discussed, as follow.

ECM-derived peptides

ECM is a collection of molecules that provide biochemical and structural support for various cells. Within ECM protein chains, amino acid sequences interact with cell membrane receptors. ECM-derived peptides have been used in many studies for surface modification.[49] Most peptides incorporated into the scaffolds contain integrin-binding RGD sequences that bind to integrins and improve cell attachment to scaffolds. Additionally, incorporating other ECM proteins like bone sialoprotein (BSP), osteopontin (OPN), or osteocalcin (OCN) has been found to enhance mineralization, accelerate bone healing, and induce angiogenesis.[46]

Collagen derived peptides

The primary mechanism for cell adhesion and subsequent cellular control is integrin-ECM protein interactions. Human osteoblasts express a variety of integrin subunits, including α1, 2, 3, 5, and β1. The α1β1- and α2β1-integrins have been identified as the primary adhesion receptors for collagen and are likely responsible for the regulatory activity of collagen.[50] One or both of these receptors, DGEA, mediate the recognition motif for α2β1 is agen on osteoblasts; this motif is unique to fibrillar collagens.[51] *In vitro*, DGEA-coated HA disks enhanced osteoblast marker expression in MSCs and encouraged cell adhesion.[52] Culpepper et al.[53] demonstrated that the E7 domain facilitates the retention of DGEA peptides for at least two months *in vivo* on dense HA and nano-HA-containing degradable matrices. Furthermore, enhanced loading and retention of E7DGEA resulted in increased cell adhesion and osteoblastic differentiation *in vitro*, as well as increased bone formation and bone-implant contact *in vivo.* The α2β1 integrin recognizes the Gly-Phe-hydroxyPro-Gly-Glu-Arg (GFOGER) motif in residues 502–507 of the α1 (I) chain of type I collagen.[51] In light of this, Reyes and Garcia developed a synthetic triple helical peptide containing the GFOGER sequence that selectively binds to α2β1-integrins. While their initial research only revealed that this peptide could support cell density-dependent adhesion and differentiation of MC3T3-E1 (mouse calvaria-derived pre-osteoblast) cells, they subsequently showed that applying GFOGER to titanium surfaces increased the expression of ALP and ECM mineralization, as well as the transcript levels of Runx2, OCN, and BSP in bone marrow stromal cells.[54] SVVYGLR peptide-containing collagen sponge or PBS (control) was used to fill standardized bone defects in rat calvaria. After 3 weeks, the peptide group had fewer TRAP-positive osteoclasts at the grafted sites. The synthetic SVVYGLR peptide suppresses osteoclast formation and could contribute to effective bone regeneration at an early stage.[55] In the following, BCSP-1™, RGD, P-15 and CTC have been discussed.

RGD

Various peptides have been isolated from type I collagen, which makes up about 90% of the organic material in bone. These peptides, including RGD, GFOGER, DGEA, BCSPTM-1, and P15, have been studied for their specific bioactivity. RGD, in particular, is a cell-binding domain commonly used to improve cell adhesion to biomaterial surfaces.[7] RGD sequences are found in several molecules and contribute to cell surface signaling. In the process of signaling at the cell surface, RGD peptides play a crucial role in enhancing the expression of ALP, Runx2, OCN, OPN, and BSP. This helps in promoting osteoblasts proliferation, differentiation, and mineralization. The RGD peptide sequence is part of the cardinal integrin-binding domain in many ECM proteins like fibronectin (FN), vitronectin (VN), and collagen. Several studies have suggested that RGD peptides enhance cell attachment and may increase angiogenesis and other fundamental cellular functions.[49] It can be categorized as linear or cyclic, with cyclic RGD (cRGD) peptides showing more significant activity. The 3D structure of cRGD peptides is thought to increase their affinity for integrin receptors and make them more resistant to proteolysis.[56] Likewise, *in vivo* studies have shown that cRGD is more effective than linear RGD for bone repair.[57] According to a model involving spinal fusion in sheep, a mineralized collagen matrix coated with cRGD was found to be as effective as rhBMP-2 in promoting osteogenesis.[58] A recent study conducted by Kim et al.[59] revealed that the use of an injectable hydrogel composed of MPEG (methoxy polyethylene glycol)—PCL-RGD can significantly enhance the osteogenic differentiation of stem cells. The researchers suggested that key proteins such as focal adhesion kinase (FAK), protein kinase B (AKT), and FAK extracellular signal-regulated kinase (ERK) are involved in the osteogenic differentiation process through the RGD-integrin-mediated pathway. Likewise, the incorporation of RGD-functionalized alginate-PEG hydrogels has been shown to lead to faster relaxation, ultimately improving the osteogenic differentiation of mesenchymal stromal cells (MSCs).[60]

BCSP-1tm

BCSP-1TM is a peptide composed of nine amino acids derived from human type I collagen, originating from the prodomain of BMP-7, a potent inducer of bone formation. Upon binding to their receptors, BMPs activate the Smad signaling pathway, specifically Smad1/5, which translocate to the nucleus to initiate the expression of essential osteogenic transcription factors such as Runx2 and Osterix. When this peptide is chemically bound to a commercial ceramic (Skelite^{TM}) made of HA and silicon-stabilized tricalcium phosphate, rat calvarial osteoprogenitor cells express specific ALP much more frequently. The researchers did not evaluate the initial attachment of the cells, although they did observe a reduction in cell count after 6 and 10 days on the modified surfaces in comparison with the standard ceramic. This could be the result of cell differentiation that lowers the proliferation rate. However, the initial attachment of the cells was not evaluated.[61]

P-15

A biomimetic bone graft PepGen P-15 (mineral-P-15 peptide) has been developed in which P15 stimulates bone regeneration. P-15 is a synthetic peptide consisting of 15 amino acids, identical to the sequence (766 GTPGPQGIAGQRGVV-780) found in the a1 (I) chain of type I collagen. Numerous studies have demonstrated its ability to promote bone regeneration by stimulating the proliferation and differentiation of osteoblasts, enhancing their attachment to bone repair materials, and increasing the production of ECM.[62] The effect of P-15 on the osteogenic potential has been effectively examined in both *in vitro* and *in vivo* investigations. ABM-P-15 scaffolds demonstrated increased adhesion, proliferation, differentiation into bone-forming cells, and production of bone tissue when compared with ABM alone. Following 8 weeks of *in vivo* implantation using a diffusion chamber model, HDPSCs (human dental pulp stem cells) cultured on ABM-P-15 scaffolds exhibited a substantial and well-organized collagen matrix deposition that positively stained for collagen I (COL-I) and osteocalcin (OCN) in contrast to ABM alone. ABM-P-15 use resulted in enhanced effectiveness in bone regeneration, suggesting the promise of using a combination of HDPSCs and ABM-P15 to improve the efficacy of bone tissue engineering for addressing challenges such as non-union fractures, critical bone defects, and implant failures in the fields of orthopedics and dentistry.[63] Moreover, the P15 peptide was covalently linked to surfaces of titanium alloy (Ti-P15 surface) and incubated with osteoblast-like cells (pre-osteocyte MLO-A5 cells (Murine Long bone Osteocyte-A5) and C3H10T1/2 (clonal mouse embryo cell line)), resulting in a notable increase in cell adhesion, spreading, expression of osteogenic genes, and differentiation. The Ti-P15 surface promotes the attachment of precursor cells involved in bone formation and facilitates their transformation.[64] Despite this, in a rabbit model of bone defect utilizing polyetheretherketone (PEEK) scaffolds, P-15 peptide administration resulted in a demonstrable reduction in the inflammatory cytokines IL-1β, IL-4, and IL-6, suggesting a potential acceleration of the transition from the inflammatory phase to the osteogenic phase of bone healing.[65]

CTC peptide

The CTC peptide derived from the α-subunit of collagen III C-terminally is known for its cryptic 12-mer structure. This peptide has been found to have a chemotactic effect

on perivascular stem cells (PSCs) and other cells. It also increases the transient expression of osteogenic markers, such as OPN or type I collagen. Interestingly, CTC peptide has also been shown to stimulate ALP activity and accelerate matrix mineralization without affecting cell proliferation rate.[66] *In vitro*, studies have demonstrated that when combined with an osteogenic medium (containing β-glycerophosphate and ascorbic acid but not dexamethasone), the CTC peptide enhances the short-term expression of osteogenic markers like type I collagen or osteopontin by PSCs. It also increases ALP activity in the cell cultures and accelerates ECM mineralization without positively affecting proliferation. Furthermore, in an adult mouse model of middle-second phalanx amputation, the CTC peptide was found to promote the formation of a bonelike nodule at the amputation site within 14 days.[67] Table 2 provides an overview of the most relevant biomimetic peptides used in bone regeneration applications.

In summery collagen derived peptides promote osteogenesis through several cascade including MAPK/ERK pathway: This pathway is crucial for cell proliferation, differentiation, and survival. Activation of ERK promotes osteoblast differentiation and bone formation. PI3K/Akt pathway: This pathway regulates cell survival, growth, and metabolism. Akt activation promotes osteoblast survival and enhances bone formation. Wnt/β-catenin pathway: This pathway plays a crucial role in osteoblast differentiation and bone formation. Some CDPs may modulate Wnt signaling, either directly or indirectly, by influencing the expression or activity of Wnt ligands or their receptors. TGF-β/SMAD pathway: Transforming growth factor-beta (TGF-β) signaling is involved in bone formation and remodeling. CDPs might modulate TGF-β signaling, influencing osteoblast differentiation and matrix production. Other pathways like the NF-κB pathway (involved in inflammation and bone remodeling) may also be influenced by CDPs, depending on the specific peptide and cellular context. These pathways lead to increased proliferation, enhanced differentiation, stimulated matrix production and improved mineralization. The specific biological effects of CDPs are highly dependent on their amino acid sequence. Slight variations in sequence can significantly alter their activity and the pathways they engage. This highlights the importance of studying individual collagen derived peptides and their specific mechanisms of action.

Laminin-derived peptides

Laminin, a major glycoprotein in basement membranes, plays a crucial role in cell adhesion and migration. It interacts with cells through at least four types of integrins (α1β1, α2β1, α3β1, and α6β1), which are transmembrane receptors that mediate cell-extracellular matrix interactions. Additionally, a 32/67 kDa laminin-binding protein acts as a receptor, further enhancing the specificity and strength of the cell-laminin interaction.[101] In a groundbreaking study by Frith et al., the impact of a non-fouling polystyrene-block-poly (ethylene oxide)-copolymer (PS-PEO) surface on the differentiation of hMSCs using cell-binding motifs (RGD, IKVAV, and RETTAWA) was explored. The hMSCs cultured on these peptides exhibited distinct morphologies and demonstrated varying capacities for differentiation into osteogenic and adipogenic lineages. Notably, IKVAV emerged as the most influential motif, significantly affecting the osteogenic and adipogenic differentiation of hMSCs compared with YIGSR and the previously mentioned α5β1 integrin-directed RRETAWA sequence.[102] Research indicates that IKVAV has the capacity to inhibit the pro-inflammatory M1 phenotype while promoting the anti-inflammatory M2 phenotype in macrophages. This shift is facilitated by its interaction with integrin α2β1; blocking this receptor reduces M1 activation and enhances the expression of M2 markers.[103] Furthermore, the potential of another laminin-derived peptide, Ln2-p3, to boost the expression of several osteogenic markers and increase the ALP activity of cells when applied to titanium surfaces has been demonstrated, opening up promising avenues for future research and applications.[104] Diverse laminin peptides and fragments potently modulate osteogenesis through integrin-mediated signaling. Specifically, LN-111, LN-211, and LN-332 stimulate osteogenic differentiation in various cell types (mesenchymal stem cells, bone marrow progenitor cells, bone-marrow-derived MSCs, and dental follicle cells) primarily via integrin-α1, αV, α6, β1, α3β1/α3β3, and α2/β1, leading to FAK/ERK pathway activation. Furthermore, LN-332 and other laminin fragments (LN-111, LN-211, iMatrix-411, iMatrix-511, LN-α2-LG1, LN-α2-LG3, and LN-α3 LG3) activate Wnt5a and BMP signaling pathways, while LN-511 and LN-521 uniquely maintain pluripotency in stem cells via integrin-α6β1/αVβ1 and PI3k/Akt pathways. These findings collectively demonstrate the significant role of laminin-derived molecules in regulating osteogenic differentiation through a complex interplay of integrin-mediated signaling and downstream pathways.[104]

Fibronectin derived peptide: PHSRN. PHSRN (Pro-His-Ser-Arg-Asn), a peptide derived from fibronectin, while ineffective on its own, has been demonstrated to work in concert with RGD to boost cellular activity through facilitating the binding of α5β1-integrin.[105] Cell attachment to FN is mediated by the α5β1-integrin, which is expressed by osteoblasts at different stages of the osteogenic process.[106] Bone marrow stromal cells are crucial for the osteogenic differentiation of these cells.[107] While the RGD sequence alone can bind α5β1, stable binding necessitates the PHSRN sequence in the 9th III repeat of fibronectin in addition to the RGD found in the 10th III type repeat.[108] The combination of

Table 2. The peptide involved in bone regeneration, sequence, and major function.

Type of peptide	Peptide group	Name and sequence	Major function	Biomaterial	Peptide amount	Type of study	References
ECM derived peptides	Collagen derived peptides	RGD	Upregulating the expression of ALP, RUNX2, osteocalcin, osteopontin and BSP; Sox9, Aggrecan, fibronectin, and collagen II	Gold-coated titanium surfaces	2 mM in deionized water	Primary rat calvarial osteoblasts cells	Huang et al.[68]
				Titanium implants	10 μmol/l of RGD	Rat model	Rammelt et al.[69]
				RGD-coupled alginate-PEG hydrogels	1500 μM of Alginate-RGD	Mesenchymal stem cells (MSCs) 3T3 fibroblasts cells	Nam et al.[60]
				MPEG (methoxy polyethylene glycol)—PCL-RGD hydrogels	A series of hydrogel formulations with 0, 0.8, 1.6, and 2.4 mM of RGD to total content of hydrogel	Bone marrow-derived mesenchymal stem cells (BMSCs) and rabbit model	Kim et al.[59]
		BCSP-1™ (NGLPGPIGP)	Increase an osteoblast differentiation marker and bone healing	BCSP™-1 covalently immobilized to the surface of Skelite™ ceramic	66 nmol/ml BCSP™-1 was dissolved in de-ionized water	Rat calvaria cells	Wang et al.[61]
		PepGen P-15 GTPGPQGIAGQRGVV	Promotes osteoblastic activity in vitro and bone regeneration in vivo	ABM-P-15	200 mg of ABM-P-15	Human periodontal ligament fibroblasts (PDLF)	Bhatnagar et al.[70]
				Hydroxyapatite (HA)–P-15 complex	200 mg of HA–P-15	Human bone marrow stromal cells and Primary human bone marrow cells	Yang et al.[62]
				HA/P-15 hydrogel	P-15 was absorbed on to algae-derived hydroxyapatite (adHA)	Pig model	Thorwarth et al.[50]
		CTC peptide IAGVGGEKSGGF	Promote migration of multiple cell types *in vitro* and formation of a bone-like nodule at the site of amputation *in vivo*	Biologic scaffolds derived from urinary bladder ECM	Concentration of 0.10^{-4} to 10^{-9} M peptide in DMEM/15 μL of 10 mM peptide inject subcutaneously *in vivo*	Perivascular stem cells, CTX cells, human adipose stem cells, C2C12 muscle myoblast cells, IEC-6 intestinal epithelial cells, RT4-D6P2T rat Schwann cells, and HMEC human microvascular endothelial cells and mice model	Agrawal et al.[66]
		GFOGER	Enhances peri-implant bone formation and mechanical osseointegration	Titanium implants	20 mg/ml in PBS	Primary bone marrow stromal cells	Reyes et al.[71]
		DGEA	Increased osteoblastic differentiation *in vitro*, and bone formation *in vivo*	Hydroxyapatite (HA) disks	1 mg/ml in ddH2O, at concentrations of 50, 100, or 200 μM.	Human bone marrow cells (MSCs) and Rat model	Culpepper et al.[53]
		SVVYGLR	Upregulation of osteogenesis and angiogenesis	Synthetic SVVYGLR peptide and collagen sponge	0.01–100 μg/ml of peptides in vitro and 10 μg SVVYGLR peptide in vivo (collagen sponge)	hMSC, hPLF, hGF, HUVEC, αvβ3-CHO, and mock-CHO cells and rat model	Egusa et al.[55]
		GFOGER	Enhances peri-implant bone formation and mechanical osseointegration	Titanium implants	20 mg/ml in PBS	Primary bone marrow stromal cells	Reyes et al.[71]
	Laminin-Derived Peptides	IKVAV and YIGSR	Increase osteoblasts differentiation and alkaline phosphatase activity	Laminin peptide (IKVAV and YIGSR)	100 μg/well laminin peptide	MC3T3-E1 cells	Vukicevic et al.[72]
		Ln2-p3	Enhance the expression of osteogenic markers and alkaline phosphatase (ALP) activity	Titanium scaffolds	23 $\mu g/cm^2$	Human osteosarcoma (HOS) cells	Min et al.[73]
	Fibronectin derived peptide	PHSRN	Increase the osteogenic differentiation and bone mineralization	RGD- and PHSRN-alginates hydrogels	peptide-modified alginates dissolved in ddH2O containing 0.2 % $(NaPO_3)_6$ to obtain a 2 % solution, Hydrogel discs (diameter 13 mm)	Normal human osteoblasts (NHOsts)	Nakaoka et al.[74]
	Other ECM derived peptide	KRSR	Promote osteoblasts adhesion and bone growth	Immobilization of KRSR onto borosilicate glass	0.01, 0.1, 1, and 2 mM modified KRSR in DMEM	Neonatal rat calvarial osteoblasts, endothelial cells (cell line CCL-209) and fibroblasts (cell line CRL-1213)	Dee et al.[75]
				Immobilization of KRSR onto nano-crystalline HA and nano amorphous calcium phosphate	KRSR, silanization	Human osteoblasts (CRL-11372)	Nelson et al.[76]
		FHRRIKA	Enhance the ability of osteoblast adhesion, spreading, and mineralization	-FHRRIKA- and -RGD- grafted surfaces	mixed FHRRIKA-RGD surfaces in ratios of 75:25 (MPS I), 25:75 (MPS II), and 50:50 (MPS III)	Primary rat calvaria osteoblast-like (RCO) cells	Rezania and Healy[77]
		MEPE peptide (AC-100) ERGDNDISPFSGDGQ	Increase number of osteoblasts and osteogenic activity	Peptide fragment of MEPE containing the RGD and SGDG motif	40 μM AC-100 in medium culture in vitro and 2, 20, or 200 μg/kg/day AC-100 in 10 μl PBS twice a day in vivo	Human primary osteoblasts (HPO cells) and mice model	Hayashibara et al.[78]

(Continued)

Table 2. (Continued)

Type of peptide	Peptide group	Name and sequence	Major function	Biomaterial	Peptide amount	Type of study	References
Peptide derived growth factors	BMP-2	KIPKASSVPTELSAISTLYL	Promote osteogenesis	BMP2/OPD + RGD-conjugated inert hydrogel	10 mM in the grafting solution	Bone marrow stromal (BMS) cells	He et al.[79]
		OPD (DWIVA)		Titanium implants	2 mg synthetic peptide in 0.5 mL PBS	Osteoblast-like cell line (MC3T3-E1) and dog model	Seol et al.[80]
	pBMP-9	RKVGKASSVPTKLSPISILYK		lipid-nucleic acid nanoparticles (LNP)	(500 ng pDNA per well) in DMEM and 30 μg pDNA/rat	C2C12 cells and Ovariectomized (OVX) rat model	Vhora et al.[81]
	BMP-7	RTVPKPSSAPTQLNAISTLYF		Polyethylene terephthatalate (PET) grafted with RGD and/or BMPs mimetic peptides	10^{-3} M BMPs/PBS	MC3T3-E1 (pre-osteoblast like) cells	Zouani et al.[82]
	Osteopointin-derived peptide	CBM (GLRSKSKKFRRPDIQYP DATDEDITSHM)	Promote osteogenic differentiation and bone mineralization	CBM	100 μl of suspension in DMEM	Human mesenchymal stem cell (hMSC)	Shin et al.[83]
		ODP (DVDVPDGRGDSLAYG)	Increase migration and cell proliferation	OPF (oligo (poly (ethylene glycol) fumarate)) hydrogels modified with an OPN-derived peptide (ODP)	0.1, 0.5, or 1.0 μM of acrylated peptide in swollen hydrogel (g)	Rat fibroblast 3T3 like cell line (CRL-1764)	Shin et al.[84]
	Bone sialoprotein derived peptide	CB (NGVFKYRPRYYLYKHA YFYPHLKRFPVQ)	Promote the osteogenic differentiation and ALP activity *in vitro* and bone formation *in vivo*	HA scaffold	6 mg/100IL PBS of peptide was applied to the HA scaffold	Human osteoblastic (HOS) cells and rabbit model	Choi et al.[85]
	FGF-2-derived peptides	F36; PDGRVD F77; KEDGRLL	Increase cell attachment and osteoblastic differentiation	Chitosan membranes	the 100-μM-peptide-immobilized chitosan	hMSCs (human mesenchymal stem cells)	Lee et al.[86]
		F105; YKRSRYT F119; KRTGQYKLGSKTGPGQK	Increase osteoblast differentiation, ALPase activity and mineralization	Peptides (F105 and F119) immobilized on the surface of cell culture dishes	100 μM peptide (F105, F119)	hBMSCs (Human bone marrow stromal cells)	Lee et al.[87]
	Calcitonin gene-related peptide	$CGRP_{8-37}$	Promote bone formation through β-catenin signaling modulation	CGRP peptide	10^{-8} M CGRP peptide	Human osteoblast-like cells (hOBs)	Mrak et al.[88]
	PTH1-34 (teriparatide)	SVSEIQLMHNLGKHLNSME RVEWLRKKLQDVHNF	Enhance bone regeneration	PEG hydrogel	20 μg/ml of PTH_{1-3} and 350 μg/ml cys-RGD	Dog model	Jung et al.[89]
				functionalization of fibrin matrices with PTH1-34	100 μl of peptide	UMR-106 cell line and sheep model	Arrighi et al.[90]
Non-ECM or non-growth factors	NBD (NEMO-binding domain)	YGRKKRRQRRR-G-TTLDWSWLQME	Improve osteoclast differentiation	Cell-permeable NEMO-binding domain (NBD) peptide	100 μM NBD in serum-free DMEM	Mouse myoblast cell line C2C12	Li et al.[91]
	CPP (Cell penetrating peptide)	LMWP; VSRRRRRRGGRRRR	Increase expression of osteoblastic genes *in vitro* and bone formation in rabbit calvarial defects	LMWP-TAZ fusion protein	LMWP-TAZ protein solution (2 mg in 100 μL PBS, pH 8.5) *in vitro* protein release, 2 mg TAZ- or LMWP-TAZ-loaded alginate gel per site *in vivo*	hMSCs and rabbit model	Suh et al.[92]
	TP508 (Chrysalin)	AGYKPDEGKRGDAC EGDSGGPFV	Promote an osteoblast differentiation	PLGA microspheres	10, 50, or 100 μg of TP508 in 10 mg of PLGA microspheres	Rabbit model	Sheller et al.[93]
	SP (Neuropeptide Substance P)	RPKPQQFFGLM	Induce osteoblastic differentiation and promote the angiogenic ability of BMSCs	Neuropeptide substance P (SP)	Concentration of SP 10^{-8} mol/l	Rat bone marrow stem cells (BMSCs)	Fu et al.[94]
	ET-1 (Endothelin-1)	CSCSSLMDKECVYFCHL DIIW	Enhance osteogenesis and chondrogenesis via AKT signaling pathway	Endothelin-1 (ET1)	Treated with 0.01 or 0.1 μM ET1 in DMEM	Human mesenchymal stem cells (hMSCs)	Tsai et al.[95]
	Histon	OGP ALKRQGRTLYGFGG	Improve bone regeneration	OGP-poly(lactic-co-glycolic) acid (PLGA) scaffolds	100 μg OGP	Rabbit model	Shuqiang et al.[96]
				-	86 ng/kg of OGP (200 μl solution in PBS), injected daily	Rat model	Gabet t al.[97]

(Continued)

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				Titanium implants	10 μmol/l of RGD	Rat model	Rammelt et al.[69]
				RGD-coupled alginate-PEG hydrogels	1500 μM of Alginate-RGD	Mesenchymal stem cells (MSCs) 3T3 fibroblasts cells	[60]
				MPEG (methoxy polyethylene glycol) —PCL-RGD hydrogels	A series of hydrogel formulations with 0, 0.8, 1.6, and 2.4 mM of RGD to total content of hydrogel	Bone marrow-derived mesenchymal stem cells (BMSCs) and rabbit model	Kim et al.[59]
		BCSP-1™ (NGLPGPIGP)	Increase an osteoblast differentiation marker and bone healing	BCSP™-1 covalently immobilized to the surface of Skelite™ ceramic	66 nmol/ml BCSP™-1 was dissolved in de-ionized water	Rat calvaria cells	Wang et al.[61]
		PepGen P-15 GTPGPQGIAGQRGVV	Promotes osteoblastic activity in vitro and bone regeneration in vivo	ABM-P-15	200 mg of ABM-P-15	Human periodontal ligament fibroblasts (PDLF)	Bhatnagar et al.[70]
				Hydroxyapatite (HA)–P-15 complex	200 mg of HA–P-15	Human bone marrow stromal cells and Primary human bone marrow cells	Yang et al.[62]
				HA/P-15 hydrogel	P-15 was absorbed on to algae-derived hydroxyapatite (adHA)	Pig model	Thorwarth et al.[50]
		CTC peptide IAGVGGEKSGGF	Promote migration of multiple cell types *in vitro* and formation of a bone-like nodule at the site of amputation *in vivo*	Biologic scaffolds derived from urinary bladder ECM	Concentration of $0,10^{-4}$ to 10^{-9} M peptide in DMEM/15 μL of 10 mM peptide inject subcutaneously *in vivo*	Perivascular stem cells, CTX cells, human adipose stem cells, C2C12 muscle myoblast cells, IEC-6 intestinal epithelial cells, RT4-D6P2T rat Schwann cells, and HMEC human microvascular endothelial cells and mice model	Agrawal et al.[66]
		GFOGER	Enhances peri-implant bone formation and mechanical osseointegration	Titanium implants	20 mg/ml in PBS	Primary bone marrow stromal cells	Reyes et al.[71]
		DGEA	Increased osteoblastic differentiation *in vitro*, and bone formation *in vivo*	Hydroxyapatite (HA) disks	1 mg/ml in ddH2O, at concentrations of 50, 100, or 200 μM.	Human bone marrow cells (MSCs) and Rat model	Culpepper et al.[53]
		SVVYGLR	Upregulation of osteogenesis and angiogenesis	Synthetic SVVYGLR peptide and collagen sponge	0.01–100 μg/ml of peptides in vitro and 10 μg SVVYGLR peptide in vivo (collagen sponge)	hMSC, hPLF, hGF, HUVEC, αvβ3-CHO, and mock-CHO cells and rat model	Egusa et al.[55]
		GFOGER	Enhances peri-implant bone formation and mechanical osseointegration	Titanium implants	20 mg/ml in PBS	Primary bone marrow stromal cells	Reyes et al.[71]
	Laminin-Derived Peptides	IKVAV and YIGSR	Increase osteoblasts differentiation and alkaline phosphatase activity	Laminin peptide (IKVAV and YIGSR)	100 μg/well laminin peptide	MC3T3-El cells	Vukicevic et al.[72]
		Ln2-p3	Enhance the expression of osteogenic markers and alkaline phosphatase (ALP) activity	Titanium scaffolds	23 $\mu g/cm^2$	Human osteosarcoma (HOS) cells	Min et al.[73]
	Fibronectin derived peptide	PHSRN	Increase the osteogenic differentiation and bone mineralization	RGD- and PHSRN-alginates hydrogels	peptide-modified alginates dissolved in ddH2O containing 0.2 % $(NaPO_3)_6$ to obtain a 2 % solution, Hydrogel discs (diameter 13 mm)	Normal human osteoblasts (NHOsts)	Nakaoka et al.[74]
	Other ECM derived peptide	KRSR	Promote osteoblasts adhesion and bone growth	Immobilization of KRSR onto borosilicate glass	0.01, 0.1, 1, and 2 mM modified KRSR in DMEM	Neonatal rat calvarial osteoblasts, endothelial cells (cell line CCL-209), and fibroblasts (cell line CRL-1213)	Dee et al.[75]
				Immobilization of KRSR onto nano-crystalline HA and nano amorphous calcium phosphate	KRSR, silanization	Human osteoblasts (CRL-11372)	Nelson et al.[76]
		FHRRIKA	Enhance the ability of osteoblast adhesion, spreading, and mineralization	-FHRRIKA- and -RGD- grafted surfaces	mixed FHRRIKA-RGD surfaces in ratios of 75:25 (MPS I), 25:75 (MPS II), and 50:50 (MPS III)	Primary rat calvaria osteoblast-like (RCO) cells	Rezania et al.[77]
		MEPE peptide (AC-100) ERGDNDISPFSGDGQ	Increase number of osteoblasts and osteogenic activity	Peptide fragment of MEPE containing the RGD and SGDG motif	40 μM AC-100 in medium culture in vitro and 2, 20, or 200 μg/kg/day AC-100 in 10 μl PBS twice a day in vivo	Human primary osteoblasts (HPO cells) and mice model	Hayashibara et al.[78]

(Continued)

Table 2. (Continued)

Type of peptide	Peptide group	Name and Sequence	Major Function	Biomaterial	Peptide Amount	Type Of Study	References
Peptide Derived Growth Factors	BMP-2	KIPKASSVPTELSAISTLYL	Promote osteogenesis	BMP2/OPD + RGD-conjugated inert hydrogel	10 mM in the grafting solution	Bone marrow stromal (BMS) cells	He et al.[79]
		OPD (DWIVA)		Titanium implants	2 mg synthetic peptide in 0.5 ml PBS	Osteoblast-like cell line (MC3T3-E1) and dog model	Seol et al.[80]
	pBMP-9	RKVGKASSVPTKLSPISILYK		lipid-nucleic acid nanoparticles (LNP)	(500 ng pDNA per well) in DMEM and 30 μg pDNA/rat	C2C12 cells and Ovariectomized (OVX) rat model	Vhora et al.[81]
	BMP-7	RTVPKPSSAPTQLNAISTLYF		Polyethylene terephthatalate (PET) grafted with RGD and/or BMPs mimetic peptides	10^{-3} M BMPs/PBS	MC3T3-E1 (pre-osteoblast like) cells	Zouani et al.[82]
	Osteopointin-derived peptide	**CBM** (GLRSKSKKFRRPDIQYP DATDEDITSHM)	Promote osteogenic differentiation and bone mineralization	CBM	100 μl of suspension in DMEM	Human mesenchymal stem cell (hMSC)	Shin et al.[83]
		ODP (DVDVPDGRGDSLAYG)	Increase migration and cell proliferation	OPF (oligo (poly (ethylene glycol) fumarate)) hydrogels modified with an OPN-derived peptide (ODP)	0.1, 0.5, or 1.0 μM of acrylated peptide in swollen hydrogel (g)	Rat fibroblast 3T3 like cell line (CRL-1764)	Shin et al.[84]
	Bone sialoprotein derived peptide	**CB** (NGVFKYRPRYYLYKHA YFYPHLKRFPVQ)	Promote the osteogenic differentiation and ALP activity *in vitro* and bone formation *in vivo*	HA scaffold	6 mg/100 IL PBS of peptide was applied to the HA scaffold	Human osteoblastic (HOS) cells and rabbit model	Choi et al.[85]
	FGF-2-derived peptides	F36; PDGRVD F77; KEDGRLL	Increase cell attachment and osteoblastic differentiation	Chitosan membranes	the 100-μM-peptide-immobilized chitosan	hMSCs (human mesenchymal stem cells)	Lee et al.[86]
		F105; YKRSRYT F119; KRTGQYKLGSKTGPGQK	Increase osteoblast differentiation, ALPase activity, and mineralization	Peptides (F105 and F119) immobilized on the surface of cell culture dishes	100 μM peptide (F105, F119)	hBMSCs (Human bone marrow stromal cells)	Lee et al.[87]
	Calcitonin gene-related peptide	$CGRP_{8-37}$	Promote bone formation through β-catenin signaling modulation	CGRP peptide	10^{-8} M CGRP peptide	Human osteoblast-like cells (hOBs)	Mrak et al.[88]
	PTH1-34 (teriparatide)	SVSEIQLMHNLGKHLNSME RVEWLRKKLQDVHNF	Enhance bone regeneration	PEG hydrogel	20 μg/ml of PTH_{1-3} and 350 μg/ml cys-RGD	Dog model	Jung et al.[89]
				functionalization of fibrin matrices with PTH1-34	100 μl of peptide	UMR-106 cell line and sheep model	Arrighi et al.[90]
non-ECM or non-growth factors	NBD (NEMO-binding domain)	YGRKKRRQRRR-G-TTLDWSWLQME	Improve osteoclast differentiation	Cell-permeable NEMO-binding domain (NBD) peptide	100 μM NBD in serum-free DMEM	Mouse myoblast cell line C2C12	Li et al.[91]
	CPP (Cell penetrating peptide)	LMWP; VSRRRRRRGGRRRR	Increase expression of osteoblastic genes *in vitro* and bone formation in rabbit calvarial defects	LMWP-TAZ fusion protein	LMWP-TAZ protein solution (2 mg in 100 μL PBS, pH 8.5) *in vitro* protein release, 2 mg TAZ- or LMWP-TAZ-loaded alginate gel per site *in vivo*	hMSCs and rabbit model	Suh et al.[92]
	TP508 (Chrysalin)	AGYKPDEGKRGDAC EGDSGGPFV	Promote an osteoblast differentiation	PLGA microspheres	10, 50, or 100 μg of TP508 in 10 mg of PLGA microspheres	Rabbit model	Sheller et al.[93]
	SP (Neuropeptide Substance P)	RPKPQQFFGLM	Induce osteoblastic differentiation and promote the angiogenic ability of BMSCs	Neuropeptide substance P (SP)	Concentration of SP 10^{-8} mol/l	Rat bone marrow stem cells (BMSCs)	Fu et al.[94]
	ET-1 (Endothelin-1)	CSCSSLMDKECVYFCHL DIIW	Enhance osteogenesis and chondrogenesis via AKT signaling pathway	Endothelin-1 (ET1)	Treated with 0.01 or 0.1 μM ET1 in DMEM	Human mesenchymal stem cells (hMSCs)	Tsai et al.[95]
	Histon	**OGP** ALKRQGRTLYGFGG	Improve bone regeneration	OGP-poly(lactic-co-glycolic) acid (PLGA) scaffolds	100 μg OGP	Rabbit model	Shuqiang et al.[96]
				-	86 ng/kg of OGP (200 μl solution in PBS), injected daily	Rat model	Gabet t al.[97]
	BMHP-1	PFSSTKT	Enhanced bone regneration	**BMHP-1**	—	—	Cao et al.[98]
			Enhanced bone regeneration than BMP-2 in silico	RADA-BMHP-1	1 mg/ml	Rat model	Tavakol et al.[99]
			Enhanced bone regeneration of two different self-assembling core	RADA-BMHP-1 KSL-BMHP-1	1 mg/ml	Rat model	Muttenthaler et al.[100]

RGD and PHSRN peptides linked to alginate hydrogels enhanced the osteogenic differentiation and mineralization tendency of normal human osteoblasts (NHOsts) as long as the RGD content was above 33%.[109] A careful biomimetic approach can yield productive results, as enhanced attachment, proliferation, and differentiation demonstrate. These effects are not only attributable to the synergistic effect of RGD and PHSRN but also to the extended RGD chain, which replicates the natural spacing between RGD and PHSRN. Notably, when the peptides were used in a more natural configuration, the synergistic effect of combining PHSRN and RGD improved the results.[4]

ECM phosphoglycoprotein peptide: MEPE peptide or AC-100

Dentonin, also known as AC-100, is a 23-amino-acid synthetic peptide that originates from the central segment of the matrix extracellular phosphoglycoprotein (MEPE) protein, specifically residues 242–264. This peptide includes an RGD, a glycosaminoglycan attachment sequence (SGDG), and a calcium-binding motif.[109] MEPE, a key regulator of bone formation, has been the focus of extensive research. Studies on MEPE-null mice have revealed that the absence of MEPE leads to significant changes in bone mass and trabecular structure, underscoring the importance of MEPE in maintaining bone health.[110] The inhibitory effect of this molecule is primarily attributed to its C-terminal. The AC-100 peptide has shown promising effects on osteoblasts with its integrin-binding RGD and SDGD, a consensus-binding site for glycosaminoglycans. Preincubation of rat calvarial osteoblasts with the AC-100 fragment has demonstrated its potential in enhancing adhesion and proliferation compared with cells treated with truncated peptide forms. This effect is further evidenced by increased differentiation, as indicated by higher ALP expression and a greater extent of ECM mineralization after 14 days in culture.[78]

Other ECM-derived peptides: FHRRIKA and KRSR

In addition to integrin binding, transmembrane heparan sulfate proteoglycans interact with heparin-binding sequences in numerous proteins linked to bone tissue to facilitate osteoblast cell attachment. Given that heparan sulphate has been found immunohistochemically on the membranes of osteogenic cells attached to the formed bone matrix, these extra interactions are also believed to have a major impact on osteoblast attachment behavior.[75] FHRRIKA and KRSR could have a clinical impact due to their positive publication histories and complementary effects. The Phe-His-Arg-Arg-Ile-Lys-Ala (FHRRIKA) is a putative heparin-binding domain of bone sialoprotein. In a report published by Gentile et al.,[111] FHRRIKA and KRSR peptides were attached to polyhedral oligomeric silsesquioxane (POSS) nano-particles crosslinked with polycarbonate-based urea urethane (PCU, UCL-Nano™), functionalized through plasma polymerization of acrylic acid and subsequent carboxyl activation via EDC/NHS. In this study, the peptides enhanced the attachment of MSCs. The FHRRIKA peptides resulted in a notable increase in ALP production, indicative of cell differentiation. However, the KRSR peptides did not result in more significant differentiation than that observed with plasma treatment alone. In particular, the combination of the two peptides demonstrated suitability for inducing osteoblastic differentiation. FHRRIKA primarily interacts with integrin receptors, particularly β1 and β5 integrins. Upon binding to these integrins, FHRRIKA initiates intracellular signaling cascades, notably the Gi-protein-MAP kinase pathway. This activation plays a crucial role in regulating various cellular functions, including the proliferation, differentiation, and mineralization of osteoblasts. As a result, the FHRRIKA peptide signaling pathway is essential for enhancing osteogenic activities through integrin-mediated signaling, making it a significant asset in regenerative medicine focused on bone repair.[49]

A heparin-binding site known as the KRSR (Lys-Arg-Ser-Arg) sequence is present in five distinct bone-related adhesive proteins: FN, VN, BSP, OPN, and thrombospondin. Notably, KRSR may boost the expression of osteogenic genes and osteoblast adhesion.[112] KRSR plays a critical role in promoting osteoblast-specific adhesion by binding to heparin on the cell surface, which is essential for its functionality. Its strong positive charge significantly enhances the adhesion of preosteoblasts. Upon interacting with integrins, KRSR activates intracellular signaling pathways that govern osteogenic processes, including the upregulation of genes that facilitate cell spreading, adhesion, and mineralization.[112,113] Currently, KRSR, BMP mimetic peptides, and FHRIKKA were successfully integrated into the outer, middle, and inner layers of PLGA and nanohydroxyapatite electrospun membranes through layer-by-layer (LbL) assembly, thereby creating peptide gradients to modulate cellular responses at the nanoscale level and induce faster bone formation by enhancing cell attachment, differentiation, and mineralization. The functionalized membrane induces a favorable *in vivo* response after implantation for 4 weeks in a non-healing rat calvaria defect model. Furthermore, the peptide incorporation enhances cellular processes with good viability, a significant increase in alkaline phosphatase (ALP) activity, and a quantitative expression of two major bone-specific proteins, osteopontin and osteocalcin.[114]

Peptide derived GFs

Bone morphogenetic protein (BMP)

Bone morphogenetic proteins (BMPs) are versatile GFs belonging to the TGF-β superfamily. They are the main osteoinductive molecules in mammals. BMPs have various multifunctional activities, such as hepatocyte proliferation,

adipogenesis, and angiogenesis. However, their primary role is in the formation and regeneration of bone and cartilage.[115] Among the BMP family, BMP-9 stands out for its unique ability to prevent the inhibition of the cellular Smad pathway induced by Noggin, a property not shared by other members like BMP-2 and BMP-7. This distinct feature of BMP-9 has shown promising results in bone healing.[116] Of the 14 known BMPs, BMP-9 is particularly promising. Its unique ability to derive osteoprogenitor cells from stem cells through differentiation is a significant advancement in the field of bone tissue engineering.[117] Research has unequivocally demonstrated the superiority of BMP-9 over its counterpart BMP-2. Its ability to facilitate the nuclear translocation of Smad 1/5/8 and its resistance to the inhibitory action triggered by Noggin are clear indicators of its potential in bone tissue engineering and regeneration.[118] Various delivery systems have been used to effectively deliver BMPs, including BMP-9. In a recent study, lipid nanoparticles incorporating histidine-modified octadecyl amine and pBMP-9 demonstrated enhanced osteogenic differentiation and bone growth in an osteoporosis-induced ovariectomized (OVX) rat model, showcasing the effectiveness of BMP-9 in bone regeneration.[81] A collagen-based 3D scaffold, nHA/Col-BMP-9/gelatin microsphere (GM), was developed by a combined fabrication method of mixing, crosslinking, and freeze-drying. The data presented that applying the nHA/Col-BMP-9/GM scaffolds with the desired mechanical strength and good biocompatibility in vitro and in vivo could be a promising strategy for bone tissue engineering to treat bone defects.[119] In a follow-up study, researchers combined a peptide derived from BMP-2 with an angiogenic peptide from osteopontin (OPD) on an RGD-conjugated inert hydrogel. This unique substrate, triple-functionalized, improved the mineralization process in rat bone marrow stromal (BMS) cells. Furthermore, the cells showed increased expression of vasculogenic markers PECAM-1 and VE-cadherin when exposed to the OPD peptide.[79] Nevertheless, specific BMPs were used by grafting small peptides covalently onto PET, which mimicked various mimetic peptides. These mimetic peptides, responsible for adhesion and osteo-induction, imitated fibronectin, BMP-2, BMP-7, and BMP-9 proteins, respectively. The biomimetic modification of the PET surface demonstrated the effectiveness of these mimetic peptides in inducing cell activity, as evidenced by the increased expression of Runx2 and the remarkable production of E. Furthermore, examining cell morphology on different surfaces revealed a correlation between cell morphology and cell function.[82]

BMP-2 derived peptide: Osteopromotive domain (OPD)

The osteopromotive domain (OPD) is a specific sequence within BMP-2 that contains the DWIVA pentamer. This sequence overlaps with the binding sites of BMP receptors I and II.[5] Lee et al.[120] demonstrated that OPD may facilitate osteogenic differentiation through an intracellular signal transduction cascade. Significantly, when attached to titanium surfaces, OPD promoted the proliferation and differentiation of MC3T3-E1 cells in vitro and showed potential for practical applications.[80] Additionally, when used to coat bovine bone mineral granules for filling calvarial defects in rabbits, the authors observed accelerated osteogenesis in the group treated with the peptide, further highlighting its practical relevance.[121]

Osteopontin-derived peptide: GRDGS

Osteopontin in humans has a specific motif known as GRDGS binding to integrin and is accompanied by a hidden sequence with angiogenic properties. The angiogenic peptides associated with this motif were documented in a previous study.[122] Although the amino acid residues flanking the GRGDS motif are mostly conserved in rat osteopontin, some variations exist. Shin et al.[84] studied the impact of peptide density on the differentiation and mineralization of MSCs in hydrogels modified with GRGDS. The proliferation of osteoblasts on these hydrogels depended on the concentration of GRGDS peptide, with higher concentrations promoting increased proliferation at earlier stages of culture. The GRGDS peptide-modified hydrogels induced osteoblast differentiation and mineralization, as shown by the expression of ALP, OPN, and calcium deposits. Notably, the peptide concentration greatly influenced the activity of ALP and the deposition of calcium, with higher concentrations accelerating the differentiation and mineralization of MSCs.

Osteopontin derived peptide: Collagen-binding motif (CBM)

The collagen-binding motif (CBM), a fragment derived from OPN, has been rigorously studied and found to promote osteogenic differentiation and migration through activating distinct signaling pathways.[123] For instance, the research conducted by Shin et al.[83] provides robust evidence that CBM enhances osteogenic differentiation in hMSCs by activating the Ca^{2+}/CaMKII/ERK/AP-1 signaling pathway. Furthermore, the role of CBM in stimulating the migration of hMSCs by suppressing cell proliferation is a testament to the scientific rigor of these findings. Remarkably, collagen-binding motifs (CBMs) in an ovariectomized rat model demonstrably increased bone mineral density and bone volume fraction. This finding holds significant implications for postmenopausal women, whose reduced estrogen levels predispose them to osteoporosis.[124]

Bone sialoprotein derived peptide: The collagen-binding peptide (CB)

The collagen-binding peptide (CB) is situated at the N-terminus of rat BSP and binds with type I collagen. It is

a sequence with 28 hydrophobic residues. This peptide has been found to enhance the process of osteogenic differentiation in human osteoblast cells by activating certain signaling pathways like ERK- and Akt-dependent.[85] It increases the mRNA level of ALP, collagen type I, OCN, and OPN, enhancing ALP activity and matrix mineralization. Furthermore, when applied to deproteinized bone particles, the CB peptide improved early bone growth in a rabbit skull defect compared with a nonfunctionalized scaffold. It is important to note that the osteogenic differentiation induced by the CB peptide was blocked when specific inhibitors for ERK1/2 and Akt were used.[85]

Fibroblast growth factor (FGF)

The FGF family, a diverse group of 22 polypeptides from FGF1 to FGF22, each with unique biological functions, includes FGF-2. This member is a dynamic player in regulating cellular processes like proliferation and differentiation, achieving this by binding to FGF receptors (FGFRs) on the cell surface in the presence of heparin proteoglycans. FGF-2, a heparin-binding GF, is a subject of ongoing exploration, with the precise location of its heparin-binding site yet to be fully uncovered, promising exciting discoveries in the future.[87] Lee et al.[86,87] investigated two potential heparin-binding sequences of FGF-2, specifically residues 105–111 and 119–135. Their peptide sequences are F105, YKRSRYT, and F119, KRTGQYKLGSKTGPGQK, respectively. Their findings have implications that are not just theoretical but also practical. When these peptides were attached to poly-L-lysine-coated culture plates, they enhanced the adhesion and proliferation of human bone marrow-derived mesenchymal stem cells (hBMSCs) and increased the expression of ALP and ECM mineralization in these cells. This suggests a potential avenue for enhancing stem cell research and applications.

Calcitonin gene-related peptides (CGRP)

Calcitonin gene-related peptides (CGRP) are present in α and β. α-CGRP is derived from the Calca gene and consists of 37 amino acids.[125] What is truly fascinating is the non-uniform distribution of CGRP in bone. It is localized in sensory nerve endings in specific areas such as the periosteum, bone marrow, and metaphysis, offering a distinct perspective on its intricate role in bone physiology and healing.[126] CGRP plays a pivotal role in bone physiology and healing. It is not just a bystander but an active participant, stimulating proliferation and differentiation and significantly reducing cell death.[88,127,128] Significantly, CGRP levels surge in patients with bone fractures, underscoring its crucial role in the inflammatory phase of bone healing and overall tissue repair.[129]

Parathyroid hormone 1–34 peptide (teriparatide)

PTH plays a crucial role in maintaining the metabolic homeostasis of calcium and phosphorus in mammals. PTH1-34, a short peptide composed of 34 amino acids, is biologically active and used for systemic osteoporosis treatment and bone formation regulation. It also stimulates bone tissue repair through various cellular mechanisms, making it a promising candidate for treating bone healing and radiation-induced bone loss.[130] A study, conducted on a murine closed fracture model, was meticulously designed to eliminate surgical artifacts. This precision allowed us to closely observe the early stages of callus formation in response to varying doses of PTH1-34. The impact of PTH1-34 on callus formation, a pivotal phase in fracture consolidation, was determined through rigorous histomorphometric and microhardness assessments over the first 4 weeks post-fracture. The remarkable findings revealed that daily administration of 40 μg/kg PTH1-34 significantly enhanced callus mineralization during the initial phases of bone healing (up to day 18). Moreover, after just 15 days of treatment, the callus microhardness mirrored that of bone. This underscores the potential of PTH1-34 in expediting the consolidation of the callus, with the highest new bone formation observed in the control group (after 18 days) occurring as early as 13 days in the treated animals.[131] Moreover, strontium-substituted hydroxyapatite (HA) composites incorporating strontium and PTH1-34 exhibit sustained teriparatide release and demonstrate significant osteogenic potential.[132] Notably, the administration of PTH1-34 has shown promising results in protecting rats from radiation-induced bone loss. This treatment has been found to preserve all trabecular elements in irradiated bone and significantly increase bone mass and strength. These findings hold significant implications for the potential use of PTH1-34 in osteoporosis treatment, instilling a sense of hope in the medical community.[133]

Other osteogenic peptides: Non-ECM or non-growth factors

NEMO-binding domain peptide (NBD)

Nuclear factor kappa B kinase inhibitor (IKK) is a large complex made up of two catalytic subunits (IKK-1 and IKK-2) and a regulatory subunit called NF-kB essential modulator (NEMO or IKK-γ). NEMO interacts with both IKK subunits through a region known as the NEMO-binding domain (NBD) located at amino acids 737–742.[134] Additionally, studies by Li et al.[91] and Jimi et al.[135] have shown that this peptide can promote cell differentiation into osteoblasts and inhibit bone resorption. In other words, it has demonstrated that NBD peptide has strong therapeutic effects in many inflammatory

and degenerative disease models in various animal models.[135–137] Li et al.[91] examined the impacts of TNF-α, NF-κB, and NBD on the differentiation of osteoblasts using C2C12 mouse myoblast cells. The findings demonstrated for the first time that NF-κB activation suppresses BMP-2-induced osteoblast differentiation by reducing Smad1 activity. Importantly, the application of NBD peptide counteracts this inhibitory effect. Additionally, the NBD peptide inhibited NF-κB activity and mitigated the suppression of osteoblast differentiation caused by TNF-α, indicating its potential use in treating inflammatory bone damage associated with rheumatoid arthritis.

Cell penetrating peptides (CPPs)

Cell-penetrating peptides (CPPs) protein composed of a small-peptide tag that promotes translocation of the protein into the cells, termed low-molecular-weight protamine (LMWP; VSRRRRRRGGRRRR), can be directly applied to target cells. LMWP with a high arginine content and significant sequence similarity to TAT (Trans-Activator of Transcription) protein, can conjugate with therapeutic anticancer protein and even with solid nanoparticulates for stem cell labeling/trafficking purposes.[138,139] CPPs show promise in transporting various cargoes, such as nanoparticles, oligonucleotides, and proteins, across cell membranes and into the cytoplasm. This ability allows CPPs to act as a delivering factor involved in hard tissue repair, such as BMP-2, into MSCs to promote bone formation.[135] CPPs can be derived from bacteria and viruses or synthesized in the laboratory.[140] In a study using a rabbit calvarial defect model, it significantly increased bone formation by treating CPPs containing a fusion protein of a transcription factor.[92] In a study the protein fusion of TAZ (A transcriptional coactivator with a PDZ-binding motif) with cell-penetrating LMWP (LMWP-TAZ) was prepared and applied in combination with alginate for specific bone formation *in vitro* and *in vivo*. The LMWP-TAZ fusion protein-loaded alginate gel matrix not only showed an ability to increased expression of osteoblastic genes and protein, but also blocked adipogenic differentiation simultaneously.[92]

Thrombin peptide 508 (chrysalin)

The synthetic peptide TP508 is a noninvasive solution made up of 23 amino acids. It represents the receptor-binding domain of thrombin and does not require proteolysis. This unique characteristic of TP508 provides reassurance about its potential safety. Furthermore, it has been found to promote the growth of human osteoblasts, bone-forming ability, and angiogenesis.[141,142] Animal studies have shown that TP508 may positively impact bone healing. Two experiments conducted on rabbits with segmental bone defects showed a significant increase in bone formation and improved torsional strength of the bone when TP508 was used in PPF composite and microsphere scaffolds.[93,143] It seems that the osteogenic effects of Chrysalin to be mediated via the Wnt/β-catenin signaling pathway.[144]

Substance P

As a natural neurotransmitter, substance P (SP) is peripherally released by the sensory neurons innervating periosteum, bone marrow, and vascular channels.[145] SP is an 11-amino acid neuropeptide that is part of the tachykinin family and primarily signals peripherally through the neurokinin 1 receptor (NK1R), which is found on various non-neuronal cell types. It activates the Wnt signaling pathway in bone marrow stromal cells (BMSCs). This activation promotes their osteoblastic differentiation, enhances their migratory capacity, and stimulates the expression of vascular endothelial growth factor (VEGF). These effects may be associated with angiogenic potential of BMSCs.[94] *In vitro* studies show that SP promotes the proliferation of osteoblast precursors in a dose-dependent manner, enhancing both cell activity and bone formation in differentiating osteoblasts. Furthermore, SP stimulation aids in the osteoclastogenesis of isolated bone marrow macrophages and increases the bone resorption activity of mature osteoclasts.[146–148] Neuropeptide SP has been shown to play a role in bone growth by promoting the proliferation and differentiation of BMSCs. Studies indicate that SP enhances BMSC differentiation by activating gene and protein expression through the Wnt signaling pathway and facilitating the translocation of β-catenin. This process can be inhibited by treatments with Wnt signaling blockers or NK-1 antagonists. Additionally, SP can elevate levels of BMP-2. It also improves the migratory capacity of BMSCs, and research has explored how SP promotes the expression of VEGF. These findings suggest that the use of vascularized and neurotized bone tissue engineering is theoretically viable.[94]

Endothelin-1 (EDN-1)

EDN-1, a multifunctional vasoconstrictor of 21 amino acids, was first isolated from porcine aortic endothelial cells.[149] Its receptors, endothelin A receptor (ETAR) and endothelin B receptor (ETBR) are structurally similar G-protein-coupled receptors. The interaction between EDN-1 and these receptors is a fascinating example of complex signaling pathways. ETAR is responsible for transmitting most of the biological effects of EDN-1, while ETBR acts as a clearance receptor, adding another layer of intricacy to this process.[150] Also, EDN-1 is known to play a fundamental role in regulating bone metabolism, particularly in stimulating new bone formation.[151] The EDN-1/ETAR pathway, a key player in bone metabolism,

regulates osteoblast activity and trabecular bone remodeling. EDN-1, in particular, is instrumental in promoting the proliferation of osteoprogenitor cells and facilitating their differentiation into mature bone matrix-secreting osteoblasts, a crucial step in the process of bone remodeling.[152] It is worth noting that ET-1 secreted by endothelial cells plays a significant role in the osteo- and chondrogenic differentiation of MSC. This is achieved through the activation of the AKT pathway, a crucial signaling pathway involved in cell survival, growth, and proliferation. The activation of this pathway by ET-1 is a key step in the regulation of bone and cartilage formation.[95]

Osteogenic growth peptide (OGP)

OGP, a native molecule, holds a primary structure that mirrors the C-terminus of histone H4. This structure encompasses a highly conserved 14-amino acid motif (NH2-ALKRQGRTLYGFGG-OH). The peptide was initially isolated by Bab et al.[153] from blood during the osteogenic remodeling of rat bone marrow regeneration post-ablation, marking a significant breakthrough in our understanding of bone regeneration. Additionally, the OGP peptide, when proteolytically cleaved, generates the C-terminal pentapeptide (NH2-YGFGG-OH), named OGP (10–14), which activates an intracellular Gi-protein-MAP kinase signaling pathway. A newly published article by Zhao et al. demonstrated the stable physical and chemical properties, excellent biocompatibility, and osteogenesis-promoting capacity of injectable OGP-based self-assembling supramolecular hydrogels. F- and G-sequence hydrogels are named after the specific sequences of amino acids in their structure. "Hydrogelator" refers to a molecule that can self-assemble into a hydrogel. In this study, the hydrogelator used was Nap-Phe-Phe-Tyr. In vitro analyses showed that hydrogels could effectively upregulate the expression of osteogenic factors, including RUNX2, BMP2, OCN, and OPN, to promote osteogenesis differentiation. Experimental outcomes in vivo illustrated the remarkable therapeutic effect of hydrogels in inducing bone formation and promoting bone regeneration in a rat bone defect model in the early stage of bone defect healing. Moreover, NapFFY-OGP, a supramolecular hydrogel (Nap-Phe-Phe-Tyr as a hydrogelator), effectively regenerated and reconstructed bone defects in situ. Human bone marrow stromal cells (HMSCs) were recruited to the defect site, and their differentiation was promoted. Following this, the bone regeneration process was initiated. Specifically, following the application of NapFFY-OGP hydrogels to the rat skull defect area, a bone regeneration rate of 37.54% bone volume fraction (BV/TV) was observed, surpassing that of the NapFFY hydrogel group (25.09%). The potential of NapFFY-OGP hydrogel in the clinical repair of bone defects is highly promising for future applications.[154]

The goal of all regenerative approaches is the enhancement of osteogenesis. Many studies are being conducted to find biological agents that can reduce healing time and increase the quality of regenerated bone. The use of biomolecules such as peptides with osteoinductive properties, which are derived from natural peptides, has been suggested to be beneficial for guided bone repair. Biological materials for tissue regeneration can be functionalized with osteogenic peptides to either mediate cell adhesion, migration, proliferation, and differentiation, or to be released as soluble ligands. These short peptides are easy to design and synthesize, facilitating their use as cost-effective and efficient scaffolds for regenerative medicine. It has been particularly noted that peptide-modified biomaterials could stimulate new bone formation more efficiently compared to non-modified materials.

Bone marrow homing peptide 1: PFSSTKT

Cao et al.[98] pioneered the investigation into the osteogenic capacity of the Bone Marrow Homing Peptide 1 (BMHP1) motif, derived from a phage display library targeting bone marrow stem cells. Their findings demonstrated that BMHP1 significantly promoted mesenchymal stem cell (MSC) adhesion, proliferation, and osteodifferentiation under both basal and osteogenic induction conditions. Conversely, a cyclic BMHP1 analog exhibited markedly diminished osteogenic potential. Subsequent research by Tavakol et al.[99] corroborated these observations in vivo, showcasing enhanced osteogenesis and bone regeneration in a rat model following the administration of BMHP1 integrated into a self-assembling peptide scaffold. Molecular docking analyses revealed that the BMHP1 sequence, particularly lysine 24, formed stronger electrostatic interactions with BMPR1A than BMP2, mediated by arginine within the RADA core sequence. Comparative studies employing an alternative KSL core structure indicated that the RADA-BMHP1 elicited superior osteogenic differentiation, characterized by augmented expression of osteogenic genes, ALP activity, and matrix mineralization. This enhanced efficacy was attributed to the RADA-BMHP1 construct's superior protein-binding affinity and hydropathy than KSL, thereby optimizing interactions with osteogenic proteins. In vivo assessments using a rat bone defect model confirmed the improved bone regeneration capacity of both peptide nanofiber constructs, with the RADA-BMHP1 conjugate demonstrating significantly enhanced bone formation compared to its KSL counterpart.[155]

In summary, this part comprehensively explores the diverse roles of biological motifs in osteogenesis, highlighting their potential to overcome limitations associated with traditional GF-based therapies. Several key signaling pathways are consistently implicated in the osteogenic effects of these peptides, demonstrating a complex interplay of molecular mechanisms. The peptides discussed

engage multiple pathways to promote bone formation. Integrin-mediated signaling is central, with peptides like RGD, GFOGER, IKVAV, and the combination of RGD and PHSRN, activating integrin receptors (α1β1, α2β1, α5β1, αVβ1, α6β1, and α3β1/α3β3) leading to downstream effects. This often involves the FAK/ERK pathway, crucial for cell adhesion, proliferation, and differentiation. The PI3K/Akt pathway, regulating cell survival and metabolism, is also frequently activated, particularly by laminin-derived peptides and RGD-containing sequences within hydrogels. Furthermore, several peptides influence the Wnt/β-catenin pathway, a master regulator of osteoblast differentiation. While not explicitly stated for all peptides, the enhanced osteoblast differentiation observed strongly suggests its involvement. The TGF-β/SMAD pathway, essential for bone formation and remodeling, is also modulated by some peptides, notably BMP-derived sequences and possibly certain ECM-derived peptides through indirect mechanisms. Finally, the MAPK/ERK pathway, promoting cell proliferation and differentiation, is consistently activated by collagen-derived peptides.

Comparing the pathways activated by different peptide groups reveals some commonalities and distinctions. Collagen-derived peptides (RGD, GFOGER, DGEA, BCSP-1™, P-15, and CTC) primarily activate the MAPK/ERK, PI3K/Akt, and potentially Wnt/β-catenin pathways, emphasizing their role in cell proliferation and differentiation. Laminin-derived peptides (IKVAV and Ln2-p3) strongly activate PI3K/Akt and potentially Wnt/β-catenin pathways, highlighting their influence on cell survival and differentiation. BMP-derived peptides (OPD) directly engage the intracellular signaling cascades downstream of BMP receptor activation, while osteopontin-derived peptides (GRDGS and CBM) demonstrate effects on integrin signaling and unique pathways like Ca^{2+}/CaMKII/ERK/AP-1, linking them to both osteogenesis and angiogenesis (Supplemental Table 1s). The synergistic effects of combining peptides, such as RGD and PHSRN, underscore the complexity of the system and the potential for enhanced osteogenic outcomes through combinatorial approaches that mimic the natural ECM environment. The success of these biomimetic peptides in preclinical studies suggests significant promise for their translation into clinical applications for bone tissue engineering and regeneration, offering a safer and more cost-effective alternative to traditional GF therapies. Further research focusing on optimizing peptide sequences, delivery methods, and understanding the precise interplay of these signaling pathways is crucial for maximizing their therapeutic potential. Current trends involve developing more stable and targeted peptides, incorporating them into biomaterials for localized delivery, and exploring their synergistic effects with other GFs. The study of osteogenic peptides is driven by the need for effective therapies to promote bone regeneration and treat various bone-related conditions, such as osteoporosis and non-union fractures

Antimicrobial peptides

The treatment of bone fractures is associated with the risk of infection. This risk can disrupt bone regeneration and impose a significant economic burden on patients and society.[156] Bone infections can arise from three main mechanisms: contiguous contamination, hematogenous spread, and vascular/neurologic failure. Depending on the type and location of the fracture, the incidence ranges from 1.8% to 27%.[157] At present, antibiotics stand as the mainstay for treating bone defect infections. However, the rampant use and inappropriate prescription of antibiotics have paved the way for the gradual rise of antibiotic-resistant bacteria, leading to recurrent infections and escalated medical expenses. This situation underscores the pressing need to develop effective antimicrobial agents that can curb the proliferation of these resistant pathogens.[158] Antimicrobial peptides (AMPs) offer a promising approach to preventing bone infections during bone defect repair. In addition to their versatile properties, AMPs are biocompatible and can be locally administered to promote regenerative effects such as cell proliferation, migration, and angiogenesis. These characteristics make them a unique and potentially powerful tool in combating bone infections.[156] AMPs are a group of oligopeptides consisting of 10 to 60 amino acids that play a vital role in the innate immune system by inhibiting the growth of bacteria, fungi, parasites, and viruses.[159] While most AMPs are cationic due to the presence of lysine, arginine, and histidine residues and carry a net positive charge ranging from +2 to +13, there are also anionic AMPs that contain acidic amino acids like aspartic acid and glutamine.[159,160] Both synthetic and natural AMPs exhibit broad-spectrum antimicrobial activity.[161] Despite their various applications, natural antimicrobial peptides (NAMPs) have limited stability, high extraction costs, short half-lives, and host toxicity. To address these challenges, synthetic antimicrobial peptides (SAMPs) are used.[162,163] As of Jan 2024, the Antimicrobial Peptide Database (APD) contains 3940 peptides, including 3146 NAMPs from the six life kingdoms (383 bacteriocins/peptide antibiotics from bacteria, 5 from archaea, 8 from protists, 29 from fungi, 250 from plants, and 2463 from animals), 190 predicted and 314 synthetic antimicrobial peptides with the main functions following activity such as: antibacterial peptides (83.42%), antifungal peptides (30.83%), anti-Candida peptides (18.68%), anti-MRSA Peptides (11.03%), antiparasital peptides (7.16%), Anticancer (antitumor) peptides (6.33%), antiviral peptides (5.14%) and etc.

Types of AMPs

Four primary categories of AMPs are based on their major secondary structures: helical, sheet-based, and coil-based (lacking both helical and β-sheet structures), and composite AMPs. In nature, various types of helical structures exist, such as α-helix, collagen triple helix, and β-spinal

helix. Most structures are based on α-helix, with 3.6 amino acids connected through hydrogen bonds per turn. These helical structures are typically rich in leucine, lysine, alanine, and glycine, which do not cause spatial interference.[164] Sheet-based structures found in nature include parallel β-sheet, anti-parallel β-sheet, and β-hairpin. Most structures are similar to β-hairpin and contain protected cysteine residues that form disulfide bonds. Extended-based AMPs are peptides that do not have a specific structural motif but are characterized by a high concentration of specific residues, such as histidine, arginine, glycine, or tryptophan.[165,166] In contrast to the previous three types of AMPs, which have distinct secondary structures, composite AMPs possess multiple structures.[156] Furthermore, while most AMPs are being investigated in clinical trials for repairing soft tissues, they show promising potential for regenerating bone tissues as well. They can influence bone repair through targeting cell structures, intracellular molecules, immunomodulation, and action on biofilms.[163]

Cell signaling by AMPs

AMPs, in the context of wound healing, play a crucial role in several vital processes. They regulate the polarization of macrophage types (M1 and M2), thereby facilitating wound healing through the expression of cytokines. AMPs also activate receptor signaling mechanisms responsible for cell proliferation and migration, aiding in re-epithelialization and wound closure. These peptides enhance wound healing and support angiogenesis by promoting the formation of endothelial cell tubes, regulating angiogenic proteins, increasing the production of vascular endothelial GF, and activating the formyl peptide receptor-like 1 pathway. Additionally, AMPs are involved in the synthesis of ECM components, such as collagen, in the vicinity of the wound area. This detailed understanding of the actions of AMPs in wound healing underscores their importance in the process.[167] Table 3 provides a comprehensive overview of the key properties of antimicrobial peptides and their relevant applications. The various mechanism of AMP are as follows.

Cell structure targeting such as membrane permeabilization. Bacterial homeostasis depends on the integrity of the membrane, and most AMPs work against bacteria by destabilizing the membrane, which has negative charge because of anionic lipids, such as lipopolysaccharides for Gram-negative bacteria or teichoic acids for Gram-positive bacteria. A cationic AMP and an anionic bacterial membrane typically initiate electrostatic interaction at first contact. A transmembrane pore model and a non-membrane pore model have been proposed to interpret antimicrobial mechanisms when AMPs aggregate on the surface of bacterial membranes.[156,192] According to transmembrane pore models, pores in bacterial membranes can be induced by AMPs. Non-membrane pore models describe AMPs as promoting micelle formation instead of pore formation.[156,192,193] In contrast to most AMPs that exert their antimicrobial activity through membrane lysis, some AMPs destroy cell wall integrity by affecting cell wall synthesis. The genetic modulation of AMPs can also affect the synthesis of cell walls. It is possible for some AMPs to target intracellular organelles as well as membranes and cell walls.[156,194,195]

Intracellular molecule targeting. In addition to damaging cell structures, especially membranes, some AMPs target intracellular molecules to kill microbes after translocation. Molecules such as nucleic acid are common targets of AMPs.[156] There is one AMP known as buforin II, which mainly binds to DNA and RNA to disrupt the functions of cells without lysing their membranes.[196,197] Furthermore, AMPs can indirectly inhibit DNA replication or transcription in addition to directly binding to DNA and causing damage.[198,199] Additionally, proteins are also important intracellular targets for AMPs. For instance, LL-37 translocates bacterial membranes and binds to Francisella cytoplasmic acyl carrier protein (AcpP), thereby changing fatty acid profiles.[200] A number of AMPs are capable of targeting intracellular enzymes in order to exert antimicrobial effects. For instance, microcin J25 (MccJ25) and capistruin (Cap) can inhibit RNA polymerase's biological action by binding to the second channel of the enzyme.[201] In E. coli, HNP1 penetrates the outer and inner membranes and represses DNA, RNA, and protein synthesis. The lethal event appears to be inner membrane permeabilization. Consequently, HNP1 interacts with lipid II and inhibits cell wall synthesis by binding to lipid II and lipid III, precursors of cell wall teichoic acid.[202,203]

Immunomodulation. AMPs can also modulate the immune system to enhance microbe clearance and reduce diverse types of inflammation. The actions of AMPs include recruiting multiple immune cells, modulating neutrophil functions, initiating antigen-presenting cells, and activating T cells and B cells, which are primarily involved in both the innate and adaptive immune systems.[204] The mechanisms of AMP-related immunomodulation can be explained by their interaction with membrane receptors and intracellular receptors on immune cells.[205] It has also been shown that AMPs can inhibit pro-inflammatory cytokines and promote anti-inflammatory cytokines to control inflammation. Inflammatory inducers are intercepted and directed to related sensors by AMPs, and inflammation-related signaling pathways and transcription factor expression can be suppressed by AMPs.[206]

Action on biofilms. Biofilms make conventional antimicrobials harder to work against. In fact, biofilm microorganisms can tolerate high concentrations of antimicrobials,

Table 3. Antimicrobial peptide properties and related applications.

AMPs Name	Sequence	Structure	Length/net Charge	Hydrophobic residue%	Activity	Related to the bone properties	Ref.
LL-37	LLGDFFRKSKEKIGKEFK RIVQRIKDFLRNLVPRTES	Helix-based	37/+6	35%	Anti-Gram+ and Gram−, Antiviral, Antifungal, candidacidal, Antiparasitic, Spermicidal, Anti-HIV, Chemotactic, Anti-MRSA, Enzyme inhibitor, anti-TB, anti-sepsis, Synergistic AMPs, Hemolytic, Antibiofilm, Wound healing, Anticancer	Osteogenesis, immunomodulation, Osteoclastogenesis Inhibition/Rat femoral defect model	Hao et al.,[156] Gudmundsson et al., [168] He et al., [169,170]
GL13K	GKIIKLKASLKLL	Sheet-based	13/+5	53%	Anti-Gram+ and Gram−, Anti-inflammatory, anti-sepsis, Antibiofilm	Osteoclastogenesis inhibition/Rabbit femoral defect model	Hao et al.,[156] Abdolhosseini et al.,[171] Fischer et al.,[172] and Chen et al.[173]
HBD-3	GIINTLQKYYCRVRGGRCAVLSC LPKEEQIGKCSTRGRKCCRRKK	Composite	45/+11	33%	Anti-Gram+ and Gram−, Antiviral, Antifungal, Anti-HIV, Chemotactic, Anti-MRSA, Anti-toxin, Anti-inflammatory, Synergistic AMPs, Antibiofilm, Wound healing, and Anticancer	Osteogenesis/osteogenic assays *In vitro*	Hao et al.,[156] Abou Alaiwa et al., [174] Hirsch et al.,[175] Liu et al.,[176] and Wang et al.[177]
Mel4	KNKRKRRRRRRGGRRRR	Composite	17/+14	82%	*Staphylococcus aureus, Pseudomonas aeruginosa*	Rabbit infected bone defect model	Zhang t al.[178] and Yasir et al.[179]
LF1-11	GRRRRSVQWCA	Helix-based	11/+4	36%	Anti-Gram+ and Gram−	osteogenic assays *In vitro*	Hao et al.,[156] He et al.,[170] and Nibbering et al.[180]
KR-12	KRIVQRIKDFLR	Helix-based	12/+5	41%	Anti-Gram−	Osteogenesis/Rat femoral defect model	Hao et al.,[156] Wang,[181] and Meng et al.[182]
Pac-525	KWRRWVRWI	Coil-based	9/+4	55%	Anti-Gram+ and Gram−	Osteogenesis/osteogenic assays *In vitro*	Wei et al.,[183] Kazemzadeh-Narbat et al.,[184] and He et al.[185]
MBD-14	FLPKTLRKFFCRIRGGRCAVLN CLGKEEQIGRCSNSGRKCCRKKK	Composite	45/+12	37%	Anti-Gram+ and Gram−, Antifungal, candidacidal, Chemotactic, Anticancer	Osteogenesis/osteogenic assays *Invitro*	Rohrl et al.[186] and Yuan et al.[187]
HHC-36	KRWWKWWRR	Sheet-based	9/+5	55%	*Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa*, MASA	Osteogenesis/Rabbit tibial Osteomyelitis model	Wang et al.,[188] Chen et al.,[189] and NategholEslam et al.[190]
Tet-213	KRWWKWWRRC	Coil-based	10/+5	50%	activity on 80% of the *S.aureus*, activity against *Pseudomonas aeruginosa*, Antibiofilm	Rabbit tibial osteomyelitis model	Kazemzadeh-Narbat et al[184] and Wu et al.[191]

thanks to transcriptional regulatory mechanisms, even though they are totally sensitive in planktonic conditions.[207] Some AMPs have been shown to inhibit the adhesion of bacteria to surfaces by decreasing certain types of motilities such as swarming and swimming. AMPs can prevent different stages of biofilm formation. Additionally, they are capable of stimulating "twitching", a type of motility known to facilitate the disassembly of biofilms.[208]

Bone regenerative functions

AMPs have been locally used in clinical settings to repair soft tissues, including diabetes-related foot ulcer, skin infection, and burn wound. Numerous studies have also examined the topical application of AMPs for wound and bone healing.[156,158,209] Ongoing research on AMPs has resulted in a significant amount of data being stored in AMP databases. Recently, AMPs have been confirmed to apply regenerative functions for bone tissue engineering by stimulating angiogenesis, osteogenesis, and inhibiting osteoclastogenesis such as LL-37,[156,168,169,170] GL13K,[156,171–173] HBD-3,[156,174–177] Mel4,[178,179] LF1-11,[156,170,108] KR-12,[156,181,182] Pac-525,[183–185] MBD-14,[186,187] HHC-36,[188–190] and Tet-213.[184,191] A unique study evaluated the effectiveness of a composite scaffold comprising PSeD/hADSCs/LL37 in repairing bone defects using a rat calvarial defect model. The concentration of LL37 was found to significantly influence the osteogenic potential of hADSCs, with the most favorable results observed at a concentration of 4 μg/ml. This optimal dose of LL37 also demonstrated potent antibacterial activity against *Escherichia coli* and *Staphylococcus anurans*. In comparison with other scaffold types, the composite scaffold showed superior osteogenic characteristics, indicating its strong potential for bone regeneration in the rat calvarial bone defect model.[210] While LL37 has shown promise in enhancing bone production when used with titanium implants, its precise role in the bone formation process remains unclear. This underscores the need for further investigation to fully understand the potential of LL37 in restoring craniofacial bone defects, paving the way for future research.[211–213] The hydrogel release system is developed to carry the antimicrobial peptide GL13K on a titanium surface. It exhibits antibacterial and anti-inflammatory properties, as well as the ability to induce a shift in macrophage polarization from the M1 to M2 phenotype is crucial in establishing a conducive environment for bone formation, especially in the presence of infections. Additionally, the modified titanium surface has been shown to decrease the expression of pro-inflammatory cytokines IL-1β, TNF-α, and iNOS while increasing the levels of anti-inflammatory cytokines Arg-1, IL-10, and VEGF-A.[214] Furthermore, bifunctional titanium materials have been developed to slow the release rate of HBD-3 and BMP-2 in treating bone fractures. This controlled release mechanism leads to prolonged resistance against biofilms and improved osteogenesis.[156,174]

Clinical trial studies and approved osteogenic and AMP peptides (2000 to present)

Tracking recent research trends (within the past 5 years) can be achieved through two primary approaches: a comprehensive review of relevant research publications, or, more efficiently, by analyzing the status and results of ongoing and completed clinical trials. 109 clinical trials have been registered in www. clinicaltrials.gov and www.irct.behdasht.gov.ir with the phrase of bone and peptide, of which 7 studies is relared to the bone fracture. Of the numerous angiogenic, osteogenic and AMP peptides discussed, only abaloparatide, RGD peptides, P-15, PTH134, TP508 (as osteogenic agents), and LL-37 and Reltecimod (as an antimicrobial peptide) have progressed to clinical investigation (Tables 4 and 5). These findings highlight the considerable translational challenges inherent in peptide therapeutics, despite extensive preclinical research. This protracted development pathway is likely attributable to the substantial research and development expenditures associated with peptide-based products. Our experience developing NMImETIC Peptide Nanofibers and NMImETIC NanoBone, encompassing over 14 years of continuous research and development, exemplifies this protracted timeline. This extended timeframe contributes to market entry barriers, prompting researchers and companies to prioritize established alternatives, such as ceramic and polymeric materials, to minimize the risk of market failure. This preference stems from the general reluctance toward novel products within the surgical community, where risk aversion is a significant factor influencing material selection.

Methods to prepare peptide-modified bone repair materials

Three principal methodologies exist for peptide synthesis: solid-phase peptide synthesis (SPPS), liquid-phase peptide synthesis (LPPS), and hybrid-phase peptide synthesis. In 2023, liquid-phase peptide synthesis (LPPS) commanded a dominant market share exceeding 45.02%, driven by the increasing demand for high-purity peptides in advanced therapeutic development. The hybrid technology segment, leveraging the combined advantages of LPPS and solid-phase peptide synthesis (SPPS), is projected to exhibit the most rapid growth. This is fueled by ongoing research and development initiatives focused on cost-effective and environmentally responsible solutions that integrate the strengths of both methodologies. The capacity of hybrid technology to mitigate impurity issues within peptide synthesis is expected to generate substantial market expansion. Furthermore, the growing prominence of hybrid

Table 4. Clinical trial investigations of osteogenic and AMP peptides.

Type of peptide	Title	Registration code	Study start	Outcome
Abaloparatide	Early Effects of Abaloparatide on Tissue-Based Indices of Bone Formation and Resorption	NCT03710889		Improved bone mineral density
	Safety and Efficacy of Abaloparatide-SC in Men With Osteoporosis (ATOM)	NCT03512262		–
	Efficacy & Safety of Abaloparatide-Solid Microstructured Transdermal System in Postmenopausal Women With Osteoporosis	NCT04064411		–
RGD	The Use of Adhesion Molecule Loaded Hydrogel With Minimally Invasive Surgical Technique in Treatment of Periodontal Intrabony Defect	NCT05653245	2021-03-15 Phases I and II	–
	Efficacy of Xenograft Alone or Mixed With Arginyl-Glycyl-Aspartic Acid (RGD) in Horizontal Ridge Augmentation With Split-crest Technique for Implant Placement	NCT06585852	2023-08-01 Not Applicable	–
P-15	ABM/P-15 Bone Graft vs Traditional Bone Graft in Adult Spinal Deformity Surgery	NCT05038527	2021-10-01 Not Applicable	–
	The Clinical Effect of i-FACTOR® Versus Allograft in Non-instrumented Posterolateral Fusion The IVANOS-study (IVANOS)	NCT02895555	2012-03 Not Applicable	–
	An Assessment of P-15 Bone Putty in Anterior Cervical Fusion With Instrumentation	NCT00310440	2006-01 Not Applicable	There was no significant difference with autograft
PTH134	Combination Risedronate - Parathyroid Hormone Trial in Male Osteoporosis (RPM)	NCT01611571	2003-12 Phase 3	Combination teriparatide and risedronate increased BMD compared to monotherapy
	Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of Oral Doses of PTH134 in Post-menopausal Women	NCT01224717	2010-09 Phase 1	–
	Safety, Tolerability, Pharmacokinetic, and Pharmacodynamic Effect of PTH134 at Increasing Doses in Healthy Postmenopausal Subjects	NCT00676312	2008-05 Phase 1	–
NMImETIC NanoBone	Clinical Trial evaluation of alveolar bone repair using self-assembling peptide nanofiber	IRCT20210526051407N2	Phases I and II	Accelerated bone regenration
TP508 **(Chrysalin)**	A Study to Evaluate the Safety and Effectiveness of Different Doses of Chrysalin in Adults Who Have a Broken Wrist	NCT00131482	2004-11 Phase 2	–
LL-37	Efficacy of LL-37 Cream on Bacteria Colonization, Inflammation Response, and Healing Rate of Diabetic Foot Ulcers	NCT04098562	2019-10 Phase 2	–
	The Role of Anti-inflammatory Cytokines and Antimicrobial Peptide LL-37 Biomarkers in the Treatment of Periodontal Disease.	NCT04404335	2021-05-17	–
Reltecimod	Phase 3 Study of Reltecimod vs Placebo in Patients With Sepsis-associated Acute Kidney Injury	NCT03403751	2018-05-24 Phase 3	It was safe
	Phase III Efficacy and Safety Study of AB103 in the Treatment of Patients With Necrotizing Soft Tissue Infections (ACCUTE)	NCT02469857	2015-12-01 Phase 3	Early administration in severe NSTI resulted in a significant improvement in the primary composite endpoint in the per protocol population

Table 5. Osteogenic peptide drugs approved (2000 to present).

Trade name	Mimic	First approval	Mode of action	Sequence
(P-15) iFactor and Cerapedics[215]	Collagen	1999	Bone regeneration; implantation	GTPGPQGIAGQRGVV
(Teriparatide) Forteo/ Forsteo®[216]	PTH1 receptor	2002	Treatment of osteoporosis	SVSEIQLMHNLGKHLNSMER VEWLRKKLQDVHNF
(Abaloparatide) Tymlos®[216]		2017		AVSEHQLLHDKGKSIQDLRRR ELLEKLLXKLHTA
NMImETIC®Peptide Nanofibers	Self-assembling peptide nanofibers	2024	Bone regeneration; implantation	-
NMImETIC®NanoBone	Self-assembling peptide nanofibers	2024	Bone regeneration; implantation	-
Reltecimod[217]	CD 28 T-lymphocyte receptor	Under approval	Antibacterial	ASPMLVAYDA

peptides in organic and biomolecular applications is a key driver of this segment's accelerated growth trajectory.[218] A significant impediment to solid-phase peptide synthesis (conventional peptide synthesizing method) is the inherent toxicity and environmental impact of the solvents traditionally employed. Recent efforts to mitigate these concerns have involved the substitution of tetrahydrofuran (THF) for dimethylformamide (DMF).[219] However, this alternative is not universally applicable to all amino acids and may compromise peptide yield in certain instances.

The application of peptides to enhance the biological activity of biomaterials has become increasingly important in recent years. When compared to naturally sourced materials, synthetic materials that are functionalized with peptides offer greater reproducibility, manageable immunogenic responses, attractive complex structures, and more predictable biological functions.[220,221] The method used to prepare peptide-modified bone repair materials influences how the peptide attaches to the substrate and greatly impacts the composite's final osteogenic activity. Common preparation techniques include physical adsorption, covalent immobilization, encapsulation within a polymer matrix.[49,222]

Physical adsorption refers to the phenomenon wherein molecules or ions are attracted and attached to the surface of substrates in liquid or gaseous environments through mechanisms such as electrostatic forces, hydrophobic interactions, van der Waals forces, or hydrogen bonding.[223] The kinetics of release for biomolecules that are immobilized through physical adsorption is contingent upon their affinity for the material of the implant and is subject to modulation by various parameters, including temperature and pH levels. To improve the adsorption of biomolecules onto biomaterials, the surfaces can be pre-treated with charged molecules, such as amino acids (e.g. serine and asparagine) or acids (e.g. pyrophosphoric acid and mercaptosuccinic acid). Surfaces of biomaterials that possess a charge can selectively attract target molecules of interest (e.g. lysozyme and BMP-2), while molecules with opposite charges are subjected to repulsion.[224–227]

A more stable attachment of biomolecule to the scaffold surface can be achieved through chemical conjugation.[57,228,229] This method relies on the creation of covalent bonds between biomolecules and biomaterials via chemical reactions such as carbodiimide-mediated amidation, esterification, or click reactions.[6,230,231] Many materials used in regenerative bone therapies, like polyesters, are inert and require surface functionalization before biomolecule attachment. The goal of surface functionalization is to introduce or reveal reactive functional groups (such as amines, carboxyls, or hydroxyls) that can form covalent bonds with the functional components of biomolecules. This functionalization can be accomplished through techniques like plasma treatment, chemical etching, and oxidation. Biomolecules can then be grafted directly onto the functionalized surfaces or attached using linker molecules (spacers), such as silane or PEG molecules.[232,233]

Biomolecules utilized in bone regeneration often face challenges related to low stability and a short half-life *in vivo*. These problems are particularly common with protein biomolecules (such as BMPs, OPN, and OC), whose bioactivity relies on their three-dimensional structure, which can be easily compromised *in vivo* due to hydrolysis, proteolysis, and endocytosis.[234] A disrupted protein structure not only results in a loss of biological activity but also increases immunogenicity and implant rejection by the host.[235] One potential strategy to extend the biological efficacy of biomolecules *in vivo* is to immobilize them within controlled/sustained release delivery systems by physically encapsulating biomolecules within a matrix material, such as PEG hydrogel, gelatin, or collagen-HA matrix. In this method, the biomolecule is incorporated into the polymer solution before scaffold fabrication, which may be followed by covalent cross-linking of the biomolecules to the polymer matrix. The ease of this approach has led to its widespread application in bone tissue engineering for entrapping biomolecules like BMPs.[236–238]

Three-dimensional (3D) printing has emerged as a flexible technique that significantly enhances our ability to

create intricate scaffolds for tissue engineering With the help of computer-aided design (CAD) and computer-aided manufacturing (CAM). This method allows for the precise placement of biomaterials, biomolecules, and even living cells in designated areas, layer by layer, while customizing their structural, mechanical, physical, chemical, and biological properties.[239,240] Successful bone regeneration relies on effective osteogenesis and vascularization. Therefore, it is highly beneficial to equip scaffolds with both strong bone-forming capabilities and favorable angiogenic properties to enhance bone regeneration alongside necessary vascular development. Research has demonstrated that 3D-printed constructs are well-suited for bone regeneration because of their outstanding pore interconnectivity, which supports the revascularization crucial for osteogenesis.[241] Traditional methods like pore-forming agent leaching, gas foaming, and phase separation often lead to significant variations in pore geometry and size distribution, as well as generally low levels of interconnectivity.[242,243] The primary 3D printing methods employed to produce these composite scaffolds include extrusion-based printing (EBP), selective laser sintering (SLS), and stereolithography (SLA).[244–246] Drawing inspiration from the natural structure of bone tissue, researchers have explored composites consisting of inorganic components like nanosilicate particles, calcium phosphate, and bioactive glasses, paired with biopolymer matrices (Natural or Synthetic polymers) as foundational materials for biofabricating bone constructs. In addition to mimicking aspects of the bone's physiological architecture, these inorganic-organic composites can be tailored for specific cohesiveness, rheological characteristics, and mechanical properties, with both the inorganic and organic components enhancing the composite's bioactivity. Incorporating inorganic fillers into soft biopolymers has ultimately improved the bioactivity and printability of composite biomaterial inks, while also allowing for adjustments to their mechanical properties.[247]

Pros, cons, and outlook for peptides

Market size value of peptide in 2023 is $4345 billion and it is estimated to reach $66,41 bilion in 2030 with a growth rate of 6.2%.[218] In other words, peptides constitute 5% of the global pharmaceutical market, with 4% specifically dedicated to osteological applications.[100] A March 2017 dataset details 484 therapeutic peptides, categorized as follows: 68 approved, 155 currently in active clinical development, 261 discontinued prior to approval, and 8 withdrawn post-approval. The rate of peptide entry into clinical trials exhibited a gradual upward trajectory from 1980 to 2010, reaching a 5-year rolling average exceeding 22 peptides per annum in 2011 (Figure 4(a)).[248] The extant literature on osteogenic peptides published within the last 5 years is limited, with research predominantly concentrating on RGD peptides, CGRP, OGP, teriparatide, cell-penetrating peptides (CPPs), substance P, laminin-derived motifs, and P-15, respectively.

According to the Food and Drug Administration database, 87 alloplastic bone graft products have been approved in the United States since 1996. The Pharmaceuticals and Medical Devices Agency database indicates 10 alloplastic bone graft products approved in Japan since 2004. The Ministry of Health and Welfare database shows 36 alloplastic bone graft products approved in South Korea since 1980. Among the products manufactured in the USA, TRICOS contains a fibrin matrix; SynOss collagen synthetic material (SynOss Putty) contains collagen; Easygraft contains PLGA; Healos dental bone graft substitute contains bovine collagen; ReOss contains PLGA; Mastergraft contains collagen; NovaBone Dental contains polyethylene glycol; PerioGlas contains gelatin; and Osteocaf contains PLGA. The polymeric components of these products include collagen, gelatin, polyethylene glycol, and PLGA that are not strongly osteoinductve and multipotential when are not applied multi-peptide form. The incorporation of peptide, synthesized purely without animal-derived sources, represents a significant advancement in the alloplastic industry. Korean products, such as Ossbone Collagen, DualPor Collagen D-Injection, and Osteon II and III Collagen, contain porcine and bovine collagen, which retain the inherent challenges associated with animal tissue extraction, including the complexities of isolation procedures and the risks of disease transmission and infection.[249] Osteoinductivity capacity of mineralized allografts, xenografts, and synthetic ceramics through the incorporation of biomimetic motifs that recapitulate the angiogenic and osteogenic properties of naturally occurring biomolecules presents a promising strategy in bone tissue engineering.

While the manufacturing costs of synthetic peptide pharmaceuticals may not undercut those of their small-molecule analogs, the overall research and development expenditure for peptides is anticipated to be lower. This stems from their inherent synthetic tractability, facilitating rapid and comprehensive structure-activity relationship (SAR) studies for lead optimization, and a reduced propensity for off-target effects, potentially resulting in enhanced clinical trial success rates. However, it is crucial to acknowledge the unique biophysical and pharmacological properties of peptides; paradigms effective for small molecules or biologics may not be directly transferable to peptide drug development. Furthermore, unmet revenue projections contribute to the often-underestimated prioritization of peptide drug development. It is nonetheless imperative to recognize that production costs constitute a relatively minor fraction (approximately 3%–5%) of the total research and development, and distribution costs associated with a novel pharmaceutical agent. Technological advancements in recombinant peptide therapeutics production, encompassing unnatural amino acids and modifications, offer a potential mitigation strategy for

this limitation. Despite recognized obstacles, a burgeoning market for peptide drugs exists, prompting pharmaceutical developers to critically assess the therapeutic domains where peptides may offer superior efficacy compared to small-molecule drugs.

Peptides possess a significant advantage over small molecules due to their increased surface area and enhanced chiral and structural complexity. This characteristic allows for exploitation in targeting scenarios demanding multi-site interactions across extended distances for effective target activation. The distinction between agonism and antagonism is a critical consideration in peptide drug development. The comparatively delayed market entry of peptide antagonists is attributable to historically elevated production costs and the inherent difference in receptor occupancy requirements: agonists necessitate only modest receptor occupancy (5%–20%) for activation, whereas antagonists must compete with endogenous ligands, requiring occupancy exceeding 50% for efficacy. More fundamentally, allosteric receptor interactions can achieve antagonism, where the expansive surface area of peptides may not confer a substantial advantage over competing small-molecule drugs. An exception arises with antagonists targeting receptors requiring extensive surface area and structural complexity for subtype selectivity and the avoidance of off-target effects. The tertiary structure of peptides frequently exhibits evolutionarily conserved motifs, including α-helices, β-sheets, β-turns, and γ-turns, which are paramount for receptor recognition, potency, selectivity, and proteolytic stability. This intricate structural and chiral complexity is often absent in small-molecule drugs, a consequence, in part, of the now-contested paradigm shift away from natural product discovery toward combinatorial libraries in the 1980s. The predominant action of peptide therapeutics involves the modulation of peripheral extracellular targets, a consequence of their limited cell membrane permeability. The engagement of intracellular targets remains a significant challenge for peptide-based drugs, notwithstanding recent encouraging advancements in cell-penetrating and stapled peptide technologies. The inherent limitations of peptide-based biomaterials, namely suboptimal bioavailability and short half-life, are mitigated by in situ application at the bone defect site. While these pharmacokinetic parameters are not critically restrictive for osteogenic peptides integrated within scaffolds, various peptide modifications exist to enhance their stability and resist proteolytic degradation. These include, but are not limited to N-terminal acetylation or C-terminal amidation, Alkylation of nitrogen atoms, α-carbon modification, carbonyl thionation, glycosylation, PEGylation, and cyclization.[216]

The development of peptide drugs, while facing challenges like higher initial production costs and the need for higher receptor occupancy for antagonists compared to agonists, offers advantages over small molecule drugs due to their larger surface area and structural complexity, enabling interaction with multiple distant sites on target receptors. This complexity, often including conserved motifs crucial for receptor binding and stability, is frequently absent in small molecules. While most peptide drugs target extracellular sites due to limited cell membrane permeability, advancements in cell-penetrating peptides are addressing this limitation. In conclusion, despite manufacturing cost considerations, the unique biophysical properties of peptides make them a promising therapeutic modality, particularly for targets requiring high selectivity and complex interactions, and ongoing technological advancements are continually expanding their therapeutic potential.

Conclusions

A significant number of alloplastic bone graft products have been approved globally, utilizing materials like collagen and PLGA, but often lacking strong osteoinductive properties unless in multi-peptide form. The field is ripe for innovation, with the incorporation of synthetic peptides offering a solution to limitations of animal-derived proteins, including disease transmission risks. While peptide drug development faces challenges including high initial production costs and the unique biophysical properties requiring specialized development strategies, the lower overall research and development costs, coupled with the potential for enhanced efficacy due to their structural complexity and ability to interact with multiple target sites, make them a promising area of research, particularly in bone tissue engineering. The past few decades have witnessed a remarkable surge in the volume of research dedicated to the use of peptides in the realm of bone tissue engineering. This upward trend in peptide-based biomaterials and scaffolds is promising for bone regeneration. The application of bioactive synthetic peptides in bone regeneration brings with it a host of benefits. These peptides, with their heightened specificity, compact size, excellent safety profile, reduced immunogenicity, impressive stability, low risk of systemic toxicity, extended half-life, and lower cost, offer a compelling case. Furthermore, a variety of bioactive peptides can be amalgamated with scaffolds and cells to trigger crucial cellular processes for bone tissue formation, such as adhesion, proliferation, migration, differentiation, angiogenesis, biomineralization, and bacteriostatic/bactericidal. It is important to note that extensive research endeavors have been dedicated to uncovering, developing, and optimizing biological motifs derived from the active sites of specialized proteins to overcome their limitations. While many bioactive peptides are currently undergoing investigation, some have shown promising results both in vitro and in vivo, thereby justifying further preclinical investigation. As a result, more studies are needed before these peptides can be used clinically to repair and regenerate bone

defects. Furthermore, there is a need for more exploration, and bioactive peptides involved in neurogenesis and immune regulation during bone regeneration must be harnessed. The ability of these substances to provide precise control over immune response and neurogenesis in terms of space and time requires further investigation. This comprehensive review brings together the most relevant information on the advancements in bioactive peptides for bone tissue regeneration, shedding light on their biological effects and potential clinical implications.

Author contributions

Shima Tavakol: Conceptualization, Supervision, and Funding acquisition and writing—review and editing. Sareh Azadi, Mohammad Ali Yazdanpanah, Ali Afshari, Nilufar Allahdad, Solmaz Chegeni, and Abdolhamid Angaji: Writing—review and editing.

Data availability

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

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by Iran University of Medical Sciences, Tehran, Iran (grant number 1401-4-99-25267).

Informed consent

N/A.

Ethical approval

N/A.

Human rights

N/A.

ORCID iDs

Sareh Azadi https://orcid.org/0000-0002-9876-1419
Shima Tavakol https://orcid.org/0000-0002-8531-7650

Supplemental material

Supplemental material for this article is available online.

Reference

1. Li R, Zhou C, Chen J, et al. Synergistic osteogenic and angiogenic effects of KP and QK peptides incorporated with an injectable and self-healing hydrogel for efficient bone regeneration. *Bioact Mater* 2022; 18: 267–283.
2. Wu A-M, Bisignano C, James SL, et al. Global, regional, and national burden of bone fractures in 204 countries and territories, 1990–2019: a systematic analysis from the Global Burden of Disease Study 2019. *Lancet Healthy Longev* 2021; 2: e580–e592.
3. Xia D, Yang F, Zheng Y, et al. Research status of biodegradable metals designed for oral and maxillofacial applications: a review. *Bioact Mater* 2021; 6: 4186–4208.
4. Bullock G, Atkinson J, Gentile P, et al. Osteogenic peptides and attachment methods determine tissue regeneration in modified bone graft substitutes. *J Funct Biomater* 2021; 12: 22.
5. Visser R, Rico-Llanos GA, Pulkkinen H, et al. Peptides for bone tissue engineering. *J Control Release* 2016; 244: 122–135.
6. Maia FR, Bidarra SJ, Granja PL, et al. Functionalization of biomaterials with small osteoinductive moieties. *Acta Biomater* 2013; 9: 8773–8789.
7. Pountos I, Panteli M, Lampropoulos A, et al. The role of peptides in bone healing and regeneration: a systematic review. *BMC Med* 2016; 14: 103.
8. Lin Z, Rios HF and Cochran DL. Emerging regenerative approaches for periodontal reconstruction: a systematic review from the AAP Regeneration Workshop. *J Periodontol* 2015; 86: S134–S152.
9. Wu M, Chen G and Li Y-P. TGF-β and BMP signaling in osteoblast, skeletal development, and bone formation, homeostasis and disease. *Bone Res* 2016; 4: 1–21.
10. Majidinia M, Sadeghpour A and Yousefi B. The roles of signaling pathways in bone repair and regeneration. *J Cell Physiol* 2018; 233: 2937–2948.
11. Chen G, Deng C and Li Y-P. TGF-β and BMP signaling in osteoblast differentiation and bone formation. *Int J Biolog Sci* 2012; 8: 272.
12. Tavakol S. The twofold role of osteogenic small molecules in Parkinson's disease therapeutics: crosstalk of osteogenesis and neurogenesis. *BioMed Res Int* 2022; 2022: 3813541.
13. Ma C, Gao J, Liang J, et al. HDAC6 inactivates Runx2 promoter to block osteogenesis of bone marrow stromal cells in age-related bone loss of mice. *Stem Cell Res Ther* 2021; 12: 1–14.
14. Manzotti G, Torricelli F, Donati B, et al. HDACs control RUNX2 expression in cancer cells through redundant and cell context-dependent mechanisms. *J Exp Clin Cancer Res* 2019; 38: 1–14.
15. Huang B, Wang W, Li Q, et al. Osteoblasts secrete Cxcl9 to regulate angiogenesis in bone. *Nat Commun* 2016; 7: 13885.
16. Tomlinson RE and Silva MJ. Skeletal blood flow in bone repair and maintenance. *Bone Res* 2013; 1: 311–322.
17. Niedzwiedzki T and 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: R23–R36.
18. Filipowska J, Tomaszewski KA, Niedźwiedzki Ł, et al. The role of vasculature in bone development, regeneration and proper systemic functioning. *Angiogenesis* 2017; 20: 291–302.
19. Percival CJ and Richtsmeier JT. Angiogenesis and intramembranous osteogenesis. *Dev Dyna* 2013; 242: 909–922.
20. Yang Y-Q, Tan Y-Y, Wong R, et al. The role of vascular endothelial growth factor in ossification. *Int J Oral Sci* 2012; 4: 64–68.

21. Fu J and Wang D-A. In situ organ-specific vascularization in tissue engineering. *Trends Biotechnol* 2018; 36: 834–849.
22. Lamalice L, Le Boeuf F and Huot J. Endothelial cell migration during angiogenesis. *Circ Res* 2007; 100: 782-794.
23. Gonçalves RC, Banfi A, Oliveira MB, et al. Strategies for re-vascularization and promotion of angiogenesis in trauma and disease. *Biomaterials* 2021; 269: 120628.
24. Senger DR and Davis GE. Angiogenesis. *CSH Perspect Biol* 2011; 3: a005090.
25. Di Maggio N and Banfi A. The osteo-angiogenic signaling crosstalk for bone regeneration: harmony out of complexity. *Curr Opin Biotechnol* 2022; 76: 102750.
26. Kim JJ, Hou L and Huang NF. Vascularization of three-dimensional engineered tissues for regenerative medicine applications. *Acta Biomater* 2016; 41: 17–26.
27. Kusumbe AP, Ramasamy SK and Adams RH. Coupling of angiogenesis and osteogenesis by a specific vessel subtype in bone. *Nature* 2014; 507: 323–328.
28. Langen UH, Pitulescu ME, Kim JM, et al. Cell–matrix signals specify bone endothelial cells during developmental osteogenesis. *Nat Cell Biol* 2017; 19: 189–201.
29. Watanabe H, Maishi N, Hoshi-Numahata M, et al. Skeletal-vascular interactions in bone development, homeostasis, and pathological destruction. *Int J Mol Sci* 2023; 24: 10912.
30. Van Hove AH and Benoit DS. Depot-based delivery systems for pro-angiogenic peptides: a review. *Front Bioeng Biotechnol* 2015; 3: 102.
31. Wang J, Peng H, Timur AA, et al. Receptor and molecular mechanism of AGGF1 signaling in endothelial cell functions and angiogenesis. *Arterioscler Thromb Vasc Biol* 2021; 41: 2756–2769.
32. Amin HD, Olsen I, Knowles J, et al. A tyrosine-rich amelogenin peptide promotes neovasculogenesis in vitro and ex vivo. *Acta Biomater* 2014; 10: 1930–1939.
33. Zhu Z, Guo L, Yeltai N, et al. Chemokine (C-C motif) ligand 2-enhanced adipogenesis and angiogenesis of human adipose-derived stem cell and human umbilical vein endothelial cell co-culture system in adipose tissue engineering. *J Tissue Eng Regen Med* 2022; 16: 163–176.
34. Santulli G, Ciccarelli M, Palumbo G, et al. In vivo properties of the proangiogenic peptide QK. *J Transl Med* 2009; 7: 1–10.
35. Sheets AR, Massey CJ, Cronk SM, et al. Matrix- and plasma-derived peptides promote tissue-specific injury responses and wound healing in diabetic swine. *J Transl Med* 2016; 14: 197.
36. Licht T, Tsirulnikov L, Reuveni H, et al. Induction of pro-angiogenic signaling by a synthetic peptide derived from the second intracellular loop of S1P3 (EDG3). *Blood* 2003; 102: 2099–2107.
37. Liu X, Wang X, Horii A, et al. In vivo studies on angiogenic activity of two designer self-assembling peptide scaffold hydrogels in the chicken embryo chorioallantoic membrane. *Nanoscale* 2012; 4: 2720–2727.
38. Liu JM, Lawrence F, Kovacevic M, et al. The tetrapeptide AcSDKP, an inhibitor of primitive hematopoietic cell proliferation, induces angiogenesis in vitro and in vivo. *Blood* 2003; 101: 3014–3020.
39. Shu Y, Hao T, Yao F, et al. RoY peptide-modified chitosan-based hydrogel to improve angiogenesis and cardiac repair under hypoxia. *ACS Appl Mater Interfaces* 2015; 7: 6505–6517.
40. Zoughaib M, Luong D, Garifullin R, et al. Enhanced angiogenic effects of RGD, GHK peptides and copper (II) compositions in synthetic cryogel ECM model. *Mater Sci Eng C Mater Biol Appl* 2021; 120: 111660.
41. Seo E, Lim JS, Jun JB, et al. Exendin-4 in combination with adipose-derived stem cells promotes angiogenesis and improves diabetic wound healing. *J Transl Med* 2017; 15: 35.
42. Tanaka S, Yasuda T, Hamada Y, et al. Synthetic peptide SVVYGLR upregulates cell motility and facilitates oral mucosal wound healing. *Peptides* 2020; 134: 170405.
43. Tavakol S, Saber R, Hoveizi E, et al. Chimeric self-assembling nanofiber containing bone marrow homing peptide's motif induces motor neuron recovery in animal model of chronic spinal cord injury; an in vitro and in vivo investigation. *Mol Neurobiol* 2016; 53: 3298–3308.
44. Tavakol S, Mousavi SMM, Tavakol B, et al. Mechanotransduction signals derived from self-assembling peptide nanofibers containing long motif of laminin influence neurogenesis in in-vitro and in-vivo. *Mol Neurobiol* 2017; 54: 2483–2496.
45. Tavakol S, Musavi SMM, Tavakol B, et al. Noggin along with a self-assembling peptide nanofiber containing long motif of laminin induces tyrosine hydroxylase gene expression. *Mol Neurobiol* 2017; 54: 4609–4616.
46. Carvalho MS, Cabral JM, da Silva CL, et al. Synergistic effect of extracellularly supplemented osteopontin and osteocalcin on stem cell proliferation, osteogenic differentiation, and angiogenic properties. *J Cell Biochem* 2019; 120: 6555–6569.
47. Devescovi V, Leonardi E, Ciapetti G, et al. Growth factors in bone repair. *La Chir Degli Organi Movimento* 2008; 92: 161–168.
48. Benoit DS and Anseth KS. The effect on osteoblast function of colocalized RGD and PHSRN epitopes on PEG surfaces. *Biomaterials* 2005; 26: 5209–5220.
49. Wang C, Liu Y, Fan Y, et al. The use of bioactive peptides to modify materials for bone tissue repair. *Regen Biomater* 2017; 4: 191–206.
50. Thorwarth M, Schultze-Mosgau S, Wehrhan F, et al. Enhanced bone regeneration with a synthetic cell-binding peptide—in vivo results. *Biochem Biophys Res Commun* 2005; 329: 789–795.
51. Staatz W, Fok K, Zutter M, et al. Identification of a tetrapeptide recognition sequence for the alpha 2 beta 1 integrin in collagen. *J Biol Chem* 1991; 266: 7363–7367.
52. Hennessy KM, Pollot BE, Clem WC, et al. The effect of collagen I mimetic peptides on mesenchymal stem cell adhesion and differentiation, and on bone formation at hydroxyapatite surfaces. *Biomaterials* 2009; 30: 1898–1909.
53. Culpepper BK, Phipps MC, Bonvallet PP, et al. Enhancement of peptide coupling to hydroxyapatite and implant osseointegration through collagen mimetic peptide modified with a polyglutamate domain. *Biomaterials* 2010; 31: 9586-9594.
54. Reyes CD and García AJ. α2β1 integrin-specific collagen-mimetic surfaces supporting osteoblastic differentiation. *J Biomed Mater Res A* 2004; 69: 591–600.

55. Egusa H, Kaneda Y, Akashi Y, et al. Enhanced bone regeneration via multimodal actions of synthetic peptide SVVYGLR on osteoprogenitors and osteoclasts. *Biomaterials* 2009; 30: 4676–4686.
56. Yang M, Zhang Z-C, Liu Y, et al. Function and mechanism of RGD in bone and cartilage tissue engineering. *Front Bioeng Biotechnol* 2021; 9: 1184.
57. Heller M, Kumar V, Pabst A, et al. Osseous response on linear and cyclic RGD-peptides immobilized on titanium surfaces in vitro and in vivo. *J Biomed Mater Res A* 2018; 106: 419–427.
58. Scholz M, Schleicher P, Sewing A, et al. Cyclic-RGD is as effective as rhBMP-2 in anterior interbody fusion of the sheep cervical spine. *Spine* 2013; 38: E59–E65.
59. Kim HJ, You SJ, Yang DH, et al. Injectable hydrogels based on MPEG–PCL–RGD and BMSCs for bone tissue engineering. *Biomater Sci* 2020; 8: 4334–4345.
60. Nam S, Stowers R, Lou J, et al. Varying PEG density to control stress relaxation in alginate-PEG hydrogels for 3D cell culture studies. *Biomaterials* 2019; 200: 15-24.
61. Wang V, Misra G and Amsden B. Immobilization of a bone and cartilage stimulating peptide to a synthetic bone graft. *J Mater Sci Mater Med* 2008; 19: 2145–2155.
62. Yang XB, Bhatnagar RS, Li S, et al. Biomimetic collagen scaffolds for human bone cell growth and differentiation. *Tissue Eng* 2004; 10: 1148–1159.
63. Mohanram Y, Zhang J, Tsiridis E, et al. Comparing bone tissue engineering efficacy of HDPSCs, HBMSCs on 3D biomimetic ABM-P-15 scaffolds in vitro and in vivo. *Cytotechnology* 2020; 72: 715–730.
64. Liu Q, Limthongkul W, Sidhu G, et al. Covalent attachment of P15 peptide to titanium surfaces enhances cell attachment, spreading, and osteogenic gene expression. *J Orthop Res* 2012; 30: 1626–1633.
65. Cheng BC, Swink IR, Cheng CT, et al. Comparing the immune response to PEEK as an implant material with and without P-15 Peptide as Bone Graft Material in a Rabbit Long Bone Model. *Bioengineering* 2024; 11: 898.
66. Agrawal V, Tottey S, Johnson SA, et al. Recruitment of progenitor cells by an extracellular matrix cryptic peptide in a mouse model of digit amputation. *Tissue Eng Part A* 2011; 17: 2435–2443.
67. Agrawal V, Kelly J, Tottey S, et al. An isolated cryptic peptide influences osteogenesis and bone remodeling in an adult mammalian model of digit amputation. *Tissue Eng Part A* 2011; 17: 3033–3044.
68. Huang H, Zhao Y, Liu Z, et al. Enhanced osteoblast functions on RGD immobilized surface. *J Oral Implantol* 2003; 29: 73–79.
69. Rammelt S, Illert T, Bierbaum S, et al. Coating of titanium implants with collagen, RGD peptide and chondroitin sulfate. *Biomaterials* 2006; 27: 5561–5571.
70. Bhatnagar RS, Qian JJ, Wedrychowska A, et al. Design of biomimetic habitats for tissue engineering with P-15, a synthetic peptide analogue of collagen. *Tissue Eng* 1999; 5: 53–65.
71. Reyes CD, Petrie TA, Burns KL, et al. Biomolecular surface coating to enhance orthopaedic tissue healing and integration. *Biomaterials* 2007; 28: 3228–3235.
72. Vukicevic S, Luyten FP, Kleinman HK, et al. Differentiation of canalicular cell processes in bone cells by basement membrane matrix components: regulation by discrete domains of laminin. *Cell* 1990; 63: 437–445.
73. Min S-K, Kang HK, Jang DH, et al. Titanium surface coating with a laminin-derived functional peptide promotes bone cell adhesion. *BioMed Res Int* 2013; 2013: 638348.
74. Nakaoka R, Hirano Y, Mooney DJ, et al. Study on the potential of RGD-and PHSRN-modified alginates as artificial extracellular matrices for engineering bone. *Journal of Artificial Organs* 2013; 16: 284-293.
75. Dee KC, Andersen TT and Bizios R. Design and function of novel osteoblast-adhesive peptides for chemical modification of biomaterials. *J Biomed Mater Res* 1998; 40: 371–377.
76. Nelson M, Balasundaram G and Webster TJ. Increased osteoblast adhesion on nanoparticulate crystalline hydroxyapatite functionalized with KRSR. *Int J Nanomed* 2006; 1: 339.
77. Rezania A and Healy KE. Biomimetic peptide surfaces that regulate adhesion, spreading, cytoskeletal organization, and mineralization of the matrix deposited by osteoblast-like cells. *Biotechnol Prog* 1999; 15: 19–32.
78. Hayashibara T, Hiraga T, Yi B, et al. A synthetic peptide fragment of human MEPE stimulates new bone formation in vitro and in vivo. *J Bone Miner Res* 2004; 19: 455–462.
79. He X, Yang X and Jabbari E. Combined effect of osteopontin and BMP-2 derived peptides grafted to an adhesive hydrogel on osteogenic and vasculogenic differentiation of marrow stromal cells. *Langmuir* 2012; 28: 5387–5397.
80. Seol YJ, Park YJ, Lee SC, et al. Enhanced osteogenic promotion around dental implants with synthetic binding motif mimicking bone morphogenetic protein (BMP)-2. *J Biomed Mater Res A* 2006; 77: 599-607.
81. Vhora I, Lalani R, Bhatt P, et al. Lipid-nucleic acid nanoparticles of novel ionizable lipids for systemic BMP-9 gene delivery to bone-marrow mesenchymal stem cells for osteoinduction. *Int J Pharm* 2019; 563: 324–336.
82. Zouani OF, Chollet C, Guillotin B, et al. Differentiation of pre-osteoblast cells on poly (ethylene terephthalate) grafted with RGD and/or BMPs mimetic peptides. *Biomaterials* 2010; 31: 8245–8253.
83. Shin MK, Kim M-K, Bae Y-S, et al. A novel collagen-binding peptide promotes osteogenic differentiation via Ca^{2+}/calmodulin-dependent protein kinase II/ERK/AP-1 signaling pathway in human bone marrow-derived mesenchymal stem cells. *Cell Signal* 2008; 20: 613–624.
84. Shin H, Zygourakis K, Farach-Carson MC, et al. Attachment, proliferation, and migration of marrow stromal osteoblasts cultured on biomimetic hydrogels modified with an osteopontin-derived peptide. *Biomaterials* 2004; 25: 895–906.
85. Choi YJ, Lee JY, Chung CP, et al. Enhanced osteogenesis by collagen-binding peptide from bone sialoprotein in vitro and in vivo. *J Biomed Mater Res A* 2013; 101: 547-554.
86. Lee JY, Choo JE, Choi YS, et al. Effect of immobilized cell-binding peptides on chitosan membranes for osteoblastic differentiation of mesenchymal stem cells. *Biotechnol Appl Biochem* 2009; 52: 69–77.

87. Lee JY, Choo JE, Choi YS, et al. Characterization of the surface immobilized synthetic heparin binding domain derived from human fibroblast growth factor-2 and its effect on osteoblast differentiation. *J Biomed Mater Res A* 2007; 83: 970–979.
88. 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: 701–708.
89. Jung RE, Cochran DL, Domken O, et al. The effect of matrix bound parathyroid hormone on bone regeneration. *Clin Oral Implants Res* 2007; 18: 319–325.
90. Arrighi I, Mark S, Alvisi M, et al. Bone healing induced by local delivery of an engineered parathyroid hormone prodrug. *Biomaterials* 2009; 30: 1763–1771.
91. Li W, Yu B, Li M, et al. NEMO-binding domain peptide promotes osteoblast differentiation impaired by tumor necrosis factor alpha. *Biochem Biophys Res Commun* 2010; 391: 1228–1233.
92. Suh JS, Lee JY, Choi YJ, et al. Intracellular delivery of cell-penetrating peptide-transcriptional factor fusion protein and its role in selective osteogenesis. *Int J Nanomed* 2014; 9: 1153.
93. Sheller MR, Crowther RS, Kinney JH, et al. Repair of rabbit segmental defects with the thrombin peptide, TP508. *J Orthop Res* 2004; 22: 1094–1099.
94. 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.
95. Tsai T-L, Wang B, Squire MW, et al. Endothelial cells direct human mesenchymal stem cells for osteo-and chondro-lineage differentiation through endothelin-1 and AKT signaling. *Stem Cell Res Ther* 2015; 6: 1–14.
96. Shuqiang M, Kunzheng W, Xiaoqiang D, et al. Osteogenic growth peptide incorporated into PLGA scaffolds accelerates healing of segmental long bone defects in rabbits. *J Plast Reconstr Aesthet Surg* 2008; 61: 1558–1560.
97. Gabet Y, Müller R, Regev E, et al. Osteogenic growth peptide modulates fracture callus structural and mechanical properties. *Bone* 2004; 35: 65–73.
98. Cao F-Y, Yin W-N, Fan J-X, et al. A novel function of BMHP1 and cBMHP1 peptides to induce the osteogenic differentiation of mesenchymal stem cells. *Biomater Sci* 2015; 3: 345–351.
99. Tavakol S, Rasoulian B, Ramezani F, et al. Core and biological motif of self-assembling peptide nanofiber induce a stronger electrostatic interaction than BMP2 with BMP2 receptor 1A. *Mater Sci Eng C Mater Biol Appl* 2019; 101: 148–158.
100. Muttenthaler M, King GF, Adams DJ, et al. Trends in peptide drug discovery. *Nat Rev Drug Discov* 2021; 20: 309–325.
101. Graf J, Iwamoto Y, Sasaki M, et al. Identification of an amino acid sequence in laminin mediating cell attachment, chemotaxis, and receptor binding. *Cell* 1987; 48: 989–996.
102. Frith JE, Mills RJ, Hudson JE, et al. Tailored integrin–extracellular matrix interactions to direct human mesenchymal stem cell differentiation. *Stem Cells Dev* 2012; 21: 2442–2456.
103. Jha A and Moore E. Laminin-derived peptide, IKVAV, modulates macrophage phenotype through integrin mediation. *Matrix Biol Plus* 2024; 22: 100143.
104. Chen C, Jiang Z and Yang GJD. Laminins in osteogenic differentiation and pluripotency maintenance. *Differentiation* 2020; 114: 13–19.
105. Mas-Moruno C, Fraioli R, Albericio F, et al. Novel peptide-based platform for the dual presentation of biologically active peptide motifs on biomaterials. *ACS Appl Mater Interfaces* 2014; 6: 6525–6536.
106. Van der Velde-Zimmermann D, Verdaasdonk M, Rademakers L, et al. Fibronectin distribution in human bone marrow stroma: matrix assembly and tumor cell adhesion via α5β1 integrin. *Exp Cell Res* 1997; 230: 111–120.
107. Moursi AM, Globus RK and Damsky CH. Interactions between integrin receptors and fibronectin are required for calvarial osteoblast differentiation in vitro. *J Cell Sci* 1997; 110: 2187–2196.
108. García A and Reyes C. Bio-adhesive surfaces to promote osteoblast differentiation and bone formation. *J Dental Res* 2005; 84: 407–413.
109. Six N, Septier D, Chaussain-Miller C, et al. Dentonin, a MEPE fragment, initiates pulp-healing response to injury. *J Dental Res* 2007; 86: 780–785.
110. Zelenchuk LV, Hedge A-M and Rowe PS. Age dependent regulation of bone-mass and renal function by the MEPE ASARM-motif. *Bone* 2015; 79: 131–142.
111. Gentile P, Ghione C, Tonda-Turo C, et al. Peptide functionalisation of nanocomposite polymer for bone tissue engineering using plasma surface polymerisation. *RSC Adv* 2015; 5: 80039–80047.
112. Sun S, Yu W, Zhang Y, et al. Increased preosteoblast adhesion and osteogenic gene expression on TiO 2 nanotubes modified with KRSR. *J Mater Sci Mater Med* 2013; 24: 1079–1091.
113. Chen Q, Zhang D, Zhang W, et al. Dual mechanism β-amino acid polymers promoting cell adhesion. *Nat Commun* 2021; 12: 562.
114. Gentile P, Ferreira AM, Callaghan JT, et al. Multilayer nanoscale encapsulation of biofunctional peptides to enhance bone tissue regeneration in vivo. *Adv Healthc Mater* 2017; 6: 1601182.
115. Tamai N, Myoui A, Hirao M, et al. A new biotechnology for articular cartilage repair: subchondral implantation of a composite of interconnected porous hydroxyapatite, synthetic polymer (PLA-PEG), and bone morphogenetic protein-2 (rhBMP-2). *Osteoarthritis Cartilage* 2005; 13: 405–417.
116. Kargozar S, Hashemian SJ, Soleimani M, et al. Acceleration of bone regeneration in bioactive glass/gelatin composite scaffolds seeded with bone marrow-derived mesenchymal stem cells over-expressing bone morphogenetic protein-7. *Mater Sci Eng C* 2017; 75: 688-698.
117. Cheng H, Jiang W, Phillips FM, et al. Osteogenic activity of the fourteen types of human bone morphogenetic proteins (BMPs). *JBJS* 2003; 85: 1544–1552.
118. Wang Y, Hong S, Li M, et al. Noggin resistance contributes to the potent osteogenic capability of BMP9 in mesenchymal stem cells. *J Orthop Res* 2013; 31: 1796–1803.

119. Li X, Zhang R, Li B, et al. Biocompatible nHA/Col-BMP-9/GM scaffold: synthesis, characterization, and effects on bone marrow mesenchymal stem cells. *J Hard Tissue Biol* 2019; 28: 175–184.
120. Lee JY, Choo JE, Park HJ, et al. Synthetic peptide-coated bone mineral for enhanced osteoblastic activation in vitro and in vivo. *J Biomed Mater Res A* 2008; 87: 688-697.
121. Park JB, Lee JY, Park HN, et al. Osteopromotion with synthetic oligopeptide–coated bovine bone mineral in vivo. *J Periodontol* 2007; 78: 157–163.
122. Yokosaki Y, Matsuura N, Sasaki T, et al. The integrin α9β1 binds to a novel recognition sequence (SVVYGLR) in the thrombin-cleaved amino-terminal fragment of osteopontin. *J Biol Chem* 1999; 274: 36328–36334.
123. Lee J-Y, Choo J-E, Park H-J, et al. Injectable gel with synthetic collagen-binding peptide for enhanced osteogenesis in vitro and in vivo. *Biochem Biophys Res Commun* 2007; 357: 68–74.
124. Park YS, Lee J-Y, Suh JS, et al. Selective osteogenesis by a synthetic mineral inducing peptide for the treatment of osteoporosis. *Biomaterials* 2014; 35: 9747–9754.
125. Amara SG, Jonas V, Rosenfeld MG, et al. Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products. *Nature* 1982; 298: 240–244.
126. Kruger L, Silverman JD, Mantyh PW, et al. Peripheral patterns of calcitonin-gene-related peptide general somatic sensory innervation: cutaneous and deep terminations. *J Comparat Neurol* 1989; 280: 291–302.
127. Villa I, Melzi R, Pagani F, et al. Effects of calcitonin gene-related peptide and amylin on human osteoblast-like cells proliferation. *Eur J Pharmacol* 2000; 409: 273–278.
128. Xu G and Jiang D. The role and mechanism of exogenous calcitonin gene-related peptide on mesenchymal stem cell proliferation and osteogenetic formation. *Cell Biochem Biophy* 2014; 69: 369–378.
129. Zheng S, Li W, Xu M, et al. Calcitonin gene-related peptide promotes angiogenesis via AMP-activated protein kinase. *Am J Physiol Cell Physiol* 2010; 299: C1485–C1492.
130. Shi R, Huang Y, Ma C, et al. Current advances for bone regeneration based on tissue engineering strategies. *Front Med* 2019; 13: 160–188.
131. Mognetti B, Marino S, Barberis A, et al. Experimental stimulation of bone healing with teriparatide: histomorphometric and microhardness analysis in a mouse model of closed fracture. *Calcif Tissue Int* 2011; 89: 163–171.
132. Zhang S, Ding L, Chen G, et al. Enhanced bone regeneration via local low-dose delivery of PTH1-34 in a composite hydrogel. *Front Bioeng Biotechnol* 2023; 11: 1209752.
133. Chandra A, Lin T, Tribble MB, et al. PTH1–34 alleviates radiotherapy-induced local bone loss by improving osteoblast and osteocyte survival. *Bone* 2014; 67: 33–40.
134. Strnad J, McDonnell PA, Riexinger DJ, et al. NEMO binding domain of IKK-2 encompasses amino acids 735–745. *J Mol Recognit* 2006; 19: 227–233.
135. Jimi E, Aoki K, Saito H, et al. Selective inhibition of NF-κB blocks osteoclastogenesis and prevents inflammatory bone destruction in vivo. *Nat Med* 2004; 10: 617–624.
136. Kornegay JN, Peterson JM, Bogan DJ, et al. NBD delivery improves the disease phenotype of the golden retriever model of Duchenne muscular dystrophy. *Skeletal Muscle* 2014; 4: 1–17.
137. Ghosh A, Roy A, Liu X, et al. Selective inhibition of NF-κB activation prevents dopaminergic neuronal loss in a mouse model of Parkinson's disease. *Proc Natl Acad Sci* 2007; 104: 18754–18759.
138. Suh JS, Lee JY, Choi YS, et al. Efficient labeling of mesenchymal stem cells using cell permeable magnetic nanoparticles. *Biochem Biophys Res Commun* 2009; 379: 669–675.
139. Park YJ, Chang LC, Liang JF, et al. Nontoxic membrane translocation peptide from protamine, low molecular weight protamine (LMWP), for enhanced intracellular protein delivery: in vitro and in vivo study. *FASEB* 2005; 19: 1555–1557.
140. Jo J, Hong S, Choi WY, et al. Cell-penetrating peptide (CPP)-conjugated proteins is an efficient tool for manipulation of human mesenchymal stromal cells. *Sci Rep* 2014; 4: 4378.
141. Li G, Cui Y, McILmurray L, et al. rhBMP-2, rhVEGF165, rhPTN and thrombin-related peptide, TP508 induce chemotaxis of human osteoblasts and microvascular endothelial cells. *J Orthop Res* 2005; 23: 680–685.
142. Vordemvenne T, Paletta JR, Hartensuer R, et al. Cooperative effects in differentiation and proliferation between PDGF-BB and matrix derived synthetic peptides in human osteoblasts. *BMC Musculoskeletal Disord* 2011; 12: 1–7.
143. Hedberg EL, Kroese-Deutman HC, Shih CK, et al. Effect of varied release kinetics of the osteogenic thrombin peptide TP508 from biodegradable, polymeric scaffolds on bone formation in vivo. *J Biomed Mater Res A* 2005; 72: 343–353.
144. Li K, Liu L, Zhang J, et al. TP508 promotes bone regeneration on distraction osteogenesis via the activation of Wnt/β-catenin signaling pathway. *Curr Pharm Biotechnol*. Epub ahead of print 11 March 2024. DOI: 10.2174/0113892010289575240306033011.
145. Imai S and 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: 61–69.
146. Grässel S. The role of peripheral nerve fibers and their neurotransmitters in cartilage and bone physiology and pathophysiology. *Arthritis Res Therapy* 2014; 16: 485.
147. Goto T, Nakao K, Gunjigake K, et al. Substance P stimulates late-stage rat osteoblastic bone formation through neurokinin-1 receptors. *Neuropeptides* 2007; 41: 25–31.
148. 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: 309–320.
149. Yanagisawa M, Kurihara H, Kimura S, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988; 332: 411–415.
150. Wilson JL, Taylor L and Polgar P. Endothelin-1 activation of ETB receptors leads to a reduced cellular proliferative rate and an increased cellular footprint. *Exp Cell Res* 2012; 318: 1125–1133.

151. Kitano Y, Kurihara H, Kurihara Y, et al. Gene expression of bone matrix proteins and endothelin receptors in endothelin-1–deficient mice revealed by in situ hybridization. *J Bone Miner Res* 1998; 13: 237–244.
152. Clines GA, Mohammad KS, Grunda JM, et al. Regulation of postnatal trabecular bone formation by the osteoblast endothelin A receptor. *J Bone Miner Res* 2011; 26: 2523–2536.
153. Bab I, Gazit D, Chorev M, et al. Histone H4-related osteogenic growth peptide (OGP): a novel circulating stimulator of osteoblastic activity. *EMBO J* 1992; 11: 1867–1873.
154. Zuo Y, Xiong Q, Li Q, et al. Osteogenic growth peptide (OGP)-loaded amphiphilic peptide (NapFFY) supramolecular hydrogel promotes osteogenesis and bone tissue reconstruction. *Int J Biol Macromol* 2022; 195: 558–564.
155. Rasouliana B, Sheikholislam Z, HoushdarTehrani MH, et al. Unveiling the superior function of RADA in bone regeneration compared to KSL as two critical cores within self-assembling peptide nanofibers: Insights from in vitro and in vivo studies. *Regen Ther* 2024; 26: 999–1009.
156. Hao Z, Chen R, Chai C, et al. Antimicrobial peptides for bone tissue engineering: diversity, effects and applications. *Front Bioeng Biotechnol* 2022; 10: 1030162.
157. Birt MC, Anderson DW, Toby EB, et al. Osteomyelitis: recent advances in pathophysiology and therapeutic strategies. *J Orthop* 2017; 14: 45–52.
158. Browne K, Chakraborty S, Chen R, et al. A new era of antibiotics: the clinical potential of antimicrobial peptides. *Int J Mol Sci* 2020; 21: 7047.
159. Huan Y, Kong Q, Mou H, et al. Antimicrobial peptides: classification, design, application and research progress in multiple fields. *Front Microbiol* 2020; 11: 2559.
160. Kumar P, Kizhakkedathu JN and Straus SK. Antimicrobial peptides: diversity, mechanism of action and strategies to improve the activity and biocompatibility in vivo. *Biomolecules* 2018; 8: 4.
161. Zhang Q-Y, Yan Z-B, Meng Y-M, et al. Antimicrobial peptides: mechanism of action, activity and clinical potential. *Mil Med Res* 2021; 8: 1–25.
162. Lima PG, Oliveira JT, Amaral JL, et al. Synthetic antimicrobial peptides: characteristics, design, and potential as alternative molecules to overcome microbial resistance. *Life Sci* 2021; 278: 119647.
163. Rima M, Rima M, Fajloun Z, et al. Antimicrobial peptides: a potent alternative to antibiotics. *Antibiotics* 2021; 10: 1095.
164. Zou P, Chen W-T, Sun T, et al. Recent advances: peptides and self-assembled peptide-nanosystems for antimicrobial therapy and diagnosis. *Biomater Sci* 2020; 8: 4975–4996.
165. Zhang L-J and Gallo RL. Antimicrobial peptides. *Curr Biol* 2016; 26: R14–R19.
166. Bin Hafeez A, Jiang X, Bergen PJ, et al. Antimicrobial peptides: An update on classifications and databases. *Int J Mol Sci* 2021; 22: 11691.
167. Thapa RK, Diep DB and Tønnesen HH. Topical antimicrobial peptide formulations for wound healing: current developments and future prospects. *Acta Biomater* 2020; 103: 52–67.
168. Gudmundsson GH, Agerberth B, Odeberg J, et al. The human gene FALL39 and processing of the cathelin precursor to the antibacterial peptide LL-37 in granulocytes. *Eur J Biochem* 1996; 238: 325–332.
169. He Y, Yang X, Yuan Z, et al. Regulation of MSC and macrophage functions in bone healing by peptide LL-37-loaded silk fibroin nanoparticles on a titanium surface. *Biomater Sci* 2019; 7: 5492–5505.
170. He Y, Mu C, Shen X, et al. Peptide LL-37 coating on micro-structured titanium implants to facilitate bone formation in vivo via mesenchymal stem cell recruitment. *Acta Biomater* 2018; 80: 412–424.
171. Abdolhosseini M, Sotsky JB, Shelar AP, et al. Human parotid secretory protein is a lipopolysaccharide-binding protein: identification of an anti-inflammatory peptide domain. *Mol Cell Biochem* 2012; 359: 1–8.
172. Fischer NG, Chen X, Astleford-Hopper K, et al. Antimicrobial and enzyme-responsive multi-peptide surfaces for bone-anchored devices. *Mater Sci Eng C* 2021; 125: 112108.
173. Chen J, Shi X, Zhu Y, et al. On-demand storage and release of antimicrobial peptides using Pandora's box-like nanotubes gated with a bacterial infection-responsive polymer. *Theranostics* 2020; 10: 109.
174. Abou Alaiwa MH, Reznikov LR, Gansemer ND, et al. pH modulates the activity and synergism of the airway surface liquid antimicrobials β-defensin-3 and LL-37. *Proc Natl Acad Sci* 2014; 111: 18703–18708.
175. Hirsch T, Spielmann M, Zuhaili B, et al. Human beta-defensin-3 promotes wound healing in infected diabetic wounds. *J Gene Med* 2009; 11: 220–228.
176. Liu H-W, Wei D-X, Deng J-Z, et al. Combined antibacterial and osteogenic in situ effects of a bifunctional titanium alloy with nanoscale hydroxyapatite coating. *Artif Cells Nanomed Biotechnol* 2018; 46: 460–470.
177. Wang B, Bian A, Jia F, et al. "Dual-functional" strontium titanate nanotubes designed based on fusion peptides simultaneously enhancing anti-infection and osseointegration. *Biomater Adv* 2022; 133: 112650.
178. Zhang S, Zhou X, Liu T, et al. The effects of Peptide Mel4-coated titanium plates on infection rabbits after internal fixation of open fractures. *Arch Orthop Trauma Surg* 2022; 142: 729–734.
179. Yasir M, Dutta D and Willcox MD. Mode of action of the antimicrobial peptide Mel4 is independent of Staphylococcus aureus cell membrane permeability. *PLOS ONE* 2019; 14: e0215703.
180. Nibbering P, Ravensbergen E, Welling M, et al. Human lactoferrin and peptides derived from its N terminus are highly effective against infections with antibiotic-resistant bacteria. *Infect Immun* 2001; 69: 1469–1476.
181. Wang G. Structures of human host defense cathelicidin LL-37 and its smallest antimicrobial peptide KR-12 in lipid micelles. *J Biol Chem* 2008; 283: 32637–32643.
182. Meng X, Zhang J, Chen J, et al. KR-12 coating of polyetheretherketone (PEEK) surface via polydopamine improves osteointegration and antibacterial activity in vivo. *J Mater Chem B* 2020; 8: 10190–10204.
183. Wei S-Y, Wu J-M, Kuo Y-Y, et al. Solution structure of a novel tryptophan-rich peptide with bidirectional antimicrobial activity. *J Bacteriol* 2006; 188: 328–334.
184. Kazemzadeh-Narbat M, Kindrachuk J, Duan K, et al. Antimicrobial peptides on calcium phosphate-coated titanium for the prevention of implant-associated infections. *Biomaterials* 2010; 31: 9519–9526.

185. He Y, Jin Y, Ying X, et al. Development of an antimicrobial peptide-loaded mineralized collagen bone scaffold for infective bone defect repair. *Regen Biomater* 2020; 7: 515–525.
186. Rohrl J, Yang D, Oppenheim JJ, et al. Identification and biological characterization of mouse β-defensin 14, the orthologue of human β-defensin 3. *J Biol Chem* 2008; 283: 5414–5419.
187. Yuan X, Ouyang L, Luo Y, et al. Multifunctional sulfonated polyetheretherketone coating with beta-defensin-14 for yielding durable and broad-spectrum antibacterial activity and osseointegration. *Acta Biomater* 2019; 86: 323–337.
188. Wang L, Wei X, Duan C, et al. Bone marrow mesenchymal stem cell sheets with high expression of hBD3 and CTGF promote periodontal regeneration. *Biomater Adv* 2022; 133: 112657.
189. Chen X, Zhou L, Wu D, et al. The effects of titanium surfaces modified with an antimicrobial peptide GL13K by silanization on polarization, anti-inflammatory, and pro-inflammatory properties of macrophages. *BioMed Res Int* 2020; 2020: 2327034.
190. NategholEslam M, Vafaei S, Nichols M, et al. Structure of the antimicrobial peptide HHC-36 and its interaction with model cell membranes. *Biophys J* 2012; 102: 397a–398a.
191. Wu P, Chen D, Yang H, et al. Antibacterial peptide-modified collagen nanosheet for infected wound repair. *Smart Mater Med* 2021; 2: 172–181.
192. Luo Y and Song Y. Mechanism of antimicrobial peptides: antimicrobial, anti-inflammatory and antibiofilm activities. *Int J Mol Sci* 2021; 22: 11401.
193. Nguyen LT, Haney EF and Vogel HJ. The expanding scope of antimicrobial peptide structures and their modes of action. *Trends Biotechnol* 2011; 29: 464–472.
194. de Leeuw E, Li C, Zeng P, et al. Functional interaction of human neutrophil peptide-1 with the cell wall precursor lipid II. *FEBS Lett* 2010; 584: 1543–1548.
195. Graf M and Wilson DN. Intracellular antimicrobial peptides targeting the protein synthesis machinery. In: *Advances in Experimental Medicine and Biology*, Springer 2019, 1117: 73–89.
196. Park CB, Kim HS and Kim SC. Mechanism of action of the antimicrobial peptide buforin II: buforin II kills microorganisms by penetrating the cell membrane and inhibiting cellular functions. *Biochem Biophys Res Commun* 1998; 244: 253-257.
197. Cardoso MH, Meneguetti BT, Costa BO, et al. Non-lytic antibacterial peptides that translocate through bacterial membranes to act on intracellular targets. *Int J Mol Sci* 2019; 20: 4877.
198. Wu C, Biswas S, Garcia De, Gonzalo CV, et al. Investigations into the mechanism of action of sublancin. *ACS Infect Dis* 2018; 5: 454–459.
199. He Y, Jin Y, Wang X, et al. An antimicrobial peptide-loaded gelatin/chitosan nanofibrous membrane fabricated by sequential layer-by-layer electrospinning and electrospraying techniques. *Nanomaterials* 2018; 8: 327.
200. Chung M-C, Dean SN and van Hoek ML. Acyl carrier protein is a bacterial cytoplasmic target of cationic antimicrobial peptide LL-37. *Biochem J* 2015; 470: 243–253.
201. Braffman NR, Piscotta FJ, Hauver J, et al. Structural mechanism of transcription inhibition by lasso peptides microcin J25 and capistruin. *Proc Natl Acad Sci* 2019; 116: 1273–1278.
202. Lehrer R, Barton A, Daher KA, et al. Interaction of human defensins with Escherichia coli. Mechanism of bactericidal activity. *J Clin Invest* 1989; 84: 553–561.
203. Ling LL, Schneider T, Peoples AJ, et al. A new antibiotic kills pathogens without detectable resistance. *Nature* 2015; 517: 455–459.
204. Mookherjee N, Anderson MA, Haagsman HP, et al. Antimicrobial host defence peptides: functions and clinical potential. *Nat Rev Drug Discov* 2020; 19: 311–332.
205. Hilchie AL, Wuerth K and Hancock RE. Immune modulation by multifaceted cationic host defense (antimicrobial) peptides. *Nat Chem Biol* 2013; 9: 761–768.
206. van der Does AM, Hiemstra PS and Mookherjee N. Antimicrobial host defence peptides: immunomodulatory functions and translational prospects. *Adv Exp Med Biol* 2019; 1117: 149–171.
207. Lebeaux D, Ghigo J-M and Beloin C. Biofilm-related infections: bridging the gap between clinical management and fundamental aspects of recalcitrance toward antibiotics. *Microbiol Mol Biol Rev* 2014; 78: 510–543.
208. De La Fuente-Núñez C, Korolik V, Bains M, et al. Inhibition of bacterial biofilm formation and swarming motility by a small synthetic cationic peptide. *Antimicrob Agents Chemother* 2012; 56: 2696–2704.
209. Mahlapuu M, Björn C and Ekblom J. Antimicrobial peptides as therapeutic agents: Opportunities and challenges. *Crit Rev Biotechnol* 2020; 40: 978–992.
210. Li L, Peng Y, Yuan Q, et al. Cathelicidin LL37 promotes osteogenic differentiation in vitro and bone regeneration in vivo. *Front Bioeng Biotechnol* 2021; 9: 638494.
211. Fan R, Tong A, Li X, et al. Enhanced antitumor effects by docetaxel/LL37-loaded thermosensitive hydrogel nanoparticles in peritoneal carcinomatosis of colorectal cancer. *Int J Nanomed* 2015: 7291–7305.
212. Chereddy KK, Her C-H, Comune M, et al. PLGA nanoparticles loaded with host defense peptide LL37 promote wound healing. *J Control Release* 2014; 194: 138–147.
213. Liu Z, Yuan X, Liu M, et al. Antimicrobial peptide combined with BMP2-modified mesenchymal stem cells promotes calvarial repair in an osteolytic model. *Mol Ther* 2018; 26: 199–207.
214. Zhou L, Xing Y, Ou Y, et al. Prolonged release of an antimicrobial peptide GL13K-loaded thermosensitive hydrogel on a titanium surface improves its antibacterial and anti-inflammatory properties. *RSC Adv* 2023; 13: 23308–23319.
215. Hasan S, Al-Jamal M, Miller A, et al. Efficacy and outcome measurement of iFactor/ABM/P-15 in lumbar spine surgery: a systematic review. *Global Spine J* 2024; 14: 1422–1433.
216. Barman P, Joshi S, Sharma S, et al. Strategic approaches to improvise peptide drugs as next generation therapeutics. *Int J Pept Res Ther* 2023; 29: 61.
217. Bulger EM, May AK, Robinson BR, et al. A novel immune modulator for patients with necrotizing soft tissue infections (NSTI): results of a multicenter, phase 3 randomized controlled trial of reltecimod (AB 103). *Ann Surg* 2020; 272: 469–478.
218. Research HGV. Global Peptide Therapeutics Market Size & Outlook, https://www.grandviewresearch.com/

horizon/outlook/peptide-therapeutics-market-size/global (2023).
219. Ferrazzano L, Corbisiero D, Martelli G, et al. Green solvent mixtures for solid-phase peptide synthesis: a dimethylformamide-free highly efficient synthesis of pharmaceutical-grade peptides. *ACS Sustain Chem Eng* 2019; 7: 12867–12877.
220. Zhu M, Zhong W, Cao W, et al. Chondroinductive/chondroconductive peptides and their-functionalized biomaterials for cartilage tissue engineering. *Bioact Mater* 2022; 9: 221–238.
221. Oliver-Cervelló L, Martin-Gómez H and Mas-Moruno C. New trends in the development of multifunctional peptides to functionalize biomaterials. *J Pept Sci* 2022; 28: e3335.
222. Szwed-Georgiou A, Płociński P, Kupikowska-Stobba B, et al. Bioactive materials for bone regeneration: biomolecules and delivery systems. *ACS Biomater Sci Eng* 2023; 9: 5222–5254.
223. Yoo HS, Kim TG and Park TG. Surface-functionalized electrospun nanofibers for tissue engineering and drug delivery. *Adv Drug Deliv Rev* 2009; 61: 1033–1042.
224. Kandori K, Oda S and Tsuyama S. Effects of pyrophosphate ions on protein adsorption onto calcium hydroxyapatite. *JPhys Chem B* 2008; 112: 2542–2547.
225. Ishihara S, Matsumoto T, Onoki T, et al. Regulation of the protein-loading capacity of hydroxyapatite by mercaptosuccinic acid modification. *Acta Biomater* 2010; 6: 830–835.
226. Lee W-H, Loo C-Y, Van KL, et al. Modulating protein adsorption onto hydroxyapatite particles using different amino acid treatments. *J R Soc Interface* 2012; 9: 918–927.
227. Boix T, Gómez-Morales J, Torrent-Burgues J, et al. Adsorption of recombinant human bone morphogenetic protein rhBMP-2m onto hydroxyapatite. *J Inorg Biochem* 2005; 99: 1043–1050.
228. Kim SE, Song S-H, Yun YP, et al. The effect of immobilization of heparin and bone morphogenic protein-2 (BMP-2) to titanium surfaces on inflammation and osteoblast function. *Biomaterials* 2011; 32: 366–373.
229. Sevilla P, Cirera A, Dotor J, et al. In vitro cell response on CP-Ti surfaces functionalized with TGF-β1 inhibitory peptides. *J Mater Sci Mater Med* 2018; 29: 1–11.
230. Biju V. Chemical modifications and bioconjugate reactions of nanomaterials for sensing, imaging, drug delivery and therapy. *Chem Soc Rev* 2014; 43: 744–764.
231. Zhang Z, Gupte MJ, Jin X, et al. Injectable peptide decorated functional nanofibrous hollow microspheres to direct stem cell differentiation and tissue regeneration. *Adv Funct Mater* 2015; 25: 350–360.
232. Rosenberg M, Shilo D, Galperin L, et al. Bone morphogenic protein 2-loaded porous silicon carriers for osteoinductive implants. *Pharmaceutics* 2019; 11: 602.
233. Oya K, Tanaka Y, Saito H, et al. Calcification by MC3T3-E1 cells on RGD peptide immobilized on titanium through electrodeposited PEG. *Biomaterials* 2009; 30: 1281–1286.
234. Vugmeyster Y, Xu X, Theil F-P, et al. Pharmacokinetics and toxicology of therapeutic proteins: Advances and challenges. *World J Biol Chem* 2012; 3: 73.
235. Jiskoot W, Randolph TW, Volkin DB, et al. Protein instability and immunogenicity: roadblocks to clinical application of injectable protein delivery systems for sustained release. *J Pharm Sci* 2012; 101: 946–954.
236. Geuze RE, Theyse LF, Kempen DH, et al. A differential effect of bone morphogenetic protein-2 and vascular endothelial growth factor release timing on osteogenesis at ectopic and orthotopic sites in a large-animal model. *Tissue Eng Part A* 2012; 18: 2052–2062.
237. Quinlan E, López-Noriega A, Thompson E, et al. Development of collagen–hydroxyapatite scaffolds incorporating PLGA and alginate microparticles for the controlled delivery of rhBMP-2 for bone tissue engineering. *J Control Release* 2015; 198: 71–79.
238. Saik JE, Gould DJ, Watkins EM, et al. Covalently immobilized platelet-derived growth factor-BB promotes angiogenesis in biomimetic poly (ethylene glycol) hydrogels. *Acta Biomater* 2011; 7: 133–143.
239. Zamani Y, Amoabediny G, Mohammadi J, et al. 3D-printed poly (Ɛ-caprolactone) scaffold with gradient mechanical properties according to force distribution in the mandible for mandibular bone tissue engineering. *J Mech Behav Biomed Mater* 2020; 104: 103638.
240. Brunello G, Sivolella S, Meneghello R, et al. Powder-based 3D printing for bone tissue engineering. *Biotechnol Adv* 2016; 34: 740–753.
241. Karageorgiou V and Kaplan D. Porosity of 3D biomaterial scaffolds and osteogenesis. *Biomaterials* 2005; 26: 5474–5491.
242. Elhaj A and Irgum K. Monolithic space-filling porous materials from engineering plastics by thermally induced phase separation. *ACS Appl Mater Interf* 2014; 6: 15653–15666.
243. Degli Esposti M, Chiellini F, Bondioli F, et al. Highly porous PHB-based bioactive scaffolds for bone tissue engineering by in situ synthesis of hydroxyapatite. *Mater Sci Eng C* 2019; 100: 286–296.
244. Turnbull G, Clarke J, Picard F, et al. 3D bioactive composite scaffolds for bone tissue engineering. *Bioact Mater* 2018; 3: 278–314.
245. Babilotte J, Guduric V, Le Nihouannen D, et al. 3D printed polymer–mineral composite biomaterials for bone tissue engineering: fabrication and characterization. *J Biomed Mater Res Part B Appl Biomater* 2019; 107: 2579–2595.
246. Bandyopadhyay A, Mitra I and Bose S. 3D printing for bone regeneration. *Curr Osteoporos Rep* 2020; 18: 505–514.
247. van der Heide D, Cidonio G, Stoddart MJ, et al. 3D printing of inorganic-biopolymer composites for bone regeneration. *Biofabrication* 2022; 14: 042003.
248. Lau JL and Dunn MK. Therapeutic peptides: historical perspectives, current development trends, and future directions. *Bioorg Med Chem* 2018; 26: 2700–2707.
249. Fukuba S, Okada M, Nohara K, et al. Alloplastic bone substitutes for periodontal and bone regeneration in dentistry: current status and prospects. *Materials* 2021; 14: 1096.