Peptide-mediated Bone Tissue Engineering

Abdullah Karadag, Hana'a Iqbal, and Hilal Yazici

Abstract Bone is a highly vascularized tissue and one of the most dynamic tissues in terms of self-renewal throughout one's life. It possesses a high regenerative capacity, which makes it possible that a majority of bone fractures will heal well without the need for major intervention. However, some large bone defects and fractures require medical intervention for bone repair and regeneration. Proteins, growth factors, and peptides have played a remarkable role in bone regeneration. However, the use of proteins and growth factors in tissue engineering has several limitations, such as cost and difficulty in production, immunogenicity, and a short half-life. In addition to these drawbacks, they have many active domains, which affect their functionality. Recently, an alternative to proteins and growth factors has emerged for the use in tissue engineering. This competent approach includes biomimetic peptides, which are amino acid sequences derived from the functional domains of soluble or extracellular matrix (ECM) proteins. Biological materials for tissue regeneration can be functionalized with these peptides to either mediate the adhesion of cells 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. In this extensive chapter, several of the peptides that have potential for bone tissue engineering, including those that facilitate cell adhesion, prompt osteogenic differentiation of progenitor cells, or those that mediate angiogenesis which is a crucial requirement for proper bone regeneration will be discussed.

Keywords Peptides · Bone regeneration · Scaffold · Osteoinduction · Biomimetic

A. Karadag · H. Yazici (✉)
TUBITAK-Marmara Research Center, Genetic Engineering and Biotechnology Institute, Kocaeli, Turkey
e-mail: hilal.yazici@tubitak.gov.tr

H. Iqbal
TUBITAK-Marmara Research Center, Genetic Engineering and Biotechnology Institute, Kocaeli, Turkey

Dr. Panjwani Center for Molecular Medicine and Drug Research (PCMD), International Center for Chemical and Biological Sciences (ICCBS), University of Karachi, Karachi, Pakistan

B. Li et al. (eds.), *Racing for the Surface*,
https://doi.org/10.1007/978-3-030-34471-9_17

Introduction

General View of Tissue Engineering

Tissue and organ injury or failure in the human body results in a heavy economic burden on health care systems worldwide. Existing therapeutic options for tissue/organ loss include: drugs, replacements through synthetic materials, and organ/tissue transplantation which have significant limitations to prevent mortality or morbidity for many patients every year. These limitations led to the creation of a new research field, tissue engineering or regenerative medicine, which focuses on assembling functional constructs that can reinstate, maintain, or repair injured tissues or whole organs. To achieve the goal, new synthetic materials (metals, polymers, ceramic-based materials, etc.) and their functionalization with various proteins, combinations of cells, and cell-instructive peptide need to be designed and fabricated [1, 2].

In tissue engineering, there are three general approaches termed as conduction, induction, and cell transplantation. Conduction includes grafting a construct at the defect site in the tissue which allows cells to penetrate through the surrounding tissue for the regeneration of the tissue. This phenomenon forms the basis of guided tissue engineering intervention used clinically in orthopedics and dentistry for bone healing [3].

The induction approach is placing a peptide or protein-based growth factor using a scaffold as a carrier, at the site of tissue defect. The cells in the surroundings are recruited to the site of injury and start proliferation. This recruitment is initiated by the peptides and proteins that bind to cell surface receptors. This approach is more attractive than the conductive approach. Inductive approaches are currently in clinical use for bone formation and angiogenesis. The third option in tissue engineering is the transplantation of particular cell types in the damaged tissues. The population is generated and multiplied outside the body by taking small starting tissue at the defect site. Multiplied cells need to be transplanted to form a new tissue replacing the lost and defective one. This strategy is preferred when inductive molecules are unknown for the tissue of interest with two applications. One requires transplanting the target cells directly at the defect site to trigger tissue regeneration. The other one requires prefabrication of the cells in vitro that are then available to patients for implantation. The latter approach is currently utilized for cartilage and skin tissue replacements [1, 4, 5] (Fig. 1).

Bone Tissue Engineering

Bone is a very dynamic tissue with well-developed vascular beds that continue to self-renew throughout an individual's lifetime.

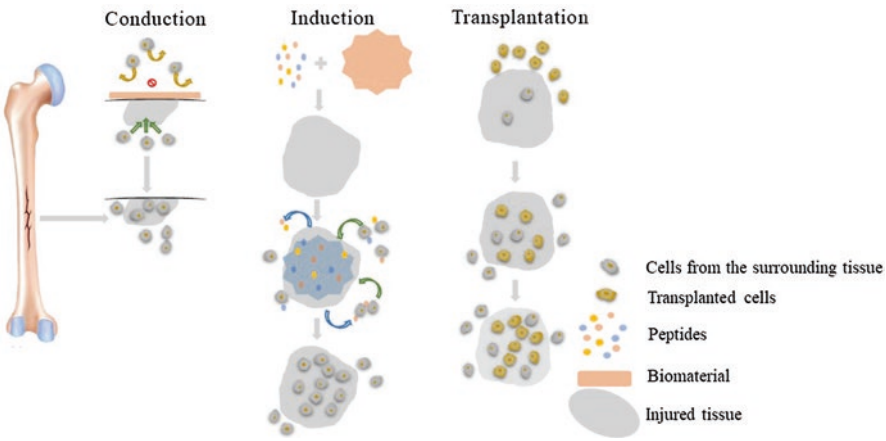

Fig. 1 Major strategies to engineer or regenerate tissues [1]

It is a composite material that is comprised of an organic matrix of type-I collagen and inorganic minerals of calcium phosphate. It plays an essential role in movement. The bone tissue also provides support to the skeleton which acts as a scaffold for the delicate soft tissue and internal organs with suitable load-bearing capacity. Besides these structural functions, bone has a storage capacity for Ca and P ions and a role in regulating the key electrolyte concentrations in the blood, making it crucial in homeostasis [5–7].

Bone Structure and Properties

The human skeleton consists of 206 bones of strikingly diverse sizes, shapes, and function. These include short bones in the ankle and writs, long bones of the limbs, irregular bones of the pelvis and vertebrae, and the flat bones in the skull and sternum. Bone tissue is composed of two layers: compact (cortical) and trabecular (cancellous) bones. Bone tissue, similar to all the organs in the human body, has a hierarchical organization spanning several folds of magnitude from the macroscale to nanoscale components (Fig. 2). For the nanoscale component, the extracellular matrix (ECM) comprises both a mineralized inorganic (carbonated apatite mineralites, 4 nm in thickness) and non-mineralized organic components (mainly collagen type I) [5].

Additionally, there are several noncollagenous matric proteins, such as sialoproteins, proteoglycans, and glycoproteins, which contribute to signaling in the immediate extracellular environment. The nanocomposite structure composed of flexible and tough collagen fibers strengthened by hydroxyapatite (HA) crystals, provide the necessary high fracture toughness and compressive strength of bone [3, 5].

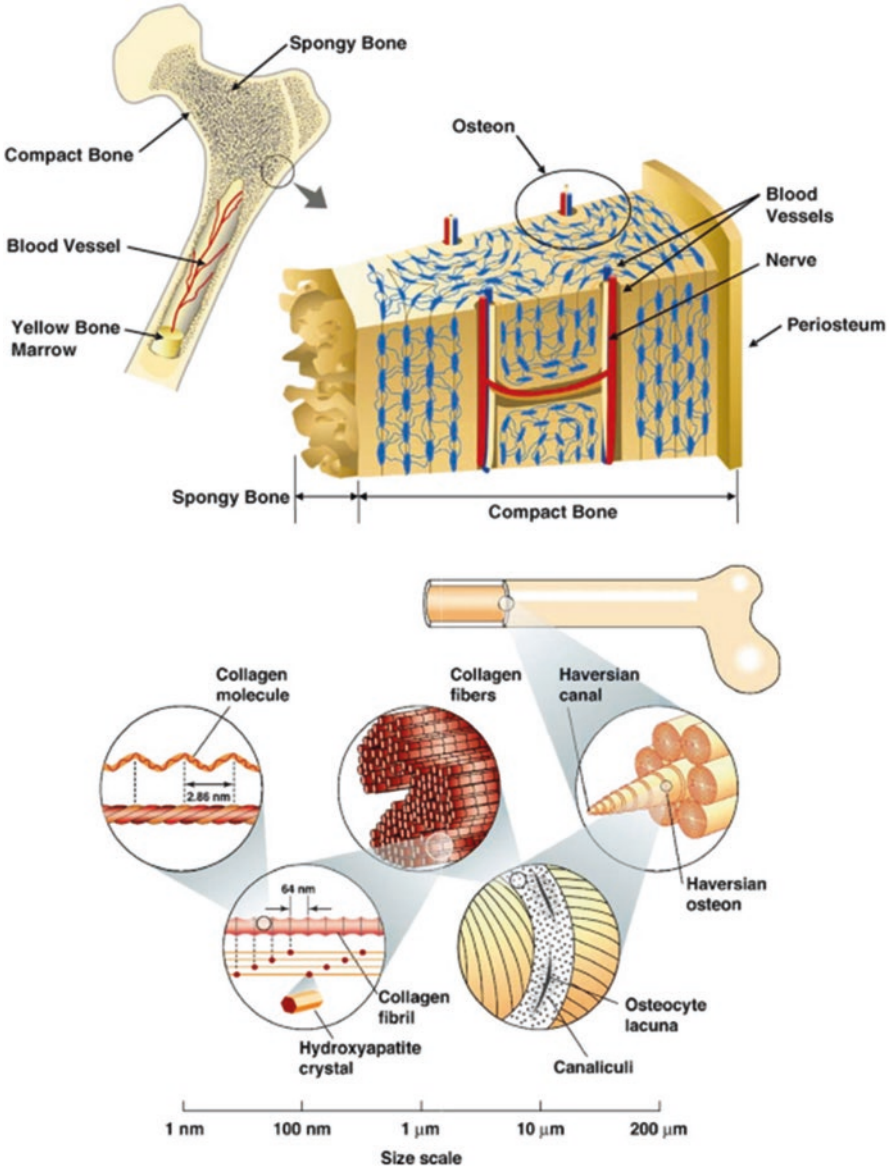

Fig. 2 The hierarchical organization of human cortical/compact bone [8]

Bone Healing

There are three major processes such as osteoconduction, osteoinduction, and osteogenesis that need to be considered in bone healing.

Osteoconduction is the process of bone formation which is marked by the passive growth of the resident cells, tissues, and blood vessels, supported by a grafted scaffold. In context to this, osteoconductive materials function as a scaffold for the response by host tissue to repair or form the new bone. Bone autograft, allograft, and inert filler structures such as calcium ceramics, and demineralized bone matrix (DBM) are some examples of osteoconductive materials. Osteoconductive scaffolds are usually composed of a structured matrix analogous to cancellous bone so as to assist the ingrowth of host cells and vasculature. Autografts comprised of cancellous bone, therefore, carry the utmost osteoconductive potential as compared to signaling molecules and growth factors, such as bone morphogenetic proteins (BMPs) when used alone, as these do not provide any specific physical supportive structure [9, 10].

Osteoinduction is bone repair and formation through the specific growth factors provided by a grafted scaffold, thus promoting the differentiation of mesenchymal stem cells (MSCs) to chondroblasts and osteoblasts. These growth factors can be of various types such as platelet-derived growth factors (PDGFs), BMPs, fibroblast growth factors (FGFs), and interleukins. Additionally, bone autografts, platelet-rich plasma (PRP), and bone marrow aspirate concentrates (BMAC) possess osteoinductive properties [4, 6] (Table 1).

The process of osteogenesis is hallmarked by new bone formation via specific cellular components within the graft. Autologous cancellous bone, as it has all the essential elements required for osteogenic stimulation, offers tremendous osteogenic potential. However, with an increase in our understanding of the molecular and cellular mechanisms involved in the bone repair and formation, alternative stimulators of osteogenesis have emerged, such as cell signaling proteins, growth factors, and cell-based treatments [4].

Table 1 Parts of bones and their specific osteoconductive, osteoinductive, and osteogenic properties [4]

	Osteoconductive	Osteoinductive	Osteogenic
Cortical autograft	+	+	+
Cancellous autograft	+++	+++	+++
Cortical allograft	+	+/−	−
Cancellous allograft	+	+/−	−
Demineralized bone matrix	+	++	−
Calcium ceramics	+	−	−
Bone marrow aspirate	−	++	+++
Bone morphogenetic protein	−	++	−
Platelet-rich plasma	−	+++	+

+ activity, − no activity, +/− activity depends on preparation process

The Role of Biomaterials in Bone Tissue Engineering

Due to the high regenerative capacity of bone, most fractures heal without the need of any surgery; however, large defects in bone require surgical intervention [5].

Currently, a bone graft is the gold standard treatment for large bone defects. Clinically, these bone grafts are classified on the basis of their origin into biological and synthetic grafts. The biological grafts comprise autografts, allografts, and xenografts. An autograft is the transplantation of bone taken from the patient's own body. This offers the greatest clinical outcome as it is compatible with the host bone and does not elicit any disease or immune-related complications. However, this approach is limited by donor site morbidity associated with the harvest of the graft bone which results in damage of the harvest site. Contrarily, an allograft is the transplantation of bone harvested from one individual and transplanted into the other individual within the same species. Allografts also have some limitations, such as the host immune response and potential transmission of pathogens. Xenografts, the bones harvested from one species and transplanted in another, have severe restrictions due to immunogenic barriers between the species. The limitations associated with biological bone transplants have directed the treatment paradigm toward the use of synthetic substitutes for bone repair, replacement, and augmentation [6, 11].

Osteoconductive Materials

The ability of the biological scaffolds to support bone formation on their surfaces, known as osteoconductivity, is one of the crucial prerequisites of biomaterials used for bone repair [12]. Materials with osteoconductive properties allow proliferation, migration, differentiation, and ECM deposition from osteoprogenitor cells in the bone defect, which are key initiators of new bone formation [13].

Biomaterial osteoconductivity during bone regeneration largely depends on their physicochemical characteristics, which include suitable chemical composition, architectural geometry, and surface properties [14]. Tricalcium phosphate (TCP) and hydroxyapatite (HA), both calcium phosphate (CaP) based ceramics, possess superior osteoconductive properties due to their similarity to natural bone mineral [15, 16]. Another osteoconductive material, bioglass, is capable of binding with the bone directly. Moreover, type-I collagen is an osteoconductive material owing to its structure and composition which is favorable for mineral deposition through noncollagenous matrix protein binding thus initiating and regulating mineralization [17].

Osteoinductive Materials

The capability of biomaterials to prompt the formation of bone at the site of the graft is known as osteoinductivity. In the past few decades, there have been tremendous advancements in revealing the role that biomaterials play in bone generation, although the precise mechanism of osteoinduction still remains to be explored [18].

Biological scaffolds possessing osteoinductive properties have been shown to influence the ectopic bone formation at many levels:

1. At the tissue level, the biomaterials actively facilitate the exchange of oxygen, nutrition, and waste between the material and the tissue. They also promote vascularization within the materials, essential for new tissue formation [19, 20].
2. At the cellular level, the differentiation of stem cells or the osteoprogenitor cells can be triggered toward the osteogenic lineage by the formation of a biological carbonated apatite layer [21, 22]. The liberated phosphate and calcium ions also serve as a potent chemotaxis for cell migration and direct cell growth at the graft site [23, 24].
3. At the molecular level, these biomaterials may play a role in enriching osteogenic proteins such as BMP-2 and BMP-7 owing to their high affinity to these biomolecules, thus promoting a series of cellular events on the surface of biomaterials [25].

Moreover, the released phosphate and calcium ions may accelerate the mineralization in the implanted site by reaching a supersaturation level in the graft void. The most extensively used osteoinductive materials are CaP-based biological ceramics. Osteoinduction has been shown on numerous types of CaP materials such as TCP, biphasic calcium phosphate, and HA. The fundamental element that confers the osteoinductive capacity to these CaP scaffolds is the presence of calcium and phosphate. Nevertheless, other materials that do not contain CaP, such as alumina ceramics, titanium, and poly (hydroxyethyl methacrylate) (PHEMA), have been found to be osteoinductive under certain conditions [17, 23].

Vascular Materials

Vascularization is a vital process during bone repair as any tissue with sizes beyond 200 μm requires formation of blood vessels for appropriate diffusion of oxygen through the tissue. Therefore, proper functional bone regeneration necessitates the close association with the vasculature, which must also properly integrate with the host blood vasculature [26].

Considering the critical requirement of establishing a functional vasculature during bone repair, biological materials that can promote various events of vascular network formation have been designed and extensively employed for bone tissue engineering. As the most used biomaterial formulation, hydrogels and scaffolds can be used as temporal matrix to facilitate progenitor cells and pericyte migration and to furnish mechanical support for vessel sprouting. Through tuning the architectural properties of scaffolds during fabrication, the influence of these on tissue-engineered constructs can be realized [27, 28].

Vascularization is also significantly influenced by the chemical composition of the biological scaffolds, as they interact with the endothelial cells directly during vessel formation [29, 30]. Though, most of the materials utilized for bone tissue engineering such as silk, PCL, collagen, and PLGA have been found to be compat-

ible with endothelial cells, there are some which have been demonstrated as proangiogenic during bone repair [31]. One of such example is the hydrogel made of dextran, which has demonstrated a remarkable ability to stimulate neovascularization resulting in skin regeneration. Akermanite, a silicate bioceramic, has been shown to effectively induce angiogenesis during bone repair by supplying appropriate Si ion concentrations, stimulating cell proliferation and gene expression of human aortic endothelial cells [32]. Other biomaterials including heparin sulfate, fibrin, and hyaluronic acid (HA) also possess the ability to regulate blood vessel formation through their high affinity to angiogenic cytokines such as endothelial growth factor (EGD), vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF) [29, 33]. These biomaterials enhance bone regeneration through vascularization by sequestering endogenous growth factors at the defect site [34].

Role of Biological Molecules in Bone Tissue Engineering

For the successful implantation of biomaterials, a favorable impact of cellular fate is a crucial requirement. There are factors including bacteria adhesion, nonspecific protein adsorption, and poor osseointegration that result in implant mobility, infection, and eventual failure of the implant. To facilitate various biological events, directed and selective cell adhesion is crucial after transplantation of the biomaterial. Therefore, for effective biomaterial implantation, new strategies are being developed to improve selective cell adhesion, recruitment of progenitor cells, and subsequent cellular differentiation. Moreover, the surfaces of the biomaterials are designed to reduce the nonspecific adhesion of the proteins. There are various bioactive molecules that have been utilized to resolve the aforementioned critical problems including: GAGs, proteins, and peptides (Fig. 3).

Proteoglycans

One of the major components of the ECM is proteoglycans, characterized by modifications with GAGs. GAGs mediate and regulate several key functions mainly through electrostatic interactions with ECM proteins. Therefore, they are a promising means to improve the biocompatibility of biomaterials. The osteogenic differentiation is influenced tremendously by the degree of sulfation of GAGs [28]. Additionally, the GAGs can regulate the functions of chemokines and growth factors by sequestering and releasing them [36]. Heparin has an anti-coagulative property, which is particularly valuable for vascular biomaterials [37]. Moreover, cell membrane proteoglycans interact synergistically with integrins, providing a potential target for multifunctional coatings. Besides these cell-attracting domains, molecules rendering cell-repelling properties to the biomaterial coatings are also incorporated.

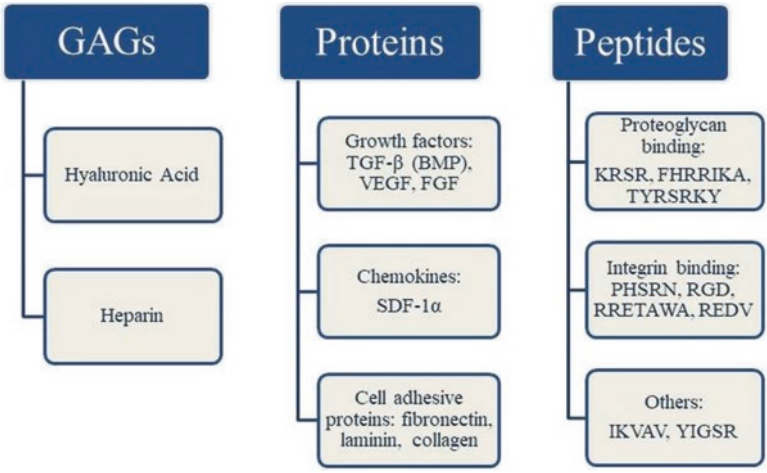

Fig. 3 Components of multifunctional coatings. Cellular function can be mediated by different glycosaminoglycans (GAGs), proteins: VEGF (vascular endothelial growth factor), BMP (bone morphogenetic protein), TGF-β (transforming growth factor), FGF (fibroblast growth factor), SDF-1 (stromal cell-derived factor 1, PDB: 1A15), or peptides (sequences are shown by the one letter code) [35]

One of the most commonly used components to prevent undesirable cell and protein adhesion is PEG [38].

Proteins

For bone tissue engineering, bone ECM proteins and peptides have been extensively used to address surface functionalization. Various proteins including vitronectin, collagen, laminin, and fibronectin are feasible mediators for cellular adhesion to biomaterials and regulate signaling events as they contain several moieties, such as proteoglycans or integrin-binding sites [39].

Moreover, growth factors are extensively used to stimulate cell proliferation and differentiation of primary and progenitor cells to osteoblasts or endothelial cells. Growth factors also induce other cellular events such as cell adhesion and proliferation or increase collagen synthesis, which favor implant integration and healing. Bone morphogenetic protein-2 (BMP-2), a growth factor of the transforming growth factor β (TGF-β) superfamily, has found its use in dental and orthopedic implants due to its potent osteoinductive effect [40]. Furthermore, the role of TGF-β1 has been implicated in the regulation of bone remodeling [41]. Vascular endothelial growth factors (VEGFs) stimulate angiogenesis and thus can be used for vascular materials and additionally for bone regeneration. The basic fibroblast growth factor (bFGF or FGF-2) and other FGFs are proangiogenic and stimulate cellular proliferation [35, 42]. A noncollagenous bone matrix protein, bone sialoprotein, also a member of SIBLING protein family, promotes migration of osteoprogenitor cells

through bridging matrix metalloproteinase (MMP)-2 with integrin αvβ3 at the cell surface [40].

Chemokines orchestrate the migration of cells to the site of injury or the biomaterial surface. The recruitment of the MSCs and stimulation of osteogenic differentiation employs this phenomenon through SDF-1-gradient [43]. Chemokines are also involved in the immune response of biomaterials. Human parathyroid hormone-related protein (PTHrP) plays a key role in the regulation of cell proliferation, differentiation, and development of skeleton. The pivotal point of application of growth factors and chemokines is their steady release from biomaterial coatings. Besides successful use in tissue engineering, growth factors have some limitations due to their short circulating half-life, low protein stability, side effects, and rapid cellular internalization [44, 45].

Peptides

Peptides have emerged as useful alternatives to proteins for bone tissue repair as evidence suggests that employing smaller growth factor fragments or peptides prompts receptor-mediated signal transduction [46]. Moreover, these small biomolecules can be custom synthesized to induce directed immobilization and multifunctional properties. This can be achieved by introducing or ligating the immobilization anchors like dihydroxyphenylalanine (DOPA), basic amino acids, nucleic acids, thiols, and phosphonic acids to bioactive peptides. Moreover, cell-attracting or repelling moieties can be integrated [35].

Cell adhesion, migration, proliferation, and differentiation are favored by binding of the peptides to the receptors in the cell membrane (as illustrated in Fig. 4). The recent vigorous research in this field has led to the discovery of numerous peptides involved in the upregulation of bone repair response. The

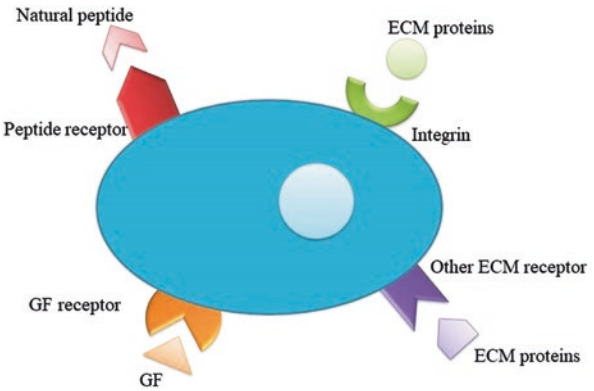

Fig. 4 Main sources of peptides for bone tissue engineering: derived from extracellular matrix proteins, soluble growth factors or are naturally occurring [47]

fragments of these peptides are typically derived from the ECM proteins, such as collagen (DGEA, GFOGER), fibronectin (RGD, PHSRN, LDV, REDV, KRSR), FGF-2 (TYRSRKY), bone sialoprotein (FHRRIKA, RGD), and laminin (YIGSR, IKVAV). The RRETAWA motif involved in selective endothelial cell binding was not derived from a protein associated with the ECM. One of the most frequently used peptides for the improvement of the tissue integration of biomaterials and to explore the integrin-mediated cell adhesion on the surfaces is the RGD motif [47].

Role of Peptides for Bone Tissue Engineering

Extensive research has established that modifying bone repair materials with suitable bioactive peptides could further improve their regenerative properties. It has been particularly noticed that peptide-modified biomaterials could stimulate new bone formation more efficiently as compared with non-modified materials.

While it has been thought earlier that biomaterials should possess a biotolerant surface so as to minimize the immune and fibrotic responses, increasingly evidence now suggests that interactive and biomimetic surfaces often demonstrate superior performances [48].

The efficacy of osseointegration relies on interactions between osteogenic macromolecules in the blood and implants. There are some biomaterials that do not readily adsorb plasma proteins to their surface, thus, these do not maintain bone-related cell activities competently and lead to inadequate bone formation [49].

Peptides Involved in Cell Adhesion

There are various insoluble proteins that reside in the ECM and form the scaffold for cells to live on. The types of biomolecules constituting the ECM and their spatial organization significantly govern cell behavior [50]. The organic fraction of the ECM is 90% type-I collagen and only 5% are noncollagenous proteins: such as osteopontin, osteocalcin, osteonectin, the adhesion proteins vitronectin and fibronectin, or the proteoglycans decorin, versican, or hyaluronan [51]. Most of these biomolecules mediate adhesive cell-ECM interactions and also play an active role in the regulation of osteoprogenitor and osteoblast proliferation, survival, and differentiation. These processes are mediated by ligand sequences in these proteins that bind to specific receptors on cell surfaces [47].

RGD Peptides

The Arg-Gly-Asp (RGD) tripeptide sequence present in fibronectin is a minimal cell adhesion peptide. It is the most studied and used peptide for biomaterial functionalization pertaining to its binding with multiple integrins stimulating in cell adhesion and differentiation. In addition to fibronectin, the RGD sequence is also present in many other ECM proteins, for instance, SIBLING proteins and vitronectin [52]. Several studies have reported enhanced osteoprogenitor cell attachment and/or differentiation with biomaterials coated with RGD peptides (Fig. 5) [53]. RGD peptides immobilized on diverse biomaterials such as alginate, titanium oxide nanotubes, or collagen sponges, have demonstrated increased cell adhesion and differentiation of MSCs or in vivo bone formation. Several variants of RGD have shown a similar osteogenesis promoting role [54].

Although categorized separately, many of the peptides discussed below contain an RGD sequence, which might be responsible partly or entirely for their biological functions.

Type-I Collagen-Derived Peptides

Osteoblastic cells consistently express major adhesion receptors for type-I collagen, the α2β1 integrins.

A synthetic triple helical peptide was engineered to contain the GFOGER sequence corresponds to 502–507 residues of the type-I collagen α1 chain. This engineered peptide selectively binds to the α2β1 integrins and promotes density-dependent cell adhesion and differentiation of MC3T3-E1 cells [55]. Coating of titanium surfaces with GFOGER increases the alkaline phosphatase (ALP) expres-

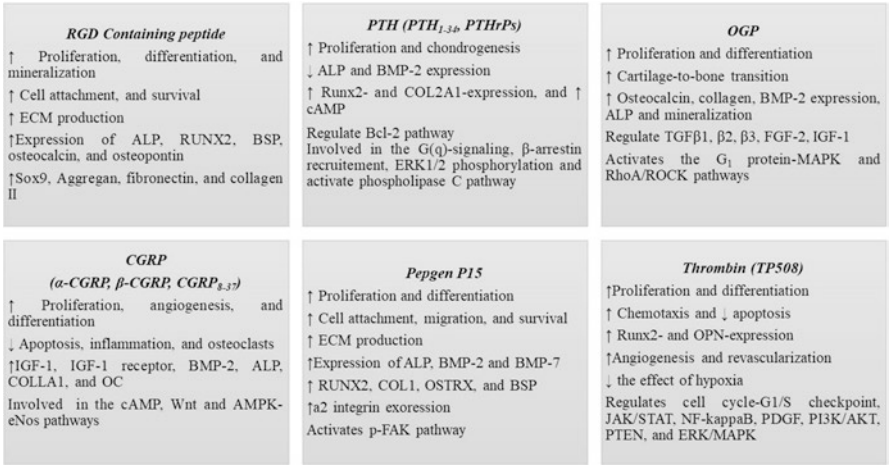

Fig. 5 Potential pathways and effect of peptides on osteoblastic cell lines [45]

sion, ECM mineralization, and the expression of osteocalcin, bone sialoprotein (BSP), and Runx2 in bone marrow stromal cells [56].

Similarly, the DGEA, a four-residue sequence initially suggested to be the recognition site of the α2β1 integrin in type-I collagen, has shown dose-dependent cell adhesion of murine MC3T3-E1 cells [57], improved attachment and spreading of hBMSCs [58], and an increased bone formation in rat tibial osteotomies when DGEA-immobilized HA was used.

P15 is another extensively studied peptide that is derived for type-I collagen. It is a 15 amino acid peptide identical to the cell-binding sequence of type-I collagen, which improves cell adhesion to bone substitutes and increases the production of ECM. P-15 significantly increases the transcript levels of BMP-2, BMP-7, and ALP when added to the scaffold (Fig. 5) [59]. It has also increased cell attachment and differentiation of human osteoblast-like HOS cells, and promoted MC3T3-E1 cell survival when adsorbed onto bovine bone-derived HA (anorganic bone matrix, ABM) [60].

PHSRN

Bone marrow stromal cells and osteoblasts express the α5β1 integrin during different stages of osteogenesis, which mediates cell adhesion to fibronectin and osteogenic differentiation. Even though only the RGD sequence can act as the ligand for α5β1, for a stable binding, both the RGD in the tenth type III repeat and the PHSRN sequence in the ninth type III repeat of fibronectin are required. A combination of RGD and PHSRN peptides conjugated to alginate hydrogels stimulated normal human osteoblasts (hOBs) for osteogenic differentiation and mineralization [61]. It is evident that the spatial configuration of the sequences in native fibronectin is crucial for assuring the correct binding as the two peptides interact with the same integrin.

FGF-2-Derived Peptides

Two peptides obtained from FGF-2 located in the cell-binding domain correspond to residue 36–41 (F36) and 77–83 (F77). When these peptides were immobilized on chitosan discs, they enhanced the cellular attachment and spreading of hBMSCs. Moreover, these peptides encourage a higher transcript level of ALP and promote ECM mineralization [54].

Laminin-Derived Peptides

Laminins are ECM proteins that bind to cell membranes through integrin receptors and other plasma membrane molecules. Peptides IKVAV and YIGSR, derived from the A and B1-chains of laminin, respectively, have shown a capability to promote

MC3T3-E1 attachment to plastic dishes coated with these peptides. YIGSR and IKVAV are peptides derived from the B1 and A-chains of laminin, respectively, have shown capability of promoting MC3T3-E1 attachment to peptide-coated plastic dishes.

The greatest osteogenic and adipogenic differentiation effects have been demonstrated by IKVAV as compared to the YIGSR peptide [62]. A more recently discovered laminin-derived peptide, Ln2-p3, has shown enhanced expression of several osteogenic markers and increased ALP activity of the cells when coated on titanium surfaces [63].

Osteopontin-Derived Peptide

Osteopontin (OPN) is a noncollagenous protein of ECM, which has demonstrated its role in bone mineralization and bone cell adhesion. The osteopontin-derived peptide (OPD) possessing RGD sequence and flanking sequences when conjugated to oligo(poly(ethylene glycol) fumarate) hydrogels showed a dose-dependent improvement of murine osteoblasts. Osteoblasts when cultured on the modified hydrogels demonstrated increased levels of secreted osteopontin, ALP activity, and ECM mineralization [64].

Heparin-Binding Peptides

Heparin-binding sequences in many ECM proteins interact with the transmembrane proteoglycans, which might have significant utility in the regulation of osteoprogenitor cell behavior.

Interactions between transmembrane proteoglycans and heparin-binding sequences found in many ECM proteins might also be of great importance to control the behavior of osteoprogenitor cells. Several studies have established that KSRS-functionalized surfaces increase the adhesion of human osteoblasts than those displaying RGD, and an increased adhesion of murine pre-osteoblasts and the expression of osteogenic markers [65].

RGD peptide and a heparin-binding domain (HBD), FHRRIKA, when functionalized on a scaffold revealed an increased ECM mineralization, and a higher degree of rat calvarial osteoblast cell and surface interactions [66].

A peptide with HBD in BMP-4 induced osteogenic differentiation of hMSCs through the ERK1/2 pathway activation. When immobilized in alginate gels, it has been demonstrated to induce a fourfold formation of new bone when grafted into a cranial defect model [67].

MEPE Peptide or AC-100

MEPE and its peptide motif, AC-100, possess an integrin-binding RGD and a consensus binding site for glycosaminoglycans SDGD. It has demonstrated significantly improved cell attachment and spreading, increased level of ECM mineralization, and superior cell differentiation with increased ALP expression in rat calvarial osteoblasts preincubated with the AC-100 fragment [68].

RRETAWA

Cyclic GA-CRRETAWAC-GA peptide-coated surfaces show an increased Runx2 and type-I collagen expression, and enhanced mineralization of ECM osteoprogenitors [69]. RRETAWA-conjugated PEG scaffolds with different stiffness profiles displayed improved MSC adhesion, higher ALP activity, and increased peptide densities. Moreover, it increased the expression of type-I collagen, osteopontin, and Runx2 [70].

Peptides Involved in Angiogenesis

During natural bone formation, vascularization is a vital process. After a bone injury, an inflammation process starts which is characterized by triggering of new blood vessel formation to recruit stem cells and soluble biomolecules that coordinate osteogenesis. In injuries where bone loss is critical, using only osteoconductive and/or osteoinductive strategies may result in graft failure due to the insufficient initial vascularization. Insufficient vascularization will lead to hypoxic conditions in the biomaterial, lacking essential osteoprogenitor cells and growth factors. Therefore, the discovery and design of proangiogenic factors and their use along with osteoinductive biomaterials are a quite appealing strategy for bone tissue engineering [26].

Osteopontin-Derived Peptide (OPD)

OPD mimics the OPN sites that are exposed during wound repair. OPD is capable of inducing the same number of newly formed blood vessels as VEGF [71]. When combined with a CO_3-apatite-collagen scaffold, OPD enhanced neovascularization in a tibial defect model [72].

Osteonectin-Derived Peptides

Osteonectin, also known as secreted protein acidic and rich in cysteine (SPARC), produces two fragments upon cleavage: SPARC113 and SPARC118. Both of these fragments contain the multifunctional tripeptide glycine-histidine-lysine (GHK) and have demonstrated potent angiogenic activity. In an in vivo model, these peptides incorporated into an MMP-degradable hydrogel prompted angiogenesis [73].

Exendin-4

Exendin-4 is an analog of glucagon-like peptide-1 (Glp-1) and it increases human umbilical vein endothelial cells (HUVECs) migration, sprouting, and tube formation in vitro and increases the sprout outgrowth from aortic rings [74].

TP508

TP508 is a 23-amino-acid peptide, commercialized as Chrysalin®. TP508, initially thought of enhancing skin wound repair when topically administered, corresponds to the receptor-binding domain of the human thrombin [75]. This peptide enhances the blood vessel formation and fracture healing. TP508 improves VEGF-stimulated angiogenesis and reduces the effects of chronic hypoxia (Fig. 5) [76].

QK Peptide

A VEGF-derived peptide, known as QK peptide, is the most studied peptide with proangiogenic properties. It binds with the Kdr and Flt-1 receptors, resulting in the stimulation of tube formation by HUVECs in Matrigel in vitro and neoangiogenesis in an ischemic hindlimb model [77].

RoY Peptide

In hypoxic conditions, the RoY peptide has demonstrated angiogenic activity by binding to a 78-KDa endoplasmic reticulum chaperone on the endothelial cell membrane, known as the glucose-regulated protein (GRP78). Moreover, a single local dose of RoY was found to normalize blood perfusion in a mouse hind limb ischemia model [78].

PBA2-1c

A multi-domain peptide, PBA2-1c, contains a heparin-binding domain and 159–163 amino acids from PDGF-BB. PBA2-1c binds with the α and β receptors of PDGF, eliciting cell proliferation, migration, and cell-induced collagen gel contraction, playing a role similar to that of which a recombinant PDGF molecule does [79].

Peptides Involved in Osteoinduction

BMPs are the main osteoinductive molecules in mammalian cells. BMPs belong to the TGF-β superfamily and interact with target cells by activating the intracellular Smad pathway directing gene expression. Out of all the identified 20 BMPs, BMP-2, BMP-4, BMP-6, BMP-7, and BMP-9 have consistently demonstrated the most osteogenic properties, being the most explored ones in the field of bone tissue engineering. A majority of the peptide molecules discussed in this section is derived from the BMPs, however, other sequences identified in the proteins of the ECM prompting osteodifferentiation have also been enlisted here.

BMP-Derived Peptides

BMPs have two sequences denoted as the "wrist" epitope, which binds to the BMP receptor type I, and the "knuckle" that binds to the BMP receptor type II. Contained within the knuckle epitope of BMP-2, a 20-mer sequence (NSVNSKIPKACCVPTELSAI) has osteogenic activity [80].

Recently, a triple functionalized substrate was generated by incorporating a BMP-2-derived peptide with an OPD angiogenic peptide on an RGD-conjugated hydrogel. This substrate induced enhanced mineralization in rat bone marrow stromal (BMS) cells, with increased transcription levels of the vasculogenic markers VE-cadherin and PECAM-1 in response to the OPD peptide. These properties made this triple functionalized substrate of particular interest for bone tissue engineering [81].

A slightly modified version of BMP-2 including the N-terminal phosphoserine and three C-terminal aspartates is P24 peptide. When this peptide was added to a porous nano-HA/collagen/poly-L-lactic acid (PLLA) scaffold with controlled release of the peptide through chitosan microspheres, it promoted the ALP activity in MSCs [82].

Another peptide of this family, BMP-9, also possess abundant osteogenic activity. A peptide derived from the knuckle epitope of the BMP-9, known as pBMP-9, has shown to elicit osteogenic marker mRNAs expression and ECM mineralization to a slight degree. These effects were not as pronounced as those of the growth factors at the equimolar concentration. A higher dose could, however, compensate its lowered activity in prompting transcription of certain markers but not all [83].

In a study, slightly different variants of BMP-2 (RKIPKASSVPTELSAISMLYL), BMP-9-derived peptide (RKVGKASSVPTKLSPISILYK), and BMP-7-derived peptide (RTVPKPSSAPTQLNAISTLYF) were implanted onto RGD-conjugated polyethylene terephthalate (PET) surfaces and compared. The BMP-2 showed the highest activity followed by the BMP-7 and the BMP-9-derived peptides in terms of the expression of osteogenic markers and the ECM thickness [84].

A BMP-2-derived peptide containing the DWIVA pentamer, which corresponds to the BMP receptors I and II binding site sequences, is known as the osteopromotive domain. This peptide has been shown to elicit the proliferation and differentiation of MC3T3-E1 cells in vitro when conjugated to titanium surfaces [85].

$PTH_{1–34}$ or Teriparatide

Teriparatide is the commercialized N-terminal 34-residue fragment of the parathyroid hormone ($PTH_{1–34}$) which retains the major activities of PTH. Teriparatide plays a major role in the regulation of the mineral ion homeostasis. It also prompts osteoblast proliferation, differentiation, and inhibits apoptosis (Fig. 5). In situ bone growth was significantly enhanced by a synthetic scaffold made of polyethyleneglycol containing $PTH_{1–34}$ [86].

Osteogenic Growth Peptide (OGP)

A naturally occurring 14-mer peptide, osteogenic growth peptide (OGP), is primarily found in serum and promotes bone anabolism, leading to increased bone formation and overall bone mass. OGP has a role in the regulation of osteoprogenitor cell proliferation, ALP activity, collagen production, differentiation, secretion of osteocalcin, and ECM mineralization (Fig. 5).

When dissociated, OGP is exposed to proteolytic cleavage, it produces a C-terminal pentapeptide YGFGG known as $OGP_{10–14}$. This pentapeptide activates the intracellular Gi-protein-MAP kinase pathway. Both OGP and $OGP_{10–14}$ enhance the early expression of various markers related to osteogenesis, such as Runx2, ALP, osteopontin, osteoprotegrin, or osteocalcin [87].

Calcitonin Gene-Related Peptide (CGRP)

Calcitonin gene-related peptides (CGRP) have two forms: α and β. α-CGRP, consisting of 37 amino acids, is derived from the Calca gene. β-CGRP is derived from Calcb gene located in close proximity to the Calca gene. CGRP is found in the sensory nerve endings in the periosteum, metaphysis, and bone marrow. CGRP enhances the proliferation and differentiation, and inhibits the apoptosis of osteoprogenitor cells [88]. It also prompts the production of osteogenic molecules such as BMP-2 and IGF-1 (Fig. 5) [89].

Collagen-Binding (CB) Peptide

The collagen-binding (CB) peptide is a 28-residue peptide corresponding to the hydrophobic sequence of the BSP N-terminal. This peptide interacts with type-I collagen and stimulates the osteogenic differentiation of osteoblastic cells through Akt- and ERK-dependent signaling. This results in the increase in type-I collagen, osteopontin, osteocalcin, and ALP mRNA levels. Moreover, an increase in the ALP activity and matrix mineralization were observed [90].

Collagen-Binding Motif (CBM) Peptide

A 28-amino-acid fragment in osteopontin, called collagen-binding motif (CBM) was identified by Lee and coauthors, which is able to bind to type-I collagen. CBM enhanced hBMS cell differentiation as shown by the ECM mineralization and ALP expression. The high level of cellular phospho-Smads due to the peptide, was due to Smad pathway activation. When implanted as a CBM-collagen gel conjugate, accelerated calvarial defect repair was noted in rabbits [91].

Substance P

Substance P (SP) has been observed to enhance type-I collagen, Runx2, and osteocalcin mRNA expression, and matrix mineralization in murine calvarial osteoblasts [92]. Moreover, it prompted dose-dependent proliferation along with enhancing the expression of osteocalcin and ALP at low concentrations and inducing ECM mineralization at higher concentrations [93].

Endothelin-1

Endothelin-1 (ET-1) promotes the proliferation and differentiation of murine osteoprogenitor cells, enhanced formation of mineralized nodules and increased the activity of ALP in cultures [94]. ET-1 secreted by endothelial cells guides the MSC osteogenic and chondrogenic differentiation through AKT signaling [95]. It also plays a crucial role in the regulation of remodeling of the postnatal trabecular bone [96]. Despite its role in osteogenesis and bone remodeling, it is crucial to exercise caution when using it in bone tissue engineering as ET-1 also plays an important part in tumor progression.

BCSP™-1

A nine-amino acid synthetic fragment from human type-I collagen is known as the bone and cartilage stimulating peptide (BCSP™-1). BCSP™-1, when covalently immobilized on a commercial HA and tricalcium phosphate ceramic, can significantly enhance ALP expression by murine calvarial osteoprogenitor cells [97].

CTC Peptide

A peptide derived from the α-subunit of the collagen III C-terminal is known as the CTC peptide. It is a 12-mer cryptic peptide, which exerts a chemotactic effect on the perivascular stem cells (PSCs) and other cells. It also increases the transient expression of various osteogenic markers, such as osteopontin or type-I collagen. CTC has also been observed to stimulate ALP activity and accelerate matrix mineralization, without an increase in proliferation rate [98].

Cathelicidins

Cathelicidins are antimicrobial peptides found in cells of innate defense systems, various epithelial cells, bone marrow stroma, and MSCs. One of the cathelicidins-derived peptide, LL-37, has shown to induce monocyte differentiation to novel bone forming cells. These cells are known as monoosteophils and possess bone forming capabilities that could enhance the repair of femoral defects in mice [69].

Advantages of Peptides

The therapeutic use of peptides in tissue engineering is growing significantly as peptides offer several advantages over proteins (Fig. 6).

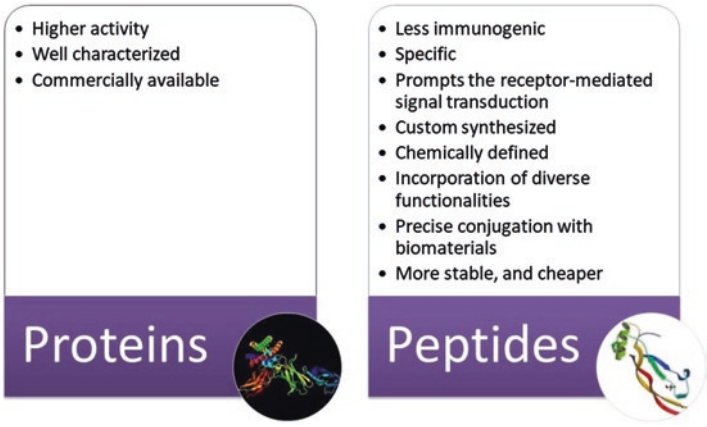

Fig. 6 Main advantages of proteins and peptides for tissue engineering [47]

Defined Chemical Properties of Peptides

Peptides are chemically defined, which is the most useful property of these biomolecules. This advantage consequently enables the refinement of their structures, corresponding experimental designs for discovering novel peptides or their combinations, and precise molecular manipulations necessary for mechanistic studies [99].

Incorporation of Non-native Chemistries and Functions into Peptides

Due to the possible synthetic methods of production, there are huge prospects of connecting moieties to peptides that are not routinely accomplished in expressed proteins or tissue-derived scaffolds. These functional groups can be natural amino acids apart from the genetically encoded ones, D-amino acids, amino acids with fluorinated side chains, polymer bioconjugates, fluorescent labels, chemical functionalities for cross-linking or polymerization, and posttranslational modifications. Chemically derived DOPA containing peptides, peptide-polymers, and peptidomimetics have been synthesized to coat and modify various synthetic biomaterials, thus, furnishing these synthetic scaffolds with adhesive properties [100].

Diverse Functions of Peptides

Peptides can possess a wide variety of functions besides cell binding, growth factor binding, surface binding, matrix-binding, self-assembly, and specific proteolytic susceptibility.

The possibility of peptide customization ensures that more efficient discovery and development of additional moieties is conceivable [99].

There are several functionalities that can be installed within a scaffold through peptides, though enzyme substrate sequences have been particularly studied, especially for hydrogels. Scaffolds can be designed that can degrade with controllable kinetics by various proteases including plasmin or matrix metalloproteinases, using peptide sequences of varying proteolytic susceptibility [101]. This enables the custom degradation of biomaterial or release of a matrix-tethered cargo through proteolysis [102].

Peptides can also be tailored to have growth factor binding abilities. This strategy has been utilized to identify VEGF-binding heparin-mimicking sulfated peptides by using bead-peptide libraries of sulfated peptides [103] or via rational design [104].

Peptides can also be engineered to self-assemble, a basic property that is native to ECM proteins. A number of peptides have been engineered and used in biomate-

rials for their self-assembling properties. These custom-made peptides include peptide amphiphiles [105], β-sheet fibrillizing peptides [33], short aromatic peptide derivatives [106], coiled coil peptides [107], β-hairpins [108], and others [109]. In engineering the custom peptides, design rules have been worked out to achieve predictable assembly into networks, fibers, gels, and tactics to decking these with functional peptide sequences which continue to be reported [28, 33].

Conjugation Capability of Peptides onto Biomaterials

Peptide conjugation onto or within synthetic scaffolds is achievable. This can be accomplished through specific chemistries, using a wide range of strategies such as the Michael addition of cysteine-containing peptides to vinyl sulfones, commercially available cross-linkers, UV-initiated cross-linking, amine/carboxylic acid coupling, acrylate or maleimide conjugation, and chemoselective chemistries such as "click" chemistries and native chemical ligation [99].

Enhancing Biofuntionality of Biomaterials Through Peptides

Materials from bioceramics and polymers to peptide-based scaffolds that enhance bone regeneration are applicable clinically for the treatment of diverse bone fractures and spinal fusions for different parts of the body. Current challenges to engineer materials for bone tissue engineering includes designing new materials mimicking the mechanical and biological context of bone tissue matrix supported with a vascularization system. Construction of new topographical features at nanoscale and functionalization of material surfaces with biological cues from the extracellular environment or any other biological environment are emerging approaches to design new biomimetic materials with desirable functions for bone tissue engineering [5].

Peptides as Coating Materials

Materials, which create a platform for tissue formation, play a significant role in nearly all tissue-engineering approaches. They are the skeleton of newly forming tissues with mechanical support. They also deliver inductive molecules or cells, which have the potential to control the structure and function of newly created tissue, to the site of interest. The interaction of biomaterials with biological systems is a very critical issue that needs to be considered in the design of any biomaterial. Features of biomaterials restricting the nonspecific adsorption of proteins on the surface result in weak interactions with cells for tissue formation. Thus, designing

new materials which enhance cell attachment and adhesion through the presentation of peptides, proteins, and GAGs that bind to cell surface receptors and trigger a desired cell response is essential [1, 35].

In addition, multifunctional coatings with increased specificity and activity are also required to enhance the function of materials via various kinds of biomolecules. To maintain multifunctionality, a diverse bio-functional surface has the potential to control interactions between the surface and the surrounding biological environment via immobilizing different cell adhesive motifs or molecules [35].

As we mentioned above, biomoleclues especially peptides, are good candidates for bio-functionalization of materials with their diverse functions. Peptides and proteins, which we also listed above, are classified according to their source. Most peptides are derived from the ECM that surrounds and organizes cells into tissues or are derived from secreted proteins or peptides by cells into surrounding fluids. Proteins purified from a natural source and recombinantly manufactured ones allow researchers to identify the function and physicochemical properties of proteins for tissue regeneration regulations. Having knowledge about their critical domains through biological assays and bioinformatics tools created the possibility of utilizing synthetic peptides. Their presenting strategy can affect their functions. They can be presented either in an immobilized form from the surface of a material or releasable form for a material to interact with cells. All these surface functionalization strategies define the quality and functionality of biomaterials. In the following section, general techniques utilized for surface functionalization will be discussed [1].

Biomaterial Functionalization Strategies

There are various applicable strategies to functionalize biomaterials with biomolecules. The major principle of immobilization strategies is to increase the stability and functionality of biomolecules. However, the random orientation and structural deformation can occur during immobilization of molecules. It can cause to diminish activity of the biomolecules. This challenge becomes critical to differentiate immobilization strategies depending on binding strength, modularity, and complexity [110].

Especially, poor binding affinity, uncontrolled release, or strong attachment are all factors to determine the efficiency of immobilization strategies. For example, uncontrolled release of the cell adhesive motif RGD from the surface because of loose attachment can block integrin-mediated adhesion and thereby provoke apoptosis [111]. Conversely, strong anchoring of biomolecules can block biological signals that cause cell recruitment by chemokines. Hence, a feasible balance between controlled release and firm attachment is essential for efficient immobilization.

Physical and chemical immobilizations are two major techniques for the biomolecular functionalization of biomaterials. In the following sections, these techniques will be discussed in detail.

Physical Immobilization

Physical immobilization or adsorption is a very simple immobilization method performed under mild conditions. Surface functionalization with this methodology occurs by dipping biomaterials into a solution of proteins. Therefore, experimental conditions such as pH, temperature, and solvent define biomolecule-binding strength. These dependencies of experimental conditions determine whether there is stable attachment or not. However, this method is hardly disruptive to the biomolecules. Intermolecular forces, mainly ionic bonds and hydrophobic and polar interactions, specify biomolecular adsorption strength on the surface. Mostly, proteins can be attached on surfaces via electrostatic, and hydrophobic or hydrogen bond interactions. The forces involved in adsorption are weak which causes reorientation of molecules through conformational and condition dependent changes [35, 112]. On the other hand, the optimal conformation for each molecule, which minimizes the repulsive forces from the surface and previously attached proteins, can regulate to form a heterogeneous and randomly oriented biomolecule layer on the surface [113].

These conformational changes can be explained through the enthalpic or entropic state of the biomolecule. For example, the net free energy change must be negative during protein adsorption at a surface. The ordered structural content of many proteins decrease during the adsorption process through conformational changes. This fact yields an entropic gain and may act as an adsorption-driving force from an enthalpic point of view. The adsorption-driving force may originate from the interactions between protein and surface. The most important ones are van der Waals, hydrophobic, electrostatic interactions, and hydrogen bonding [114].

Surface properties of the inorganic materials lead to physical attachment of proteins. The surface hydrophobicity, charge, morphology, and topography are physical parameters which have control over a wide range of protein adsorption. For example, hydrophilic interactions are more favorable for protein adsorption on to the surface due to the nature of the protein. In terms of surface physical features, cells like rough surface topographies and morphologies for their attachment and adhesion [112, 114, 115].

Among all of the inter- and intramolecular forces we mentioned above, electrostatic interactions that are defined via the charge of biomolecules and the surfaces, can directly control adsorption as the most efficient forces to anchor positively charged molecules to negatively charged biomaterial surfaces or vice versa. Therefore, the affinity of electrostatic interactions can be tuned by raising the number of charged residues such as in poly-lysine or other charged amino acids. Other such cooperative effects could be coordinative and hydrogen bond interactions [35, 116] (Fig. 7).

Covalent Immobilization for Surface Functionalization

Covalent attachment is frequently utilized and is the most preferable for the immobilization of peptides, enzymes, and adhesive proteins onto material surfaces [1].

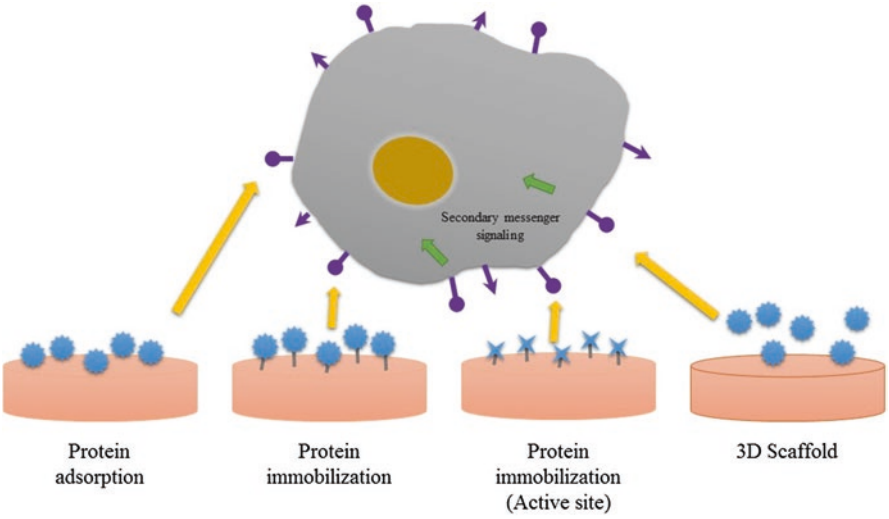

Fig. 7 Surface functionalization for biomolecules [1]

In this section, various chemistries and treatments have been applied so far in terms of covalent coupling. Alkanethiol, silane, carbodiimide, phenyl azides, acrylate, DOPA are well-known coupling agents used through chemical and photo immobilization, plasma treatment, and click chemistry as a treatment method and have been applied on biomaterial surfaces for the attachment of biomolecules [117].

Among them, carbodiimide-coupling chemistry is one of the most preferable approaches for conjugating proteins covalently to other molecules. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) is used as a bioconjugation agent for the intermediate conjugation reaction to form amide bonds in between the carboxylic functional groups and the amino groups [117–119].

Silanization is another well-known strategy to introduce functional groups of molecules to the surface. A surface modified with an alkoxysilane such as (3-aminopropyl) triethoxysilane (APTES) covered by hydroxyl groups, which will be attractive for the coupling reaction with amino groups. Subsequently, the surface can be modified directly or thiol-containing biomolecules as a cross-linker can be used to enable the immobilization. This covalent strategy has disadvantages as a complex and sensitive surface treatment and potential hydrolysis of the siloxane bond, which would result in release of the bioactive moiety [35, 120].

Click chemistry is a new approach other than traditional esterification and amidation conjugation reactions. The basis of the click chemistry relies on regiospecificity. Small units together with heteroatom links were introduced to the surface through simple reaction conditions with high yield, and stereospecificity to produce newly designed materials. This chemistry triggers the reactions that can be selectively performed in the presence of natural occurring functional groups (bioorthogonal). The reaction proceeds mostly in water and it results in high yields [121].

Side and cross-reactions define whether the reactions are carried out simultaneously in one pot or stepwise. The reaction conditions and high yields make this chemistry highly suitable to covalently ligate molecules for biomaterial coatings [35, 117, 121].

To functionalize a surface with biomolecules is not only dependent on properties of the coupling agent and biomaterial surface but also functional groups in biomolecules that can be reacted by cross-linking or its derivative reagent. For peptide conjugation to any biomaterial surface, amino acid side chains are the most significant entity for covalent coupling. For example, peptides and proteins are composed of over 20 amino acids, which are identified by their side chain chemical structure, charge, hydrogen bonding, and reactivity properties, polymerized together through the formation of peptide bonds. The side chain of amino acids is free to interact and react with their environment. However, this interaction is limited by features of the side chains. For example, the aliphatic and aromatic residues are often located at the interior of the protein molecules due to their hydrophobic nature. The other amino acids (Asn: asparagine, Thr: Threonine, and Ser: Serine) are hydrophilic and contain relatively polar residues located near surfaces where they can be interacting with the surrounding aqueous environment. However, modifying Asn, Thr, and Ser, which are often posttranslationally modified with carbohydrates, with common reagent systems under aqueous conditions is difficult. The difficulty of this modification relies on the same nucleophilicity for hydroxyl and amide portions [1].

The other amino acids such as aspartic acid (Asp), glutamic acid (Glu), lysine (Lys), arginine (Arg), cysteine (Cys), histidine (His), and tyrosine (Tyr) have ionizable side chains that are suitable for covalent coupling. Each of these side chains can be in nucleophile to place in a reaction when they are in an unprotonated state. For example, Asp and Glu contain carboxylate groups that have ionization capability similar to C-terminal alpha carboxylate. Derivatization of carboxylate groups can be made through the use of amide bond forming agents or through active ester or reactive carbonyl intermediates. Lys, Arg, and His are good candidates for alkylation and acylation reactions due to their ionizable amine containing side chains. The imidazole ring of His makes it reactive species for electrophilic reactions. Cys is the only amino acid containing a sulfhydryl group, which gives a critical role to Cys in protein stabilization. The most important modification reaction of peptides and proteins is the derivatization of the side chain sulfhydryl of cysteine. The functional groups of side chains together with the N-terminal α-amino and the C-terminal α-carboxylate form the full complement of polypeptide reactivity [1].

These approaches are more complicated and time consuming than other immobilization methods. A major limitation of this methodology is the loss of protein mobility when they are immobilized on the surfaces, which is directly affected by possible representation of unfamiliar protein conformation on the surface. Remains of toxic monomer residues on the surface may cause biocompatibility problems in the area of implantation. Toxic monomer residues, which are directly caused by the instability of the molecules on the surface, are the most challenging issues in the task of chemical immobilization. Physical adsorption techniques can be addressed to reduce toxic residues. While it may help reduce the effect of toxic residues, its

weak immobilization capability brings more problems to surface functionalization [122, 123].

Therefore, there is a need for alternative methodologies for surface functionalization in bone tissue engineering. In the next section, a new strategy based on peptide-based surface functionalization through material recognition capability will be discussed more in detail.

Material-Binding Peptides for Surface Functionalization

In recent years, material-binding peptides (MBPs) have shown remarkable potential in various application areas, taking advantage of molecular biomimetics. The idea behind this is to discover and design MBPs mainly inspired by nature and a biomimetic approach. This approach is revealed at the intersection of different disciplines such as material science and molecular biology. It mainly covers understanding the interactions between materials and biomolecules by learning from nature [124, 125].

All biological materials are highly organized often in a hierarchical manner from the molecular to the nanoscale, with complex nano-architectures [126, 127]. The contribution of biomacromolecules such as lipids, proteins, glycoproteins, and phosphoproteins, etc. is a key aspect of this architecture. For example, proteins control the formation of hard tissues like teeth, bone, and many other hard tissues occurring in different organisms. Due to the role of proteins for the fabrication of materials in nature, MBPs can be good candidates to fabricate, design, assemble, and functionalize many materials from various fields due to their molecular recognition and binding capabilities [123, 128–130].

Especially in bone tissue engineering, inorganic surface and protein/peptide or any biomolecule interactions is a key aspect due to bone structure which shows composite material features. The major concern in bone tissue engineering and implants is the uncontrolled interactions between synthetic materials and human tissues. The most successful approach to this issue is functionalization of the biomaterial surfaces with different molecules with desired functions including anti-fouling polymers or cell growth factors. To date, physical and covalent immobilization methods have been applied onto varieties of biomaterial surfaces. Covalent immobilization requires the presence of specific functional groups and synthetic pathways. Moreover, the functional groups used in these strategies have low selective properties, which restrict their use as we discussed in previous sections [110].

On the other hand, the behavior, stability, and cytotoxicity of the modified surfaces for all strategies under physiological conditions are not well understood. Therefore, material selectivity, coupling efficiency, and flexibility remain a challenge at the biomaterial interface. MBPs can provide a new platform for surface functionalization via immobilization of biomolecules with controlled attachment and assembly on solid surfaces [110, 123, 131].

During the last decade, MBPs have been selected using phage and cell surface display techniques possessing affinity and specificity to select inorganic surfaces [132, 133].

Their potential use was shown in many disciplines including surface functionalization, and biomineralization for tissue engineering and regenerative medicine [125]. There has been a great deal of interest in identifying and characterizing peptides that bind to various materials, such as TiO_2 [134], Au [133], SiO_2, and HAP utilized especially in tissue engineering and regenerative medicine [135].

In this section, two case studies will be demonstrated to give basic perspectives for the promising potential of MBPs in implantation and hard tissue engineering as molecular linker and material synthesizer.

In the first example, the capability of MBPs as molecular linkers will be given. Yazici et al. selected titanium binding peptides (TiBPs) through cell surface display and conjugated them with RGDS, which is a cell adhesive peptide to show their molecular linker capability for implant surface functionalization [110, 123].

These peptides can be conjugated with a variety of bioactive molecules to enhance cell attachment, cell proliferation, cellular spreading, and other cell behaviors or creating antimicrobial or anti-fouling surfaces. Therefore, these peptide-based molecular linkers provide a new platform to conjugate domains with different functionality. In this case study, TiBP was conjugated with biologically active signaling molecules (RGDS) while retaining their remarkable binding and selectivity to a solid substrate in the absence of cytotoxicity properties [123]. This study proved that a TiBP-RGDS bifunctional peptide enhanced osteoblast attachment and adhesion on a titanium implant surface. The proposed peptide-based surface coating can be applied on various materials to induce various desired biological activities on any biomaterial using an easily adaptable single-step biologically relevant set of conditions [110, 123, 128, 129].

In another example, the capability of MBPs as a synthesizer in the biomineralization process will be given. In nature, biomineralization, which occurs under mild conditions to form bone, teeth, sponge spicules, and similar tissues with nanoarchitecture in various organisms, has attracted attention in the field of bionanotechnology. Biological organisms have a capability to synthesize their inorganic structures or hard tissues with unique morphological, structural, and functional properties. This biological mineralization process usually involves a large number of proteins with various temporal and spatial distributions. To understand the exact role of proteins in the biomineralization process and biological material synthesis, the traditional approach, which involves extracting and purifying proteins from the organism of interest, has been utilized [136]. Although there are exciting examples for performing biomineralization using isolated proteins, this approach is limited because of the difficulties involved in the extraction and purification steps of these proteins from biological systems. De novo design is another approach that stems from the prediction of functional sequence of proteins using computational methods. Biomineralization through extracted proteins or de novo designed peptide sequences remains elusive due to impractical identification of all proteins and their sequences [137].

MBPs offer a unique and a more practical approach in the biomineralization process. MBPs have a recognition capability to solid materials; this recognition capability may influence the fabrication process of inorganic materials as well. To

prove this, Gungormus et al. have explored the possibility of HA-binding peptides to regulate calcium phosphate formation in vitro. They found that the formation of calcium phosphate mineral could be controlled via a strong-binding peptide HABP1. The rate of mineralization decreased by the addition of HABP1, resulting in the formation of much larger plate-like particles compared to control samples. Meanwhile, the rate of transformation of the amorphous phase to the crystalline phase increased. The transition between the two phases can happen via interactions of HABP1 in the amorphous mineral surface. This interaction may stabilize the crystal structure by lowering surface energy, therefore, resulting in a growth-dominated mineralization pathway [137, 138].

As given in the examples above, MBPs have a great potential in biomaterial surface functionalization and the biomineralization process. Biofunctionalization of biomaterials through peptides with various methodologies are summarized in Table 2.

Peptides as Scaffold Materials

Self-Assembled Peptides

Self-assembling proteins and peptides have a remarkable potential due to their unique features as scaffolds for applications in tissue engineering. Their self-organization capability from basic building blocks to forming supramolecular structures and simulating the native ECM, make them as preferable scaffold materials for tissue engineering applications. Favorable properties of self-assembling peptides are mainly based on their modification capability at the sequence level. Moreover, their synthesis does not rely on difficulties of a recombinant protein expression and purification system. They can be easily produced through solid phase peptide synthesis. On the other hand, recombinant technologies can be the only alternative to solve any homogeneity and standardization issues necessary for applications [139, 140].

The unique structural properties of the self-assembled peptides are relevant with alternating hydrophilic and hydrophobic amino acid residues. Under physiological conditions or mild conditions, these residues spontaneously adopt a β-sheet structure when exposed to monovalent cation solutions. This process finalizes with the formation of self-assembled matrices with certain geometries. The RADA-16 series, which is commercially available (PuraMatrix), ELK, and EAK are well-known examples for self-assembled scaffolds in the literature. The RADA series is also the best example for the commercialization of self-assembled peptide-based scaffolds [141, 142].

Among self-assembling biomolecules, peptide amphiphiles are another class of peptide-based scaffolds that can enhance osteoprogenitor cells and prompt their differentiation. Many studies in the literature revealed the role of peptide amphiphiles through mineralized matrixes in promoting osteogenic differentiation of hMSCs

Table 2 Peptides and their assembling methodology and function [35, 110, 123]

Bioactive	Surface	Immobilization	Assembly	Favored cellular functions
BMP 4	Ti	Carbodiimide mediated	Covalent	Proliferation, mineralization
BMP-2	PLGA	Acylate-NHS-PEG	Covalent	Mineralization
BMP-2 derivative	Alginate	Carbodiimide mediated	Covalent	Mineralization
OGP	Si	Click Chemistry	Covalent	Mineralization
KRSR	Ti	Silanization	Covalent	Spreading, adhesion, mineralization
RGD + FHRRIKA	Si	Silanization	Covalent	Spreading, mineralization
RGD + PHSRN	Ti	Thiol	Covalent	Spreading, proliferation
RGD + BMP-2 + hydroxyapatite	Ti	DOPA	Covalent/ electrostatic	Adhesion, mineralization
RGD + bFGF	Si	Spin coating/ thermal annealing	Covalent/ electrostatic	Spreading, focal adhesion
OGP + fibronectin	Ti	Adsorption/ co-precipitation	Electrostatic	Adhesion, proliferation, differentiation
Heparin + 1 aminin + bFGF	PLLA	Covalent	Covalent/ electrostatic	Neurite outgrowth
Heparin + BMP-2	Ti	Silanization	Covalent/ electrostatic	Anti-inflammatory, proliferation, mineralization
Heparin + VEGF + fibronectin	Ti	Electrostatic	Layer by layer electrostatic	Anti-coagulative, adhesion, proliferation
Heparin + SDF-1	PGS	Electrostatic	Electrostatic	Progenitor cell recruitment
GFOGER	PEG	Covalent	Covalent	Bone regeneration, osseointegration
Chitosan + BMP-2	Ti	Covalent	Electrostatic	Differentiation
Hyaluronic acid + collagen	Ti	Silanization	Layer by layer covalent	Adhesion, proliferation, differentiation
Hyaluronic acid + collagen	Ti	Electrostatic	Electrostatic	Non-pathological smooth muscle cell phenotype
Collagen binding motif	Ti	Electrostatic		Osteogenic differentiation

(continued)

Table 2 (continued)

Bioactive	Surface	Immobilization	Assembly	Favored cellular functions
Collagen + lactoferrin	Ti	Electrostatic	Electrostatic	Adhesion, proliferation, differentiation
Collagen + CS + BMP-4	PLGA	Electrostatic	Electrostatic	Increase of bone-implant contact
Fibronectin-derived	Ti	Covalent, electrostatic	Covalent/ electrostatic	Cell spreading, adhesion

BMP bone morphogenetic proteins, *OGP* osteogenic growth peptide, *FGF* fibroblast growth factor, *VEGF* vascular endothelial growth factor, *SDF-1* stromal cell derived factor, *CS* chondroitin sulfate, *PEG* polyethylene glycol, *PGS* poly(glycerol sebacate), *PLGA* poly(lactic-*co*-glycolic acid), *PLLA* poly(lactic-*co*-glycolic acid)

and bone formation. On the other hand, peptide amphiphile matrices functionalized with MSCs and platelet-rich plasma were demonstrated to encourage bone formation and improve angiogenesis [105, 143, 144].

Peptide-Based Biomaterial Scaffolds

Synthetic biomaterials are a necessity for the controlled release of drugs, tissue restoration, and tissue engineering. One of the most common advantages shared by all synthetic scaffolds is reducing the possibility of carrying biological pathogens or contaminants [10]. Moreover, synthetic biomaterials can be engineered to meet particular needs with their promising in vivo biocompatibility. Recently, newly designed biomaterials have displayed a tremendous enhancement for in vivo biocompatibility.

Each synthetic material is composed of different substitutes, such as calcium phosphate and amino acids are substitutes for ceramics and for peptides, respectively. Some synthetic scaffolds are comprised of molecules that are not found in vivo such as ceramics or metal-based materials. However, they display desired features such as high tensile strength (e.g., bone tissue replacement materials). Other classes of materials that were discussed here are mainly peptide-based materials that are composed of spontaneously self-assembling oligopeptides and were discovered recently. One of the advantages of peptide-based scaffolds is their design flexibility that will allow us to conjugate them with various molecules that have different functions. For example, biological functional domains that enhance cell adhesion such as the cell attachment motif RGD, an integrin receptor-binding ligand or any peptide with mineralization and cell differentiation capacity, can be easily incorporated during synthesis of these peptides. This function allows researchers to gain various functions in one material. On the other hand, amino acids as a monomer of peptide-based scaffolds display outstanding physiological compatibility and minimal cytotoxicity. Having a biological substitute is an advantage for a scaffold

design due to their breakdown products of biologically derived biomaterials and can be incorporated into synthesized biomolecules or metabolized in the host organism [142].

As all peptide-based scaffolds, the peptide amphiphiles are also composed of a unique sequence of amino acids which comprise of repeating units of positively charged (lysine or arginine) and negatively charged (aspartate or glutamate) amino acids with hydrophobic residues (alanine or leucine) in between. These self-complementary peptides consist of 50% charged amino acids. Therefore, their self-complementary properties rely on the type and sequence of their amino acid substitute [142, 145, 146].

Among peptide-based scaffolds, RAD16-I, which has the sequence AcN-RADARADARADARADA-CNH_2, and RAD16-II, which has the sequence AcN-RARADADARARADADA-CNH_2, are well-known examples in terms of their clinical use. Though both of these peptides have the same length and number of residues, RD16-I possesses a spacing modulus of one based on the formula (RADA)n, contrarily, RAD16-II has a spacing modulus of two based on the formula (RARADADA)n, where n denotes any number of repeats.

The self-assembly of peptides depends on various factors, such as peptide and salt concentration, which may determine the geometry and dimensions of the macroscopic matrices either as tapes, strings, or sheets. Circular dichroism (CD) is one common technique to define the structure of peptide-based scaffolds. It is a very important technique to define structure related parameters, which are very crucial techniques to design peptide-based scaffolds. CD spectroscopy revealed that RAD-, EAK-, and ELK-based peptides with their representative periodicities displayed strong β-sheet secondary structure in aqueous solutions. These secondary structures displayed two distinctive polar and nonpolar surfaces with simple rules of amino acid sequence and type, which give the structural property of the scaffold [147–149]. Having defined structural properties through this simple rule should be explained with some predictions or paradox. For example, the measured β-sheet secondary structure of RAD, EAK, and ELK peptides opposed anticipations based on the Chou-Fasman statistical predictions for protein helical preferences. Glutamate, leucine, and lysine all have high α-helical tendency in the Chou-Fasman model [13].

The secondary structure of self-assembling synthetic peptides is an outcome of when local and nonlocal intramolecular influences compete. Generally, local influences for defining secondary structures include the intrinsic helical propensity of amino acids. Nonlocal influences are illustrated by the periodicity and positioning of amino acids in the context of the peptide sequence—periodicity and amino acid positioning determined secondary structures for all synthetic peptides. Thus, nonlocal effects predominated over local effects [142, 149].

Interestingly, the role of local and nonlocal forces can be affected by environmental conditions in which the self-assembly occurs. Amphiphilic peptides, such as RAD16 and EAK16, can be solubilized at low millimolar concentrations in salt-free aqueous solutions. However, the amphiphilic peptides spontaneously form hydrogel-like matrices when the peptide solutions are exposed to salt solutions or physiologi-

cal media. The ordered matrix formation is due to millimolar levels of monovalent cations. The ordered biomatrix comprises a hydrogel with a water content of >99%.

Contrary to the ordered biomaterial matrix that forms when exposed to monovalent cations, EAK16 and related peptides form highly disordered materials in the presence of millimolar levels of divalent cations. This demonstrates that the concentration of salt is critical to trigger a molecular switch to form a matrix. One explanation for this can be that the matrix formation is triggered due to the electrostatic interactions that occur through the salt between the negatively and positively charged amino acids of the adjacent peptides. Consequently, promoting the staggered configuration of the individual peptide. Alternatively, as these peptides are self-complementary in aqueous solutions, monomeric peptides might undergo folding to form intramolecular electrostatic interactions. However, if salt is added, it could disrupt the intramolecular electrostatic interactions, directing the peptides to adopt a configuration that favors intermolecular hydrophobic interactions between adjacent peptides.

Although salt concentration effects matrix formation, the length of the peptide and degree of hydrophobicity of the aliphatic amino acids are also critical. For example, amphiphilic peptides containing alanine (such as EAK16) form a salt-induced stable matrix when at least 16-mer peptides are present [9]. Amphiphilic peptides containing leucine (such as ELK8), by contrast, require eight-mer peptides to form salt-induced stable matrices. These observations show that increasing the hydrophobicity of the aliphatic residue contributes to matrix formation. A third hierarchal model includes features of both earlier models. The matrix could be stabilized as a result of the electrostatic intermolecular interactions between the charged amino acids of two adjacent peptides after the intermolecular hydrophobic interactions are formed. Moreover, other conditions of the process, such as the temperature and pH, can be adjusted to direct the resulting self-assembling matrix geometry. All of these models of peptide-based matrix formation and stabilization require further direct experimental confirmation to understand the function of each experimental condition [17, 150]. The sequence of the peptide may not have the only role for gel formation. For example, the RAD-based amphiphilic peptide sequence shares similarity to the ligand that binds with the cell adhesion receptor integrin RGD. Some of the ECM proteins contain RAD sequences that can bind to the isoforms of integrin [151]. The first hypothesis that was tested was if the cells can adhere and grow on peptide-based scaffolds in an integrin-dependent manner. Cell adhesion to RAD- and EAK-based scaffolds do not involve integrin binding [152], and both of these matrices promote cell adhesion and growth. Though the RAD sequence can bind to certain integrin receptors, the EAK does not bind to integrins. Moreover, high concentrations of RGD peptides do not affect cell attachment to RAD- and EAK-based matrices, which confirms that integrin-based attachment is not crucial for cell adhesion to these peptide-based matrices. The RAD- and EAK-peptide scaffolds support cell adhesion of diverse types of mammalian and avian primary and transformed cells [7, 17, 142, 150].

Conclusion

The expanding need of interventions for bone tissue regeneration can be met with the use of biomimetic peptides. These peptides have various advantages that render them useful for tissue engineering including their compact size. These small molecules with simple structures can be customized to include many properties such as directed immobilization. The peptides can also be used to functionalize the osteoinductive biomaterials which can enhance the cell attachment, differentiation, and phenotype development. These biomimetic peptides can confer bioactivity that synthetic scaffold lack, which can lead to better biomaterial–host interaction. Numerous peptides have been developed and explored for bone repair. However, inadequate verification from clinical trials limits the use of these peptides. These peptides have potential for tissue engineering, which needs to be explored more. Further investigations will result in biological molecules that can be utilized in the clinical settings for bone tissue engineering. The areas that need to be explored more in this regard include one of the most common problem of stability of the peptides resulting in low bioavailability and short duration of activity due to proteolysis. This can be achieved by improving the peptide designs incorporating cyclization, nonnatural amino acids, and stable peptide bonds. Moreover, polytherapy, i.e., combining several distinct peptides targeting specific bone repair phase or a specific population of cells involved in bone healing, should be explored further. This strategy can be used to couple osteoconductive peptide with another osteoinductive peptide or a vascularization inducing peptide to increase the bone healing. Another field to be expanded is the scaffolding technologies to incorporate controlled release of peptides to provide correct signal at the precise stage of the repair pathway. Therefore, further research in the peptide design and scaffolding technologies aiming for the upregulation of osteoinduction and osteoconduction is desirable to lead to future treatment involving biomimetic peptides in clinical setups.

Acknowledgments Dr. Hana'a Iqbal is jointly supported by institutional post-doctoral fellowship program, International Center for Chemical and Biological sciences (ICCBS) in Pakistan and the TUBITAK Marmara Research Center (MAM) in Turkey. The authors also thank TUBITAK-MAM for research funding (Project Code: 5183402).

References

1. Hirano Y, Mooney DJ (2004) Peptide and protein presenting materials for tissue engineering. Adv Mater 16(1):17–25. https://doi.org/10.1002/adma.200300383
2. Agarwal R, García AJ (2015) Biomaterial strategies for engineering implants for enhanced osseointegration and bone repair. Adv Drug Deliv Rev 94:53–62
3. Ho-Shui-Ling A, Bolander J, Rustom LE, Johnson AW, Luyten FP, Picart C (2018) Bone regeneration strategies: engineered scaffolds, bioactive molecules and stem cells current stage and future perspectives. Biomaterials 180:143–162
4. Calcei JG, Rodeo SA (2019) Orthobiologics for bone healing. Clin Sports Med 38(1):79–95

5. Stevens MM (2008) Biomaterials for bone tissue engineering. Mater Today 11(5):18–25
6. Roseti L, Parisi V, Petretta M, Cavallo C, Desando G, Bartolotti I, Grigolo B (2017) Scaffolds for bone tissue engineering: state of the art and new perspectives. Mater Sci Eng C 78:1246–1262
7. Chan K, Zhuo S, Ni M (2013) Natural and synthetic peptide-based biomaterials for bone tissue engineering. OA Tissue Eng 1(1):6
8. Nalla RK, Kruzic JJ, Kinney JH, Balooch M, Ager JW, Ritchie RO (2006) Role of microstructure in the aging-related deterioration of the toughness of human cortical bone. Mater Sci Eng C 26(8):1251–1260. https://doi.org/10.1016/j.msec.2005.08.021
9. Finkemeier CG (2002) Bone-grafting and bone-graft substitutes. JBJS 84(3):454–464
10. Giannoudis PV, Dinopoulos H, Tsiridis E (2005) Bone substitutes: an update. Injury 36(3):S20–S27
11. Pan P, Chen X, Metavarayuth K, Su J, Wang Q (2018) Self-assembled supramolecular systems for bone engineering applications. Curr Opin Colloid Interface Sci 35:104–111
12. Rezwan K, Chen Q, Blaker J, Boccaccini AR (2006) Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering. Biomaterials 27(18):3413–3431
13. LeGeros RZ (2008) Calcium phosphate-based osteoinductive materials. Chem Rev 108(11):4742–4753
14. Rodrigues C, Serricella P, Linhares A, Guerdes R, Borojevic R, Rossi M, Duarte M, Farina M (2003) Characterization of a bovine collagen–hydroxyapatite composite scaffold for bone tissue engineering. Biomaterials 24(27):4987–4997
15. Chen K-Y, Shyu P-C, Dong G-C, Chen Y-S, Kuo W-W, Yao C-H (2009) Reconstruction of calvarial defect using a tricalcium phosphate-oligomeric proanthocyanidins cross-linked gelatin composite. Biomaterials 30(9):1682–1688
16. Yu X, Tang X, Gohil SV, Laurencin CT (2015) Biomaterials for bone regenerative engineering. Adv Healthc Mater 4(9):1268–1285
17. Habibovic P, Yuan H, van der Valk CM, Meijer G, van Blitterswijk CA, de Groot K (2005) 3D microenvironment as essential element for osteoinduction by biomaterials. Biomaterials 26(17):3565–3575
18. Chai YC, Carlier A, Bolander J, Roberts SJ, Geris L, Schrooten J, Van Oosterwyck H, Luyten FP (2012) Current views on calcium phosphate osteogenicity and the translation into effective bone regeneration strategies. Acta Biomater 8(11):3876–3887
19. Li X, Liu H, Niu X, Fan Y, Feng Q, Cui F z, Watari F (2011) Osteogenic differentiation of human adipose-derived stem cells induced by osteoinductive calcium phosphate ceramics. J Biomed Mater Res B Appl Biomater 97(1):10–19
20. Ripamonti U (2006) Soluble osteogenic molecular signals and the induction of bone formation. Biomaterials 27(6):807–822
21. Barradas AM, Fernandes HA, Groen N, Chai YC, Schrooten J, van de Peppel J, van Leeuwen JP, van Blitterswijk CA, de Boer J (2012) A calcium-induced signaling cascade leading to osteogenic differentiation of human bone marrow-derived mesenchymal stromal cells. Biomaterials 33(11):3205–3215
22. Liu H, Yazici H, Ergun C, Webster TJ, Bermek H (2008) An in vitro evaluation of the Ca/P ratio for the cytocompatibility of nano-to-micron particulate calcium phosphates for bone regeneration. Acta Biomater 4(5):1472–1479
23. Breitwieser GE (2008) Extracellular calcium as an integrator of tissue function. Int J Biochem Cell Biol 40(8):1467–1480
24. Murphy WL, Peters MC, Kohn DH, Mooney DJ (2000) Sustained release of vascular endothelial growth factor from mineralized poly (lactide-co-glycolide) scaffolds for tissue engineering. Biomaterials 21(24):2521–2527
25. Krishnan L, Willett NJ, Guldberg RE (2014) Vascularization strategies for bone regeneration. Ann Biomed Eng 42(2):432–444
26. Kanczler JM, Oreffo RO (2008) Osteogenesis and angiogenesis: the potential for engineering bone. Eur Cell Mater 15(2):100–114

27. Salbach-Hirsch J, Ziegler N, Thiele S, Moeller S, Schnabelrauch M, Hintze V, Scharnweber D, Rauner M, Hofbauer LC (2014) Sulfated glycosaminoglycans support osteoblast functions and concurrently suppress osteoclasts. J Cell Biochem 115(6):1101–1111
28. Sun G, Zhang X, Shen Y-I, Sebastian R, Dickinson LE, Fox-Talbot K, Reinblatt M, Steenbergen C, Harmon JW, Gerecht S (2011) Dextran hydrogel scaffolds enhance angiogenic responses and promote complete skin regeneration during burn wound healing. Proc Natl Acad Sci 108(52):20976–20981
29. Jabbarzadeh E, Starnes T, Khan YM, Jiang T, Wirtel AJ, Deng M, Lv Q, Nair LS, Doty SB, Laurencin CT (2008) Induction of angiogenesis in tissue-engineered scaffolds designed for bone repair: a combined gene therapy–cell transplantation approach. Proc Natl Acad Sci 105(32):11099–11104. https://doi.org/10.1073/pnas.0800069105
30. Unger RE, Peters K, Huang Q, Funk A, Paul D, Kirkpatrick CJ (2005) Vascularization and gene regulation of human endothelial cells growing on porous polyethersulfone (PES) hollow fiber membranes. Biomaterials 26:3461–3469. https://doi.org/10.1016/J.BIOMATERIALS.2004.09.047
31. Madden LR, Mortisen DJ, Sussman EM, Dupras SK, Fugate JA, Cuy JL, Hauch KD, Laflamme MA, Murry CE, Ratner BD (2010) Proangiogenic scaffolds as functional templates for cardiac tissue engineering. Proc Natl Acad Sci 107(34):15211–15216. https://doi.org/10.1073/pnas.1006442107
32. Zhai W, Lu H, Chen L, Lin X, Huang Y, Dai K, Naoki K, Chen G, Chang J (2012) Silicate bioceramics induce angiogenesis during bone regeneration. Acta Biomater 8:341–349. https://doi.org/10.1016/j.actbio.2011.09.008
33. Rudra JS, Tian YF, Jung JP, Collier JH (2010) A self-assembling peptide acting as an immune adjuvant. Proc Natl Acad Sci 107(2):622–627
34. Hubbell JA, Chilkoti A (2012) Nanomaterials for drug delivery. Science 337(6092):303–305. https://doi.org/10.1126/science.1219657
35. Pagel M, Beck-Sickinger AG (2017) Multifunctional biomaterial coatings: synthetic challenges and biological activity. Biol Chem 398(1):3–22
36. Hintze V, Samsonov SA, Anselmi M, Moeller S, Becher J, Schnabelrauch M, Scharnweber D, Pisabarro MT (2014) Sulfated glycosaminoglycans exploit the conformational plasticity of bone morphogenetic protein-2 (BMP-2) and alter the interaction profile with its receptor. Biomacromolecules 15(8):3083–3092
37. Liu T, Zeng Z, Liu Y, Wang J, Maitz MF, Wang Y, Liu S, Chen J, Huang N (2014) Surface modification with dopamine and heparin/poly-L-lysine nanoparticles provides a favorable release behavior for the healing of vascular stent lesions. ACS Appl Mater Interfaces 6(11):8729–8743
38. Pagel M, Hassert R, John T, Braun K, Wießler M, Abel B, Beck-Sickinger AG (2016) Multifunctional coating improves cell adhesion on titanium by using cooperatively acting peptides. Angew Chem Int Ed 55(15):4826–4830
39. Siebers MC, ter Brugge PJ, Walboomers XF, Jansen JA (2005) Integrins as linker proteins between osteoblasts and bone replacing materials. A critical review. Biomaterials 26(2):137–146. https://doi.org/10.1016/j.biomaterials.2004.02.021
40. Karadag A, Fisher LW (2006) Bone sialoprotein enhances migration of bone marrow stromal cells through matrices by bridging MMP-2 to αvβ3-integrin. J Bone Miner Res 21(10):1627–1636
41. Tang Y, Wu X, Lei W, Pang L, Wan C, Shi Z, Zhao L, Nagy TR, Peng X, Hu J (2009) TGF-β1–induced migration of bone mesenchymal stem cells couples bone resorption with formation. Nat Med 15(7):757
42. Liu T, Liu S, Zhang K, Chen J, Huang N (2014) Endothelialization of implanted cardiovascular biomaterial surfaces: the development from in vitro to in vivo. J Biomed Mater Res A 102(10):3754–3772. https://doi.org/10.1002/jbm.a.35025
43. Eman RM, Öner FC, Kruyt MC, Dhert WJ, Alblas J (2013) Stromal cell-derived factor-1 stimulates cell recruitment, vascularization and osteogenic differentiation. Tissue Eng Part A 20(3–4):466–473

44. Guo J, Liu M, Yang D, Bouxsein ML, Thomas CC, Schipani E, Bringhurst FR, Kronenberg HM (2010) Phospholipase C signaling via the parathyroid hormone (PTH)/PTH-related peptide receptor is essential for normal bone responses to PTH. Endocrinology 151(8):3502–3513. https://doi.org/10.1210/en.2009-1494
45. Pountos I, Panteli M, Lampropoulos A, Jones E, Calori GM, Giannoudis PV (2016) The role of peptides in bone healing and regeneration: a systematic review. BMC Med 14(1):103
46. Mitchell AC, Briquez PS, Hubbell JA, Cochran JR (2016) Engineering growth factors for regenerative medicine applications. Acta Biomater 30:1–12
47. Visser R, Rico-Llanos GA, Pulkkinen H, Becerra J (2016) Peptides for bone tissue engineering. J Control Release 244:122–135
48. Yi J, Xiong F, Li B, Chen H, Yin Y, Dai H, Li S (2016) Degradation characteristics, cell viability and host tissue responses of PDLLA-based scaffold with PRGD and β-TCP nanoparticles incorporation. Regen Biomater 3(3):159–166
49. Punet X, Mauchauffé R, Rodríguez-Cabello JC, Alonso M, Engel E, Mateos-Timoneda MA (2015) Biomolecular functionalization for enhanced cell-material interactions of poly(methyl methacrylate) surfaces. Regen Biomater 2(3):167–175. https://doi.org/10.1093/rb/rbv014
50. Rosso F, Giordano A, Barbarisi M, Barbarisi A (2004) From cell-ECM interactions to tissue engineering. J Cell Physiol 199(2):174–180
51. Shekaran A, Garcia AJ (2011) Extracellular matrix-mimetic adhesive biomaterials for bone repair. J Biomed Mater Res A 96(1):261–272. https://doi.org/10.1002/jbm.a.32979
52. Fisher LW, Fedarko NS (2003) Six genes expressed in bones and teeth encode the current members of the SIBLING family of proteins. Connect Tissue Res 44(Suppl 1):33–40
53. Bilem I, Chevallier P, Plawinski L, Sone E, Durrieu M, Laroche G (2016) RGD and BMP-2 mimetic peptide crosstalk enhances osteogenic commitment of human bone marrow stem cells. Acta Biomater 36:132–142
54. Rezania A, Healy KE (2000) The effect of peptide surface density on mineralization of a matrix deposited by osteogenic cells. J Biomed Mater Res 52(4):595–600
55. Reyes CD, García AJ (2004) α2β1 integrin-specific collagen-mimetic surfaces supporting osteoblastic differentiation. J Biomed Mater Res A 69(4):591–600
56. Reyes CD, Petrie TA, Burns KL, Schwartz Z, García AJ (2007) Biomolecular surface coating to enhance orthopaedic tissue healing and integration. Biomaterials 28(21):3228–3235. https://doi.org/10.1016/j.biomaterials.2007.04.003
57. Gilbert M, Giachelli CM, Stayton PS (2003) Biomimetic peptides that engage specific integrin-dependent signaling pathways and bind to calcium phosphate surfaces. J Biomed Mater Res A 67(1):69–77
58. Hennessy KM, Pollot BE, Clem WC, Phipps MC, Sawyer AA, Culpepper BK, Bellis SL (2009) The effect of collagen I mimetic peptides on mesenchymal stem cell adhesion and differentiation, and on bone formation at hydroxyapatite surfaces. Biomaterials 30(10):1898–1909
59. Nguyen H, Qian JJ, Bhatnagar RS, Li S (2003) Enhanced cell attachment and osteoblastic activity by P-15 peptide-coated matrix in hydrogels. Biochem Biophys Res Commun 311(1):179–186
60. Hanks T, Atkinson BL (2004) Comparison of cell viability on anorganic bone matrix with or without P-15 cell binding peptide. Biomaterials 25(19):4831–4836
61. Nakaoka R, Hirano Y, Mooney DJ, Tsuchiya T, Matsuoka A (2013) Study on the potential of RGD-and PHSRN-modified alginates as artificial extracellular matrices for engineering bone. J Artif Organs 16(3):284–293
62. Frith JE, Mills RJ, Hudson JE, Cooper-White JJ (2012) Tailored integrin–extracellular matrix interactions to direct human mesenchymal stem cell differentiation. Stem Cells Dev 21(13):2442–2456
63. Min S-K, Kang HK, Jang DH, Jung SY, Kim OB, Min B-M, Yeo I-S (2013) Titanium surface coating with a laminin-derived functional peptide promotes bone cell adhesion. Biomed Res Int 2013:638348

64. Shin H, Zygourakis K, Farach-Carson MC, Yaszemski MJ, Mikos AG (2004) Modulation of differentiation and mineralization of marrow stromal cells cultured on biomimetic hydrogels modified with Arg-Gly-Asp containing peptides. J Biomed Mater Res A 69(3):535–543
65. Palchesko RN, Romeo JD, McGowan KA, Gawalt ES (2012) Increased osteoblast adhesion on physically optimized KRSR modified calcium aluminate. J Biomed Mater Res A 100(5):1229–1238
66. Rezania A, Healy KE (1999) Biomimetic peptide surfaces that regulate adhesion, spreading, cytoskeletal organization, and mineralization of the matrix deposited by osteoblast-like cells. Biotechnol Prog 15(1):19–32
67. Choi YJ, Lee JY, Park JH, Park JB, Suh JS, Choi YS, Lee SJ, Chung CP, Park YJ (2010) The identification of a heparin binding domain peptide from bone morphogenetic protein-4 and its role on osteogenesis. Biomaterials 31(28):7226–7238. https://doi.org/10.1016/j.biomaterials.2010.05.022
68. Sprowson AP, McCaskie AW, Birch MA (2008) ASARM-truncated MEPE and AC-100 enhance osteogenesis by promoting osteoprogenitor adhesion. J Orthop Res 26(9):1256–1262
69. Fromigué O, Brun J, Marty C, Da Nascimento S, Sonnet P, Marie PJ (2012) Peptide-based activation of alpha5 integrin for promoting osteogenesis. J Cell Biochem 113(9):3029–3038
70. Gandavarapu NR, Alge DL, Anseth KS (2014) Osteogenic differentiation of human mesenchymal stem cells on α5 integrin binding peptide hydrogels is dependent on substrate elasticity. Biomater Sci 2(3):352–361
71. Hamada Y, Yuki K, Okazaki M, Fujitani W, Matsumoto T, Hashida MK, Harutsugu K, Nokihara K, Daito M, Matsuura N (2004) Osteopontin-derived peptide SVVYGLR induces angiogenesis in vivo. Dent Mater J 23(4):650–655
72. Hamada Y, Egusa H, Kaneda Y, Hirata I, Kawaguchi N, Hirao T, Matsumoto T, Yao M, Daito K, Suzuki M (2007) Synthetic osteopontin-derived peptide SVVYGLR can induce neovascularization in artificial bone marrow scaffold biomaterials. Dent Mater J 26(4):487–492
73. Van Hove AH, Burke K, Antonienko E, Brown E III, Benoit DS (2015) Enzymatically-responsive pro-angiogenic peptide-releasing poly (ethylene glycol) hydrogels promote vascularization in vivo. J Control Release 217:191–201
74. Kang H-M, Kang Y, Chun HJ, Jeong J-W, Park C (2013) Evaluation of the in vitro and in vivo angiogenic effects of exendin-4. Biochem Biophys Res Commun 434(1):150–154
75. Norfleet AM, Bergmann JS, Carney DH (2000) Thrombin peptide, TP508, stimulates angiogenic responses in animal models of dermal wound healing, in chick chorioallantoic membranes, and in cultured human aortic and microvascular endothelial cells. Gen Pharmacol 35(5):249–254
76. Olszewska-Pazdrak B, Carney DH (2013) Systemic administration of thrombin peptide TP508 enhances VEGF-stimulated angiogenesis and attenuates effects of chronic hypoxia. J Vasc Res 50(3):186–196
77. Santulli G, Ciccarelli M, Palumbo G, Campanile A, Galasso G, Ziaco B, Altobelli GG, Cimini V, Piscione F, D'Andrea LD (2009) In vivo properties of the proangiogenic peptide QK. J Transl Med 7(1):41
78. Hardy B, Battler A, Weiss C, Kudasi O, Raiter A (2008) Therapeutic angiogenesis of mouse hind limb ischemia by novel peptide activating GRP78 receptor on endothelial cells. Biochem Pharmacol 75(4):891–899
79. Lin X, Takahashi K, Liu Y, Derrien A, Zamora PO (2007) A synthetic, bioactive PDGF mimetic with binding to both α-PDGF and β-PDGF receptors. Growth Factors 25(2):87–93
80. Suzuki Y, Tanihara M, Suzuki K, Saitou A, Sufan W, Nishimura Y (2000) Alginate hydrogel linked with synthetic oligopeptide derived from BMP-2 allows ectopic osteoinduction in vivo. J Biomed Mater Res 50(3):405–409. https://doi.org/10.1002/(sici)1097-4636(20000605)50:3<405::Aid-jbm15>3.0.Co;2-z
81. He X, Yang X, Jabbari E (2012) Combined effect of osteopontin and BMP-2 derived peptides grafted to an adhesive hydrogel on osteogenic and vasculogenic differentiation of marrow stromal cells. Langmuir 28(12):5387–5397. https://doi.org/10.1021/la205005h

82. Niu X, Feng Q, Wang M, Guo X, Zheng Q (2009) Porous nano-HA/collagen/PLLA scaffold containing chitosan microspheres for controlled delivery of synthetic peptide derived from BMP-2. J Control Release 134(2):111–117
83. Bergeron E, Senta H, Mailloux A, Park H, Lord E, Faucheux N (2009) Murine preosteoblast differentiation induced by a peptide derived from bone morphogenetic proteins-9. Tissue Eng Part A 15(11):3341–3349
84. Zouani OF, Chollet C, Guillotin B, Durrieu M-C (2010) Differentiation of pre-osteoblast cells on poly (ethylene terephthalate) grafted with RGD and/or BMPs mimetic peptides. Biomaterials 31(32):8245–8253
85. Seol YJ, Park YJ, Lee SC, Kim KH, Lee JY, Kim TI, Lee YM, Ku Y, Rhyu IC, Han SB (2006) Enhanced osteogenic promotion around dental implants with synthetic binding motif mimicking bone morphogenetic protein (BMP)-2. J Biomed Mater Res A 77(3):599–607
86. Jung RE, Hämmerle CH, Kokovic V, Weber FE (2007) Bone regeneration using a synthetic matrix containing a parathyroid hormone peptide combined with a grafting material. Int J Oral Maxillofac Implants 22(2):258–266
87. Pigossi SC, de Oliveira GJ, Finoti LS, Nepomuceno R, Spolidorio LC, Rossa C Jr, Ribeiro SJ, Saska S, Scarel-Caminaga RM (2015) Bacterial cellulose-hydroxyapatite composites with osteogenic growth peptide (OGP) or pentapeptide OGP on bone regeneration in critical-size calvarial defect model. J Biomed Mater Res A 103(10):3397–3406
88. Xu G, Jiang D (2014) The role and mechanism of exogenous calcitonin gene-related peptide on mesenchymal stem cell proliferation and osteogenetic formation. Cell Biochem Biophys 69(2):369–378
89. Millet I, Vignery A (1997) The neuropeptide calcitonin gene-related peptide inhibits TNF-α but poorly induces IL-6 production by fetal rat osteoblasts. Cytokine 9(12):999–1007
90. Choi YJ, Lee JY, Chung CP, Park YJ (2013) Enhanced osteogenesis by collagen-binding peptide from bone sialoprotein in vitro and in vivo. J Biomed Mater Res A 101(2):547–554
91. Lee J-Y, Choo J-E, Choi Y-S, Park J-B, Min D-S, Lee S-J, Rhyu HK, Jo I-H, Chung C-P, Park Y-J (2007) Assembly of collagen-binding peptide with collagen as a bioactive scaffold for osteogenesis in vitro and in vivo. Biomaterials 28(29):4257–4267
92. Goto T, Nakao K, Gunjigake K, Kido M, Kobayashi S, Tanaka T (2007) Substance P stimulates late-stage rat osteoblastic bone formation through neurokinin-1 receptors. Neuropeptides 41(1):25–31
93. Wang L, Zhao R, Shi X, Wei T, Halloran BP, Clark DJ, Jacobs CR, Kingery WS (2009) Substance P stimulates bone marrow stromal cell osteogenic activity, osteoclast differentiation, and resorption activity in vitro. Bone 45(2):309–320
94. Von Schroeder H, Veillette C, Payandeh J, Qureshi A, Heersche J (2003) Endothelin-1 promotes osteoprogenitor proliferation and differentiation in fetal rat calvarial cell cultures. Bone 33(4):673–684
95. Tsai T-L, Wang B, Squire MW, Guo L-W, Li W-J (2015) Endothelial cells direct human mesenchymal stem cells for osteo- and chondro-lineage differentiation through endothelin-1 and AKT signaling. Stem Cell Res Ther 6(1):88
96. Clines GA, Mohammad KS, Grunda JM, Clines KL, Niewolna M, McKenna CR, McKibbin CR, Yanagisawa M, Suva LJ, Chirgwin JM (2011) Regulation of postnatal trabecular bone formation by the osteoblast endothelin A receptor. J Bone Miner Res 26(10):2523–2536
97. Wang V, Misra G, Amsden B (2008) Immobilization of a bone and cartilage stimulating peptide to a synthetic bone graft. J Mater Sci Mater Med 19(5):2145–2155
98. Agrawal V, Tottey S, Johnson SA, Freund JM, Siu BF, Badylak SF (2011) Recruitment of progenitor cells by an extracellular matrix cryptic peptide in a mouse model of digit amputation. Tissue Eng Part A 17(19–20):2435–2443
99. Collier JH, Segura T (2011) Evolving the use of peptides as components of biomaterials. Biomaterials 32(18):4198–4204
100. Lee H, Dellatore SM, Miller WM, Messersmith PB (2007) Mussel-inspired surface chemistry for multifunctional coatings. Science 318(5849):426–430. https://doi.org/10.1126/science.1147241

101. Patterson J, Hubbell JA (2011) SPARC-derived protease substrates to enhance the plasmin sensitivity of molecularly engineered PEG hydrogels. Biomaterials 32(5):1301–1310
102. Tokatlian T, Shrum CT, Kadoya WM, Segura T (2010) Protease degradable tethers for controlled and cell-mediated release of nanoparticles in 2- and 3-dimensions. Biomaterials 31(31):8072–8080
103. Maynard HD, Hubbell JA (2005) Discovery of a sulfated tetrapeptide that binds to vascular endothelial growth factor. Acta Biomater 1(4):451–459
104. Kim SH, Kiick KL (2007) Heparin-mimetic sulfated peptides with modulated affinities for heparin-binding peptides and growth factors. Peptides 28(11):2125–2136
105. Cui H, Webber MJ, Stupp SI (2010) Self-assembly of peptide amphiphiles: from molecules to nanostructures to biomaterials. Pept Sci 94(1):1–18
106. Zhou M, Smith AM, Das AK, Hodson NW, Collins RF et al (2009) Self-assembled peptide-based hydrogels as scaffolds for anchorage-dependent cells. Biomaterials 30(13):2523–2530
107. Woolfson DN, Mahmoud ZN (2010) More than just bare scaffolds: towards multi-component and decorated fibrous biomaterials. Chem Soc Rev 39(9):3464–3479
108. Rajagopal K, Lamm MS, Haines-Butterick LA, Pochan DJ, Schneider JP (2009) Tuning the pH responsiveness of β-hairpin peptide folding, self-assembly, and hydrogel material formation. Biomacromolecules 10(9):2619–2625
109. Jung JP, Gasiorowski JZ, Collier JH (2010) Fibrillar peptide gels in biotechnology and biomedicine. Pept Sci 94(1):49–59
110. Khatayevich D, Gungormus M, Yazici H, So C, Cetinel S, Ma H, Jen A, Tamerler C, Sarikaya M (2010) Biofunctionalization of materials for implants using engineered peptides. Acta Biomater 6(12):4634–4641
111. Mas-Moruno C, Rechenmacher F, Kessler H (2010) Cilengitide: the first anti-angiogenic small molecule drug candidate design, synthesis and clinical evaluation. Anticancer Agents Med Chem 10(10):753–768
112. Rabe M, Verdes D, Seeger S (2011) Understanding protein adsorption phenomena at solid surfaces. Adv Colloid Interf Sci 162(1–2):87–106
113. Rusmini F, Zhong Z, Feijen J (2007) Protein immobilization strategies for protein biochips. Biomacromolecules 8(6):1775–1789
114. Gray JJ (2004) The interaction of proteins with solid surfaces. Curr Opin Struct Biol 14(1):110–115
115. Nakanishi K, Sakiyama T, Imamura K (2001) On the adsorption of proteins on solid surfaces, a common but very complicated phenomenon. J Biosci Bioeng 91(3):233–244
116. Chen C, Zhang S-M, Lee I-S (2013) Immobilizing bioactive molecules onto titanium implants to improve osseointegration. Surf Coat Technol 228:S312–S317
117. Hajimiri M, Shahverdi S, Kamalinia G, Dinarvand R (2015) Growth factor conjugation: strategies and applications. J Biomed Mater Res A 103(2):819–838
118. Chiu LL, Weisel RD, Li RK, Radisic M (2011) Defining conditions for covalent immobilization of angiogenic growth factors onto scaffolds for tissue engineering. J Tissue Eng Regen Med 5(1):69–84
119. Liu L, Deng D, Xing Y, Li S, Yuan B, Chen J, Xia N (2013) Activity analysis of the carbodiimide-mediated amine coupling reaction on self-assembled monolayers by cyclic voltammetry. Electrochim Acta 89:616–622
120. Aissaoui N, Bergaoui L, Landoulsi J, Lambert J-F, Boujday S (2011) Silane layers on silicon surfaces: mechanism of interaction, stability, and influence on protein adsorption. Langmuir 28(1):656–665
121. Kolb HC, Finn MG, Sharpless KB (2001) Click chemistry: diverse chemical function from a few good reactions. Angew Chem Int Ed Engl 40(11):2004–2021
122. Tang L, Thevenot P, Hu W (2008) Surface chemistry influences implant biocompatibility. Curr Top Med Chem 8(4):270–280
123. Yazici H, Fong H, Wilson B, Oren E, Amos F, Zhang H, Evans J, Snead M, Sarikaya M, Tamerler C (2013) Biological response on a titanium implant-grade surface functionalized with modular peptides. Acta Biomater 9(2):5341–5352

124. Sarikaya M, Tamerler C, Jen AK-Y, Schulten K, Baneyx F (2003) Molecular biomimetics: nanotechnology through biology. Nat Mater 2(9):577
125. Sarikaya M, Tamerler C, Schwartz DT, Baneyx F (2004) Materials assembly and formation using engineered polypeptides. Annu Rev Mater Res 34:373–408
126. Mayer G, Sarikaya M (2002) Rigid biological composite materials: structural examples for biomimetic design. Exp Mech 42(4):395–403
127. Sakiyama-Elbert S, Hubbell J (2001) Functional biomaterials: design of novel biomaterials. Annu Rev Mater Res 31(1):183–201
128. Yazici H, Habib G, Boone K, Urgen M, Utku FS, Tamerler C (2019) Self-assembling antimicrobial peptides on nanotubular titanium surfaces coated with calcium phosphate for local therapy. Mater Sci Eng C 94:333–343
129. Yazici H, O'Neill MB, Kacar T, Wilson BR, Oren EE, Sarikaya M, Tamerler C (2016) Engineered chimeric peptides as antimicrobial surface coating agents toward infection-free implants. ACS Appl Mater Interfaces 8(8):5070–5081
130. So CR, Hayamizu Y, Yazici H, Gresswell C, Khatayevich D, Tamerler C, Sarikaya M (2012) Controlling self-assembly of engineered peptides on graphite by rational mutation. ACS Nano 6(2):1648–1656
131. Shiba K (2010) Exploitation of peptide motif sequences and their use in nanobiotechnology. Curr Opin Biotechnol 21(4):412–425
132. Donatan S, Yazici H, Bermek H, Sarikaya M, Tamerler C, Urgen M (2009) Physical elution in phage display selection of inorganic-binding peptides. Mater Sci Eng C 29(1):14–19
133. Hnilova M, Oren EE, Seker UO, Wilson BR, Collino S, Evans JS, Tamerler C, Sarikaya M (2008) Effect of molecular conformations on the adsorption behavior of gold-binding peptides. Langmuir 24(21):12440–12445
134. Sano K-I, Shiba K (2003) A hexapeptide motif that electrostatically binds to the surface of titanium. J Am Chem Soc 125(47):14234–14235
135. Naik RR, Brott LL, Clarson SJ, Stone MO (2002) Silica-precipitating peptides isolated from a combinatorial phage display peptide library. J Nanosci Nanotechnol 2(1):95–100
136. Ajikumar PK, Vivekanandan S, Lakshminarayanan R, Jois SD, Kini RM, Valiyaveettil S (2005) Mimicking the function of eggshell matrix proteins: the role of multiplets of charged amino acid residues and self-assembly of peptides in biomineralization. Angew Chem Int Ed 44(34):5476–5479
137. Gungormus M, Oren EE, Horst JA, Fong H, Hnilova M, Somerman MJ, Snead ML, Samudrala R, Tamerler C, Sarikaya M (2012) Cementomimetics—constructing a cementum-like biomineralized microlayer via amelogenin-derived peptides. Int J Oral Sci 4(2):69
138. Gungormus M, Fong H, Kim IW, Evans JS, Tamerler C, Sarikaya M (2008) Regulation of in vitro calcium phosphate mineralization by combinatorially selected hydroxyapatite-binding peptides. Biomacromolecules 9(3):966–973
139. Loo Y, Goktas M, Tekinay AB, Guler MO, Hauser CA, Mitraki A (2015) Self-assembled proteins and peptides as scaffolds for tissue regeneration. Adv Healthc Mater 4(16):2557–2586
140. Matson JB, Zha RH, Stupp SI (2011) Peptide self-assembly for crafting functional biological materials. Curr Opinion Solid State Mater Sci 15(6):225–235
141. He B, Zhao J, Ou Y, Jiang D (2018) Biofunctionalized peptide nanofiber-based composite scaffolds for bone regeneration. Mater Sci Eng C 90:728–738
142. Holmes TC (2002) Novel peptide-based biomaterial scaffolds for tissue engineering. Trends Biotechnol 20(1):16–21
143. Gelain F, Horii A, Zhang S (2007) Designer self-assembling peptide scaffolds for 3-D tissue cell cultures and regenerative medicine. Macromol Biosci 7(5):544–551
144. Stupp SI (2010) Self-assembly and biomaterials. Nano Lett 10(12):4783–4786
145. Wu EC, Zhang S, Hauser CAE (2012) Self-assembling peptides as cell-interactive scaffolds. Adv Funct Mater 22(3):456–468. https://doi.org/10.1002/adfm.201101905
146. Schachner M (2000) Neurobiology. Nervous engineering. Nature 405(6788):747–748. https://doi.org/10.1038/35015648

147. Aggeli A, Bell M, Boden N, Keen JN, Knowles PF, McLeish TC, Pitkeathly M, Radford SE (1997) Responsive gels formed by the spontaneous self-assembly of peptides into polymeric beta-sheet tapes. Nature 386(6622):259–262. https://doi.org/10.1038/386259a0
148. West MW, Wang W, Patterson J, Mancias JD, Beasley JR, Hecht MH (1999) De novo amyloid proteins from designed combinatorial libraries. Proc Natl Acad Sci U S A 96(20):11211–11216. https://doi.org/10.1073/pnas.96.20.11211
149. Xiong H, Buckwalter BL, Shieh HM, Hecht MH (1995) Periodicity of polar and nonpolar amino acids is the major determinant of secondary structure in self-assembling oligomeric peptides. Proc Natl Acad Sci U S A 92(14):6349–6353. https://doi.org/10.1073/pnas.92.14.6349
150. Lashuel HA, Labrenz SR, Woo L, Serpell LC, Kelly JW (2000) Protofilaments, filaments, ribbons, and fibrils from peptidomimetic self-assembly: implications for amyloid fibril formation and materials science. J Am Chem Soc 122(22):5262–5277. https://doi.org/10.1021/ja9937831
151. Prieto AL, Edelman GM, Crossin KL (1993) Multiple integrins mediate cell attachment to cytotactin/tenascin. Proc Natl Acad Sci U S A 90(21):10154–10158. https://doi.org/10.1073/pnas.90.21.10154
152. Zhang S, Holmes TC, DiPersio CM, Hynes RO, Su X, Rich A (1995) Self-complementary oligopeptide matrices support mammalian cell attachment. Biomaterials 16(18):1385–1393