



# Recent Advances of Biphasic Calcium Phosphate Bioceramics for Bone Tissue Regeneration

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## Abstract

Biphasic calcium phosphate bioceramics consist of an intimate mixture of hydroxyapatite (HA) and beta-tricalcium phosphate ( $\beta$ -TCP) in varying ratios. Due to their biocompatibility, osteoconductivity, and safety in in vitro, in vivo, and clinical models, they have become promising bone substitute biomaterials and are recommended for use as alternatives for or as additives in bone tissue regeneration in various orthopedic and dental applications. Many studies have demonstrated the potential uses of BCP bioceramics as scaffolds for tissue engineering. Here, we highlight the recent advances in the uses of BCP bioceramics and functionalized BCPs for bone tissue regeneration.

## Keywords

Marked BCP · Injectable BCP/polymer · Osteoinductive growth factors · Drug delivery · Osseointegration

## 12.1 Introduction

Bone tissue regeneration is a complex and well-orchestrated process of biological events of bone induction and conduction to optimize skeletal repair and restore skeletal function [1]. Currently, autografts and allografts are commonly performed and are considered as the gold standard for bone replacement surgery. However, their clinical applications are still challenging due to several disadvantages, such as the limited supply of donor bone graft, a secondary trauma for autograft, and the immune reactions against the allograft [2].

Synthetic or natural bioceramics with properties similar to native bone have been developed as alternatives to autografts or allografts for bone replacement. The most common bioceramics are calcium phosphate (CaP)-based biomaterials, including hydroxyapatite (HA),  $\alpha$ - and  $\beta$ -tricalcium phosphates ( $\alpha$ -TCP,  $\beta$ -TCP), octacalcium phosphate (OCP), amorphous calcium phosphate (ACP), and biphasic calcium phosphates (BCP) [3, 4]. Due to their biocompatibility, safety, availability, low morbidity, and cost-effectiveness over autografts and allografts,

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these CaP bioceramics are commonly used for medical and dental applications, such as treatment of bone defects and fracture, joint replacement, dental implants, and periodontal therapy [5].

BCPs consist of a more stable hydroxyapatite [HA,  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ] and a more soluble beta-tricalcium phosphate [ $\beta$ -TCP,  $\text{Ca}_3(\text{PO}_4)_2$ ] in different proportions. Among the CaP biomaterials, BCPs have significant advantages over other CaP materials. Variations in the HA/ $\beta$ -TCP ratio can modulate their bioactivity and balance between resorption and solubilization which guarantees the stability of the biomaterials while promoting bone ingrowth [6]. However, their inherent brittleness is not suitable in load-bearing bone applications [7]. Despite their brittleness, depending on the ratio of HA and  $\beta$ -TCP, various BCP ceramics can be obtained for the application to large bone defects as well as customized pieces [8]. Additionally, owing to their suitable degradation rates and chemical similarity to the mineral phase of the bone, they have common clinical applications in the fields of dental and orthopedic surgery [6, 9, 10]. Previous studies have shown that BCP-based materials have osteoconductive properties in a specific HA/ $\beta$ -TCP ratio, leading to enhanced osteoblast proliferation and osteogenic differentiation [11–14]. In addition to osteoconductivity, BCP-based materials have osteoinductivity, that is, the property of graft materials in which it induces de novo bone formation with biomimetic substances such as bone morphogenic proteins (BMPs). Indeed, recent study suggested that the optimization of the material characteristics can endow biomaterials with osteoinductive ability [15]. Indeed, the researchers showed that BCP (30% HA and 70%  $\beta$ -TCP) promoted much greater expression of BMP-2 and showed higher osteoinductivity in vivo than BCP (70% HA and 30%  $\beta$ -TCP), pure  $\beta$ -TCP, and HA [15]. However, BCP-based scaffolds still have a limitation toward new bone formation due to their lack of intrinsic osteoinductivity.

To enlarge the application of BCPs in bone tissue regeneration, many researches have focused on the development of the functionalized BCP scaffolds by combining with various polymers

and adding bioactive factors. In this chapter, we describe the characteristics of BCPs and marketed BCP products. Also, we review the latest advances in the study of various functionalized BCPs for enhancing bone tissue regeneration.

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## 12.2 Characteristics of BCPs

BCPs are composed of two phases such as a more stable HA and a more soluble  $\beta$ -TCP in different ratios. As a first phase, HA is ideal material for bone substitute and is commonly used because of its similarity to the mineral phase of the bone and better mechanical properties. However, the bioresorption of a more stable HA is slower than other CaP such as TCP. These non-resorbable and bioinert properties of HA lead to incomplete remodeling of the bone [16]. Thus, HA is usually combined with other bioresorbable bone phases at an appropriate ratio because its bioresorption rates can be controlled by the HA/TCP ratio. As a second phase,  $\beta$ -TCP is generally selected because it has a higher chemical stability and biodegradation rate [17]. The composite BCP ceramics, comprising a mixture of HA with good osteoconductivity and  $\beta$ -TCP with high resorption, proved to be highly biocompatible with good osteoconductive properties in specific ratios of HA/ $\beta$ -TCP [11, 18]. By manipulating the HA/ $\beta$ -TCP composition ratios, it is possible to optimize the biodegradation rate of BCPs [19]. Therefore, biodegradation kinetics of BCPs depends on the types of chemical phases (HA/ $\beta$ -TCP) and their percentage ratios, where the higher the TCP ratio, the higher is the biodegradation of the BCPs. The biodegradation process of BCPs is also influenced by several other factors. For instance, a lower porosity and surface area or a higher crystallinity and larger particle sizes exhibited a slower biodegradation rate [6, 20]. Importantly, the higher biodegradation of BCPs facilitates an increase of calcium ( $\text{Ca}^{2+}$ ) and phosphate ( $\text{HPO}_4^{2-}$ ,  $\text{PO}_4^{3-}$ ) ion concentration in the vicinity of the bone cells. This results in osteogenic differentiation and the subsequent mineralization of extracellular matrix (ECM) in the newly generated bone [21–23].

### 12.3 Marketed BCP Products

Due to these characteristics, many BCP bone substitute products, with suitable composition ratios of HA and TCP, are commercially available for various orthopedic and maxillofacial applications, as summarized in a previous review article [6]. Some of the marketed BCP products are as follows. For alternative autogenous bone grafts, Graftys BCP® (Latin American Solutions (LAS), Brazil) has been marketed in several forms such as granules, sticks, cylinders, and wedges. This product is a micro-, meso-, and macro-porous two-phase CaP ceramic consisting of 60% HA and 40%  $\beta$ -TCP, which facilitates long-term volume stability by decelerating the overall resorption capabilities and promoting a more stable and uniform bone growth [24]. Bicara™ (60% HA and 40%  $\beta$ -TCP, Wiltrom Co., Ltd.) shows good biocompatibility in vivo without side effects such as abnormal inflammation at implantation sites. Although complete absorption and replacement were not observed after 6 months of implantation, new bone regeneration was seen to occur effectively on the surface of the periphery in the specimens with the use of Bicara™. Another synthetic BCP, OSOPIA (>90% TCP and <10% HA), is manufactured by NextGen Biomaterials (London, UK) and is much closer to biologically derived bone grafts than the standard synthetic materials [25]. OSOPIA features higher bone ingrowth rates than BCP grafts and a more controlled cellular-induced resorption pattern compared to standard TCP materials [26].

Based on these results, most studies have reported that, regardless of the HA/ $\beta$ -TCP ratio, BCP scaffolds generally enhance the rate and quality of bone tissue regeneration compared to empty control groups. In this regard, the various types of BCP bioceramics are considered as the best materials for bone tissue regeneration because they possess the inorganic phase of the bone ECM with mineral composition similar to that of natural bone. It has been reported that only BCPs with HA/ $\beta$ -TCP ratios of 65/35, 60/40, and 50/50 have been successfully applied in human clinical trials [27–29]. For an example, Artzi et al. reported that the combination of BCP (50%

HA and 50%  $\beta$ -TCP) with particulate autogenous bone chips in a 1 to 1 ratio showed osteoconductive properties and promoted newly formed bone [27]. More recently, clinical trials have been studied to compare the new bone formation of two BCPs (B1, 60.28% HA and 39.72%  $\beta$ -TCP; B2, 78.21% HA and 21.79%  $\beta$ -TCP) and BoneCeramic (61% HA and 39%  $\beta$ -TCP) in fresh dental sockets after 6 months [30]. In this study, the B1 group showed the greatest amount of newly formed bone compared to other groups after 6 months. Although BCPs have shown biocompatibility, osteoconductivity, and new bone formation, currently, there is no general agreement on an ideal HA/ $\beta$ -TCP ratio for BCPs in clinical applications. Therefore, various HA/ $\beta$ -TCP ratios have been evaluated by researchers to determine the best ratio for optimum bone regeneration.

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### 12.4 Injectable BCPs/Polymer Scaffolds

Porous scaffolds provide the structural support for cell adhesion, proliferation, and differentiation. They have been used as substrates for promoting new bone formation at the defect through surgical procedures [31, 32]. From a clinical perspective, injectable scaffold systems are one of the best options for bone tissue regeneration of irregular-shaped bone defects.

Porous microspheres have been used as vehicles for sustained drug or protein delivery due to their inherently small size, small volume, large surface area, and high drug loading efficiency [33]. Recently, Song and coworkers developed an injectable BCP (60% HA and 40%  $\beta$ -TCP)/porous microsphere scaffold system through the surface immobilization of BCP nanoparticles modified with heparin on porous poly(lactic-co-glycolic acid) (PLGA) microspheres modified with positively charged L-lysine via electrostatic interactions [34]. Although this system showed enhanced ALP activity, calcium deposition, and expression of osteogenic differentiation genes (i.e., osteocalcin and osteopontin), only in vitro

results of this study were not enough to demonstrate its enhanced osteogenesis effects.

Poly(methyl methacrylate) (PMMA) bone cement has been used in bone defects caused by osteoporosis due to its excellent mechanical properties [35, 36]. However, its bioinertness and non-degradation have limited its extensive application in bone regeneration. To enhance its biological activity, HA could be incorporated into PMMA bone cement [37]. Although the formulation of HA/PMMA bone cement has some advantages, such as the excellent mechanical properties and injectability, it still has some limitations due to the slow biodegradation of HA *in vivo*, consequently leading to a weakening of the interfacial integration between host bone tissue and cement [38]. Recently, Quan et al. fabricated a series of bioactive BCP (40%)/PMMA bone cements containing different BCP contents (up to 40%) to achieve an adjustable resorption rate and to accelerate osteogenesis *in vivo* [39]. The increase of  $\beta$ -TCP content (30%, 50%, and 70% of  $\beta$ -TCP per BCP contents) in BCP/PMMA cements induced more mineralization and was seen to promote cell adhesion, proliferation, and differentiation of rat bone marrow mesenchymal stem cells (rBMSCs) and osteogenesis. Furthermore, micro-computed tomography and histological studies have demonstrated that the growth rate of new bone was accelerated by increasing the  $\beta$ -TCP content in such BCP/PMMA cements.

Multichannel BCP granule (MCG, 60% HA and 40%  $\beta$ -TCP) is also an appropriate bone graft material due to its unique morphology, optimal porosity with interconnected pores, good mechanical strength, biocompatibility, osteoconductivity, and biodegradability [40]. Additionally, its porous structure is much like the osteon of a natural bone and allows bone cells to attach, migrate, and proliferate in the defect site [41, 42]. However, its low weight and repellent nature make it difficult to handle them in clinical applications. To solve this problem, MCGs are mixed with a material that can hold them by its cohesive force and induce bone formation at the defect site. As one of the main ECM components, hyaluronic acid (HA) has been previously evaluated in conjunction with CaP granules for improving

injectability and stimulating bone regeneration due to its non-toxicity, non-immunogenicity, viscosity, and good biodegradability, as well as for its wound healing and drug delivery capabilities [43–46]. Recently, the addition of HA to MCG (0.7 mm, 60% HA and 40%  $\beta$ -TCP) resulted in injectable granules and allowed for their easy handling during the implantation [47]. Without a significant change in porosity, the injectable HA/MCG exhibited greater cell viability and proliferation *in vitro*, as well as better *in vivo* bone tissue growth at critical sized defects after 4 weeks of implantation compared to MCG alone.

Calcium phosphate cement (CPC) with biocompatibility and osteoconductivity is considered as the most promising injectable filler material due to its identical composition to the mineral part of the bone [48]. However, the lack of interconnected porosity and inadequate pore size distribution in CPC cements may adversely affect bone growth. To improve biocompatibility, CPC has been incorporated with sucrose granules and/or different amounts of  $\text{NaHCO}_3$  and  $\text{Na}_2\text{HPO}_4$  to achieve the desired size distribution and interconnected pores [49]. Additionally, to promote cell adhesion and differentiation through specific interactions with ligands and adhered cells, collagen has been adsorbed onto the surface of these bioceramics because it is a major component of bone ECM [50–52]. Moreover, to further accelerate tissue regeneration, a potent osteoinductive growth factor, such as bone morphogenetic protein-2 (BMP-2), is also incorporated into bioceramics [53]. Recently, Lee et al. developed an advanced injectable CPC bone cement system by incorporating 15% of the functionalized MCGs (60% HA and 40%  $\beta$ -TCP) with collagen coating and BMP-2 loaded into CPC to enhance bone tissue regeneration [40]. The incorporation of the functionalized MCGs into CPC achieved a sustained BMP-2 release for 1 month, as well as implant degradation behavior, resulting in boosted bone tissue growth as compared to CPC matrix alone, in a rabbit femur head defect model after 2 and 4 weeks of implantation.

## 12.5 Functionalized BCPs with Bioactive Molecules

### 12.5.1 Functionalized BCPs for the Delivery of Osteoinductive Growth Factors

As described above, BCP-based scaffolds have gained attention in the fields of dental and orthopedic surgery due to their excellent biocompatibility and biodegradability. However, BCP-based scaffolds alone are not sufficient to stimulate adequate revascularization, cellular reconstitution, or osteogenesis. Many researchers have tried to incorporate bioceramics into polymer scaffolds to improve bioactivity [54], but due to the lack of bioactive signaling molecules, bare BCP or bare BCP/polymer scaffold systems are not effective in promoting cell proliferation, osteogenic differentiation, and tissue regeneration. In particular, BCPs/polymer composite scaffolds do not have appropriate pore structures and interconnectivity for cell accommodation [55]. Therefore, a combination of an appropriate scaffold and bioactive molecules has been suggested which play an extensive role in the stimulation of cell growth, migration, differentiation, and angiogenesis [56–58].

Among the osteoinductive factors, BMP-2 has been widely used in bone tissue engineering because of its superior osteoinductive activity, which stimulates the gene expression of osteogenic markers such as osteocalcin, osteopontin, bone sialoprotein, and alkaline phosphatase during osteoblast differentiation *in vitro* [59, 60]. To enhance bone tissue regeneration *in vivo*, several growth factors containing BCP ceramics were evaluated in animal models. Cho et al. found that BMP-2-loaded BCP (20% HA and 80%  $\beta$ -TCP) effectively induced new bone formation in the rat calvarial defect model [61]. Although new bone formation in 10  $\mu$ g and 20  $\mu$ g BMP-2/BCP groups was seen to be greater at 8 weeks than at 2 weeks, a statistically significant difference depending on the BMP-2 dose was not observed. On the other hand, bone replacement via alloplast needs to be accompanied by ample vascularization since it is one of the most important prerequisites for bone

healing [62]. Recently, Arisan and coworkers showed that vascular endothelial growth factor (VEGF)-incorporated BCP (60% HA and 40%  $\beta$ -TCP) alloplast enhanced early-term new bone formation in femoral defect models [63]. However, although VEGF seemed to significantly contribute to recovery and osteogenesis in the early stages of bone defect healing, the new bone of the VEGF/BCP alloplast did not show a statistically significant difference compared to that of bare BCP alloplast. Based on these results, it was evident that the simple mixing and incorporation of growth factors into BCP ceramics may not significantly improve new bone formation *in vivo*. It may be possible that the growth factors were not released to the defect in a sustained manner due to the short retention of growth factors *in vivo*. When they are loaded into BCP scaffolds by soaking, all of growth factors are rapidly released at once and disappear completely within 2 days of scaffold implantation [64]. Due to the short *in vivo* half-life of growth factors such as BMP-2, clinicians tried using a large dose of BMP-2. However, a high loading amount of BMP-2 onto a defect site may cause side effects like bone overgrowth and may also illicit an immune response [65].

To overcome these limitations, gradually degradable scaffolds with a sustained release of bioactive molecules are necessary to reduce the dose for clinical applications as well as to induce successful bone formation. By simply mixing BCP nanoparticles with heparin-alendronate (Hep-ALN), Kim and Park modified the surface of the BCPs (60% HA and 40%  $\beta$ -TCP) through the intense interactions between the phosphate groups of ALN and the calcium ions of BCP [66]. This modification prevents the BCPs from forming large particles due to the repulsion of negatively charged heparin molecules. Additionally, Hep-ALN/BCPs extended the release profile of osteoinductive BMP-2 up to 30 days in a sustained manner, as a result of the strong electrostatic interactions between Hep and BMP-2. This sustained release of BMP-2 from BCPs promoted the *in vitro* osteogenic differentiation of human adipose-derived stem cells (hADSCs) and the *in vivo* bone tissue regeneration in a rat calvarial defect model. This proposed simple bio-functionalization tech-



nique is applicable to CaP-based bioceramics and has potential in bone tissue engineering via the effective and sustained delivery of osteoinductive growth factors. As another example, Lee et al. recently developed a new 3D scaffold system with the sustained release of dual growth factors such as BMP-2 and VEGF [67], since dual delivery of BMP-2 and VEGF exhibited a better and more efficient bone regeneration than that of single growth factor delivery [68, 69]. The new 3D scaffold system (BNBV) was prepared by loading a sponge BCP (25% of nano-sized BCP powder) scaffold with 0.5% nano-cellulose (NC) containing BMP-2 and VEGF. This system resulted in the sustained release of the dual growth factors over a period of 30 days. This sustained release of the BNBV system better induced the cell attachment, proliferation, and differentiation of the rat bone marrow mesenchymal stem cells (rBMSCs) as compared to scaffold systems loaded with single growth factor. The use of cellulose in scaffolds, with its high density of hydroxyl groups, facilitates the immobilization of cell adhesive proteins [70]. BNBV scaffolds showed a higher amount of bone formation than BCP scaffolds, suggesting that the released dual growth factors from scaffolds resulted in accelerated bone healing mechanism. In particular, an increase in vasculature of the newly deposited bone and connective tissue inside the pores demonstrated the angiogenic effect of the released VEGF. As a chemoattractant, VEGF promoted differentiation of osteoblasts and thereby promoted BMP-2-induced bone formation [71, 72]. Therefore, stem cell-loaded BNBV scaffolds increased the extent of bone and vessel formation in the orthotopic site at 4 weeks.

Healing of bone defects is based on various biological cascade processes such as the recruitment and activation of cell lineages, regulation by molecular mediators (i.e., chemokines, growth factors, and cytokines), and cooperation in a cascade of events to fill the gap of bone fractures [73]. Considering the complicated bone healing cascade depends on a wide range of growth factors, it has been suggested that incorporation of various growth factors would be a more rational approach compared to using a specific growth factor for bone tissue engineering [74]. For more efficient

bone repair, platelet-rich plasma (PRP) is a promising alternative approach because it contains various growth factors, including platelet-derived growth factor (PDGF), transforming growth factor- $\beta$  (TGF- $\beta$ ), epidermal growth factor (EGF), insulin growth factor (IGF), and VEGF [75, 76]. Although PRP can be used alone, the combination of PRP with scaffolds containing polymers and ceramics has been suggested to enhance bone healing process. Previous studies have reported that the combination of PRP with various biomaterials and cell sources showed positive effects on bone regeneration [77] and led to improved osteogenesis in ADSCs [78]. Recently, Chen et al. developed the thermo-gelling hydrogel scaffold by incorporating PRP and BCPs (60% HA and 40%  $\beta$ -TCP) in the thermo-gelling hydrogel, hyaluronic acid-*g*-chitosan-*g*-poly(N-isopropylacrylamide) (HA-CPN) [79]. This thermo-gelling HA-CPN/PRP/BCP hydrogel scaffold exhibited highly efficient cell proliferation and enhanced osteogenic differentiation. In vitro results revealed that PRP/BCP boosts osteoblastic differentiation and ECM mineralization of ADSCs in a HA-CPN scaffold. Additionally, in vivo CT and histological analyses confirmed that ADSCs and HA-CPN/PRP/BCP system showed successful bone formation in a rabbit calvarial defect model. Taken together, combining osteoinductive PRP and osteoconductive BCP with a HA-CPN hydrogel system could promote the osteogenesis of ADSCs for bone tissue engineering.

### 12.5.2 Functionalized BCPs for the Delivery of Small Molecular Drugs

As mentioned previously, osteoinductive growth factors have been incorporated in appropriate BCP scaffolds to render scaffolds with good osteoinductivity. However, the most commonly used osteoinductive proteins, including BMPs and TGF- $\beta$ , can readily lose their bioactivity during the preparation of these scaffolds [80]. To minimize denaturation and to maintain their bioactivity, growth factors are usually incorporated into BCP scaffolds by physical adsorption.

However, the initial burst release of physically adsorbed growth factors is inevitable and cannot be retained in vivo at the implantation site for a long period [81]. Alternatively, osteoinductive small molecular drugs have drawn much attention for incorporation in scaffolds for bone tissue engineering due to their relatively high stability even in tough chemical environments [82].

Alendronate (ALN) can effectively inhibit bone resorption and induce osteogenic differentiation of osteoblasts, BMSCs, and ADSCs [83–85]. However, due to its high hydrophilic property, its oral bioavailability is only about 0.9%–1.8% [86]. Many researchers have tried to find appropriate delivery carriers that can provide an osteoconductive matrix for implantation at the bone repair sites [87, 88]. Song et al. prepared BCP (60% HA and 40%  $\beta$ -TCP) scaffolds that maintained ALN concentrations at the repair site long enough to allow the bone-forming cells to migrate to the defect site, proliferate, and differentiate in response to ALN [89]. This ALN/BCP scaffold significantly enhanced osteogenesis and mineralization in vitro, and the locally delivered ALN might affect the remodeling of newly regenerated bone in vivo, thus promoting osteogenesis in a rat tibia defect model.

Dexamethasone (DEX) is one of the low-molecular weight osteoinductive factors for bone tissue regeneration [82]. DEX and HA nanoparticles were hybridized with gelatin and poly(L-lactide) (PLLA) to construct a HA/DEX/PLLA/gelatin composite scaffold by the electrospinning technique [90]. However, the problem of initial burst release and a short release period of DEX still needed to be solved. Recently, composite scaffold systems of collagen and DEX-loaded BCP nanoparticles were prepared for a sustained release of DEX, together with the calcium and phosphorous ions [91]. DEX-loaded BCP nanoparticles were homogeneously distributed on the walls of the collagen scaffolds, enhancing the mechanical properties and roughness of the scaffolds. The sustained and prolonged release of DEX from the scaffolds was achieved for up to 35 days. This scaffold system, with good biocompatibility, enhanced the osteogenic differentiation of human BMSCs in vitro depending on the concentration of DEX, by increasing ALP activity and gene expres-

sion of *ALP*, runt-related transcription factor-2 (*RUNX2*), bone sialoprotein 2 (*IBSP*), and *BMP-2* both in vitro and in vivo. Furthermore, the scaffold increased the concentration of collagen I and osteocalcin in the in vivo environment.

### 12.5.3 Functionalized BCPs for Immunomodulation

Osseointegration is a direct contact between bone and the implanted biomaterials. During the osseointegration process, inflammation and immune reactions can be observed at the implanted sites [92, 93]. Although TGF- $\beta$ 1 plays many important roles in regeneration processes, it is mostly related to the increased production of fibrotic tissue [94, 95]. Therefore, recent studies suggested that the inhibition of TGF- $\beta$ 1 is an alternative strategy for enhancing osseointegration around medical implants by preventing several fibrotic reactions [96–98]. Among TGF- $\beta$ 1 inhibitors, it has been known that P144 (TGF- $\beta$ 1 inhibitor peptide) blocks the binding of TGF- $\beta$ 1 with its receptor [99–101]. Previous results showed that P144-biofunctionalized CP-Ti surfaces reduced fibrotic differentiation and increased osteoblastic differentiation [96]. Also, the inhibiting TGF- $\beta$ 1 can prevent the formation of fibrotic tissues or induce osseointegration around the implanted biomaterials [97, 98]. In a recent study, Gil group demonstrated that P144-biofunctionalized BCPs (60% HA and 40%  $\beta$ -TCP) in the hemimandibles of beagle dogs after tooth extraction maintained a stable membranous bone formation and showed the constant presence of vascular structures in the alveolar space compared to bare BCPs [102]. These results suggested that immunomodulation using TGF- $\beta$ 1 inhibitor peptide can be an alternative strategy for enhancing osseointegration of the implanted biomaterials.

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## 12.6 Conclusion

In summary, due to the excellent biocompatibility, biodegradability, bioactivity, safety, and cost-effectiveness over autografts and allografts, BCP

**Table 12.1** Various types of BCPs and functionalized BCPs for bone tissue regeneration

Types of BCPs	Ratio of HA to $\beta$ -TCP	Bioactive factors	References
Nano-BCP/porous PLGA microspheres	60:40	–	[34]
BCP/PMMA	30:70, 50:50, 70:30	–	[39]
Multichannel BCP (MCG)	60:40	–	[40–42]
Hyaluronic acid (HA)/MCG	60:40	–	[47]
Collagen/MCG/CPC	60:40	BMP-2	[40]
BCP	20:80	BMP-2	[61]
BCP alloplast	60:40	VEGF	[63]
Heparinized BCP	60:40	–	[66]
3D scaffold containing nano-BCP/ nano-cellulose	60:40	BMP-2, VEGF	[67–69]
HA-CPN/BCP	60:40	PRP	[79]
BCP	60:40	Alendronate	[89]
Collagen/Nano BCP	60:40	Dexamethasone	[91]
BCP	60:40	P144 (TGF- $\beta$ 1 inhibitor peptide)	[102]

scaffolds ranging from nanoparticles to granules are very attractive biomaterials with preclinical and clinical applications for bone tissue engineering. Although BCP scaffolds have good osteoconductivity, bare BCP scaffolds alone are not enough to significantly improve osteogenic differentiation in vitro and new bone formation in vivo. As summarized in Table 12.1, to achieve more effective bone tissue regeneration, osteoinductive molecules, including growth factors or small molecular drugs, have been combined with BCP scaffolds or BCP/hydrogel polymer systems. By combining these bioactive molecules with BCPs or BCP/hydrogel polymer scaffolds, their initial burst release and short retention time in vitro and in vivo could be overcome, thereby promoting cell proliferation, osteogenic differentiation, and new bone tissue regeneration. However, efforts are still required to find the optimal BCP-based scaffolds with the most effective clinical outcomes.

**Acknowledgments** This research was supported by the Bio and Medical Technology Development Program of the NRF funded by the Korean government, MSIP (NRF-2017M3A9B3063640).

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