*European Cells and Materials* Vol.56 2026 (pages 64–81) DOI: 10.22203/eCM.v056a05 ISSN 1473-2262

*Review*

# BONE REGENERATION BY AN OCTACALCIUM PHOSPHATE/GELATIN COMPOSITE IN CONNECTION WITH ITS BIODEGRADABLE PROPERTIES

Osamu Suzuki[1,*], Yu Mori[2], Ryo Hamai[1], Ryuichi Kanabuchi[2] and Toshimi Aizawa[2]

[1]Division of Biomaterials Science and Engineering (Division of Craniofacial Function Engineering), Tohoku University Graduate School of Dentistry, 980-8575 Sendai, Japan

[2]Department of Orthopaedic Surgery, Tohoku University Graduate School of Medicine, 980-8574 Sendai, Japan

**Abstract**

Bone grafting is a widely utilized treatment for bone defects and augmentation of bone fusion, and the development of bone substitute materials with superior osteogenic potential is of significant interest as an alternative to autogenous bone grafting. Octacalcium phosphate (OCP) has been investigated as a bone substitute material due to its capacity to promote replacement with new bone more effectively than hydroxyapatite (HA) and $\beta$-tricalcium phosphate ($\beta$-TCP) in preclinical studies. The solubilities of OCP and $\beta$-TCP are higher than that of HA under the physiological conditions, according to the thermodynamic stability of these crystal structures. However, OCP promotes osteoblastic differentiation and osteoclast formation compared to $\beta$-TCP and HA, which is associated with the difference in the dissolution behaviors of these calcium phosphates. OCP has been combined with natural polymers such as collagen and gelatin to enhance its handling properties and clinical applicability. Among these composites, OCP/gelatin (OCP/Gel) has demonstrated superior osteoconductivity and biodegradability compared to $\beta$-TCP in the long bone defect model. Therefore, OCP/Gel composites should provide an optimal balance between new bone formation and material resorption, addressing a critical limitation of $\beta$-TCP in clinical applications. This review article focuses on the intrinsic biodegradable property of OCP and summarizes the development and preclinical findings of OCP and OCP/Gel composites, and exploring the potential for orthopedic application.

**Keywords:** Octacalcium phosphate, gelatin, $\beta$-tricalcium phosphate, biodegradation, bone regeneration.

***Address for correspondence**: Osamu Suzuki, Division of Biomaterials Science and Engineering (Division of Craniofacial Function Engineering), Tohoku University Graduate School of Dentistry, 980-8575 Sendai, Japan. E-mail: suzuki-o@tohoku.ac.jp.

**Copyright policy**: © 2026 The Author(s). Published by Forum Multimedia Publishing, LLC. This article is distributed in accordance with Creative Commons Attribution Licence (http://creativecommons.org/licenses/by/4.0/).

## Introduction

Synthetic calcium phosphate materials, such as hydroxyapatite (HA), have been widely used as bone substitutes in orthopedic and oral bone defects due to their excellent osteoconductive property, which can directly bond to living bone [1,2]. Biodegradable calcium phosphate materials have attracted the attention of many researchers for repairing bone defects because they can be expected to be replaced by the patient's own tissue [3–5]. $\beta$-tricalcium phosphate ($\beta$-TCP), carbonate apatite ($CO_3Ap$), and octacalcium phosphate (OCP) have been classified as biodegradable materials [1,6]. These biodegradable calcium phosphate materials show specific solubilities depending on their structures and the chemical compositions under physiological pH [6–9]. It seems likely that focusing on the solubility of calcium phosphate materials is of interest in terms of exploring their bioactivity [10].

OCP has been reported to be involved as a precursor phase in the formation of HA from supersaturated solutions with respect to HA and advocated as a precursor of bone apatite crystals [11–14]. In fact, the presence of OCP has been detected in human and rat bone tissues under high-resolution transmission electron microscopic (HRTEM) observation [15]. In recent years, there has been growing interest in OCP as a biomaterial: OCP shows bone regeneration capacities in oral and orthopedic bone defects [10,15–17] and works as a drug delivery carrier [10,16–19]. OCP has higher osteoconductive properties in some material forms, such as granules [20], coating [21–25], thin film [26], and blocks [27–29].

OCP is known as a metastable phase in physiological conditions as well as amorphous calcium phosphate (ACP) [7,30]; therefore, it has also been studied from the perspective of mineralization or the interaction with matrix proteins [31–34]. The participation of OCP in biomineralization has been studied from the viewpoint of the advancement of hydrolysis to explain the characteristics of biological apatite crystals [35,36]. The importance of OCP participation in biomineralization has also been investigated to explain the incorporation of carbonate ions into bone apatite crystals [37], the plate-like crystal morphology of bone apatite crystals [13], and the bonds between bone apatite crystals mineralized in bone matrices [38]. It has been reported that human plasma is supersaturated with respect to HA but almost saturated with respect to OCP [39]. Therefore, it is believed that OCP does not simply dissolve in the physiological conditions [39], and in fact, equilibrium is reached in a slightly supersaturated state in a buffer solution containing $Ca^{2+}$ and inorganic phosphate (Pi) ions in a chemical experiment [40]. However, it has been demonstrated that OCP implanted onto mouse calvaria or within rat calvaria bone defect indeed undergoes a progressive transformation into an apatitic phase over time [10,20,40,41]. Furthermore, it was confirmed that osteoconduction of OCP is induced when OCP undergoes a phase transformation to HA, suggesting that the metastability of OCP is an important factor determining its performance as a biomaterial [10,40,41].

OCP composites with tissue-derived polymers such as collagen [42] and gelatin [43], as well as composites with synthetic polymers such as poly (lactide-*co*-glycolide) (PLGA) [44] and polycaprolactone (PCL) [45], have been developed as bone substitute materials, and their bone tissue responses have been studied from the viewpoint regarding whether they can be used as filling bone defects in dental or orthopedic fields. Two of these composite materials, OCP/collagen (OCP/Col) [42] and OCP/gelatin (OCP/Gel) [43], have recently been approved in Japan for clinical use in dentistry and orthopedics, respectively. Because OCP contains a large number of water molecules in the structure ($Ca_8H_2(PO_4)_6 \cdot 5H_2O$), this characteristic makes it difficult to mold, which is different from sintered HA ($Ca_{10}(PO_4)_6(OH)_2$) and $\beta$-TCP ($Ca_3(PO_4)_2$) materials and therefore combining OCP with polymeric materials is one of the effective methods [42–45]. These composites exhibit porous form and have the advantage of being easy to handle and fill oral bone defects [42] and orthopedic bone defects [43]. These composite materials exhibit biodegradability as a composite [46] as well as the single use of OCP [42], which could be related to the biodegradable property of OCP [47]. In this review article, we focus on introducing the bone formation coupled with the biodegradation of an OCP/Gel composite in comparison with a typical biodegradable material, porous $\beta$-TCP, and discuss its potential application as a bone substitute in the field of orthopedics, with a special emphasis on the chemical, structural, and bioactive performance of OCP.

## Structural and chemical properties of HA, $\beta$-TCP, and OCP

### *Structural properties*

Crystal structures of HA, $\beta$-TCP, and OCP (Fig. 1) belong to the hexagonal system with space group P63/m6, triclinic, and rhombohedral systems, respectively [12,48,49]. The stoichiometric chemical compositions of HA, $\beta$-TCP, and OCP are $Ca_{10}(PO_4)_6(OH)_2$, $Ca_3(PO_4)_2$, and $Ca_8H_2(PO_4)_6{\cdot}5H_2O$, respectively. The positions of $Ca^{2+}$, $PO_4{}^{3-}$, and $OH^-$ in the HA crystal structure can be substituted with another cation and anion [50–53]. The bone apatite contains carbonate ion, which is substituted with a part of $PO_4{}^{3-}$ and $OH^-$ of the HA structure. In wet synthesis, the nonstoichiometric composition of HA, such as Ca-deficient HA (CDHA, $Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$), is able to be synthesized [54–57]. The synthesis of the nonstoichiometric composition of OCP ($Ca_{16}H_{4+x}(PO_4)_{12}(OH)_x{\cdot}(10\text{-x})H_2O$) by the direct precipitation method is also reported [12,41,47]. The position of $Ca^{2+}$ in the OCP structure is able to be substituted with other metal ions, such as $Ag^+$, $Sr^{2+}$, $Mg^{2+}$, and $Mn^{2+}$ [28,58]. Mathew *et al*. [12] identified the crystal structure of OCP, which consists of a CDHA-like layer and a dicalcium phosphate dihydrate-like hydrated layer. These layers stack alternatively toward the *a*-axis of the OCP lattice. The hydrogen phosphate ions are present either in the hydrated layer or at the junction of the CDHA-like and hydrated layers. The hydrogen phosphate ions in the hydrated layers is also able to be substituted with the organic molecules of dicarboxylate [59–61]. The lattice parameters toward the *c*-axis for HA and OCP are almost the same ($c$ = 6.883 Å for HA, $c$ = 6.835 Å for OCP) [12,48], and the parameter toward the *a*-axis for HA is approximately half the value for OCP with collapsed hydrated layer ($a$ = 9.4214 Å for HA, $a$ =18.86 Å for collapsed OCP, $a$ = 19.692 Å for OCP) [62]. The presence of the $Ca_9(PO_4)_6$ cluster within the crystal structure of HA and the CDHA-like layer of OCP is assumed [63]. The $Ca_9(PO_4)_6$ cluster is a structure model of ACP proposed by Posner *et al*. [64]. The ACP cluster is detected during the nucleation of calcium phosphate in aqueous solution [65–67]. This structural similarity suggests that ACP and OCP are precursors of HA when HA is precipitated in the aqueous solution [31,63]. TCP is synthesized through the solid reaction at high temperatures. The $\beta$ phase is the low-temperature phase of TCPs and transforms to the $\alpha$ phase (monoclinic) at 1125ºC [68].

### *Chemical properties*

The solubility of calcium phosphates depends on the thermodynamic stability of their crystal structure under the physiological pH and temperature [8]. The solubility of HA is lower than that of OCP and $\beta$-TCP, and OCP shows lower solubility than $\beta$-TCP [8,70–72]. However, the solubilities of calcium phosphates are also controlled by chemical composition, such as $Ca^{2+}$ and Pi ions of the surrounding

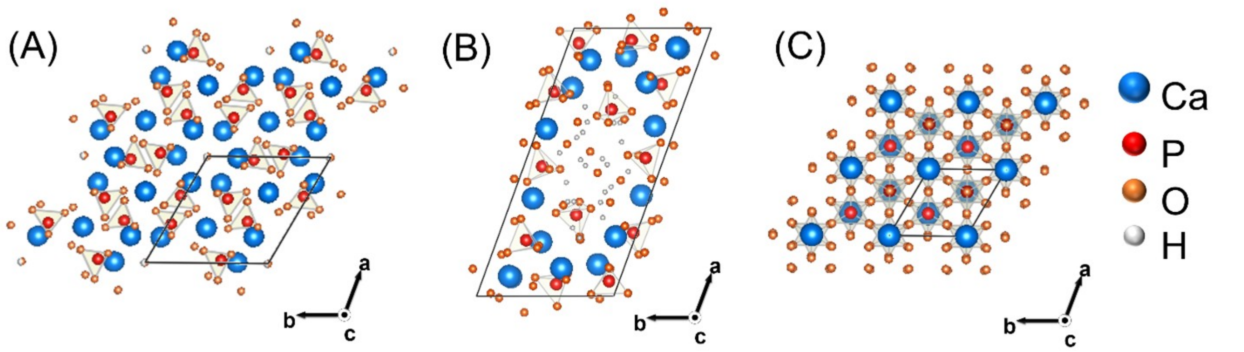


**Fig. 1. Schematic images showing the unit cells of HA (A), OCP (B), and $\beta$-TCP (C) on the (001) plane.** Crystal structures of these calcium phosphates are drawn by VESTA [69]. Reproduced from Suzuki O *et al*.[10], Dent Mater J 2020; 39: 187–199 with permission.

environment [10]. The serum is supersaturated and saturated with respect to HA and OCP, respectively [39]. In addition, simulated body fluid (SBF) [73,74] is slightly saturated with respect to OCP and $\beta$-TCP after the incubation of OCP and $\beta$-TCP granules, respectively [10]. SBF incubated with these granules is supersaturated with respect to HA. The degree of supersaturation with respect to calcium phosphates suggests that OCP and $\beta$-TCP do not simply dissolve and become resorbed in the physiological conditions expected chemically. However, OCP gradually transforms into a stable phase of HA through the hydrolysis reaction in physiological environments. In the hydrolysis reaction, OCP crystals release Pi ions and uptake $Ca^{2+}$ from the surrounding environment [7,10]. The dissolution-precipitation reaction and ion diffusion-crystallization process have been proposed as the transformation mechanisms [7,10,75]. In the dissolution-reprecipitation reaction, a part of the OCP crystal is dissolved, and de novo HA crystals are formed on its surface [7]. When the OCP hydrolysis progresses through the ion diffusion-crystallization process, $HPO_4{}^{2-}$ ions and $H_2O$ in the hydrated layer are diffused, and HA structures are formed epitaxially due to the structural similarity between OCP and HA [10,75]. The higher degree of supersaturation with respect to HA is a driving force of the progress of transformation. Fluoride ion acts as a promoter for the hydrolysis of OCP [76,77], whereas $Mg^{2+}$ [76], pyrophosphate [78], and polyacrylic acid [79] are inhibitors. Although increasing the amounts of serum protein adsorbed tended to inhibit the OCP hydrolysis, serum-derived albumin and fetuin assist in the formation of de novo crystals on the surface of OCP at a suitable concentration [80,81]. The HA formed from OCP through the hydrolysis reaction has a nonstoichiometric composition, which corresponds to that of CDHA [41,47].

### *Bioactivity synergy*

After the implantation of OCP onto the mouse calvaria or in the mammal subcutaneous pouches, de novo apatite crystals formed on the surface of plate-like crystals of OCP were observed by transmission electron microscope [10,82]. The $\beta$-TCP also forms de novo apatite after implantation in the rat subcutaneous pouches, whereas part of the $\beta$-TCP structure remains [10]. The de novo apatite formation of $\beta$-TCP could be induced by increasing the degree of supersaturation with respect to HA through the partial dissolution of $\beta$-TCP. The formation of de novo crystals on the CDHA surface is also observed after the incubations [82]. Although stoichiometric HA is a stable phase in physiological conditions, nonstoichiometric composition, such as deficient $Ca^{2+}$ and substitution of $CO_3{}^{2-}$, increases the solubility of HA [6,83]. The solubilities of calcium phosphates are associated with crystal structure and chemical composition. However, the degree of supersaturation indicates that the unstable calcium phosphates of OCP and $\beta$-TCP remain and form a stable phase of the apatitic structure via their dissolution. Therefore, biodegradation of OCP and $\beta$-TCP could be induced through the mechanisms of both self-dissolution of the crystal and osteoclastic-mediated resorption.

## Performance comparison of HA, $\beta$-TCP, and OCP in bone repair

The first study to observe the osteoconductive property of OCP compared the appearance of bone tissue following the subperiosteal implantation of granules of OCP, dicalcium phosphate anhydrous (DCPA), amorphous calcium phosphate (ACP), and non-sintered two HA materials (CDHA and stoichiometric HA) into the mouse calvaria [20]. Although DCPA, OCP, and ACP induced bone tissue earlier appearance than HA materials did, OCP enhanced the most among these precursor calcium phosphates to the apatite phase [20]. Further studies clarified that OCP enhances bone formation more than sintered HA in the rabbit femur defect [41] and in the mouse calvarial defect [84], and non-sintered CDHA prepared through the hydrolysis of OCP in the rat calvarial defect [41], and that OCP is resorbed more rapidly than $\beta$-TCP in the rat tibia defect [47] and in the mouse calvarial defect [84]. OCP accumulated tartrate-resistant acid phosphatase (TRAP)-positive multinucleated cells in the rabbit [41] and alkaline phosphatase-

**Table 1. Newly formed bone and remaining implants in the rat and mouse calvarial bone defects treated with calcium phosphate materials were quantified by histomorphometric analysis.**

| Implanted materials | Rat calvarial bone defect (6 months) | | Mouse calvarial bone defect (10 weeks) | |
|---|---|---|---|---|
| | New bone area (%) | Remaining implants (%) | New bone area ($\times 10^5$ $\mu m^2$) | Remaining implants ($\times 10^5$ $\mu m^2$) |
| OCP | 63.3 | 6.58 | 13.3 | 9.1 |
| $\beta$-TCP | 42.8 | 23.4 | 8.7 | 6.8 |
| HA | 24.2 | 39 | 5.6 | 13.2 |

The numerical values were reproduced from Kamakura S *et al.* [87], J Biomed Mater Res, 2002; 59:29–34 and Sato T *et al.* [84], Acta Biomater, 2019; 88: 477–490 with permission. OCP, octacalcium phosphate; $\beta$-TCP, $\beta$-tricalcium phosphate; HA, hydroxyapatite.

positive cells on the mouse calvaria [85] and osteocalcin-positive cells in the bone marrow of mouse tibia [82] around OCP itself during their bone formation and resorption, suggesting that osteoblastic cells and osteoclastic cells around OCP are activated [10,40]. OCP also stimulated osteocyte differentiation than CDHA in a critical-sized defect of rat calvaria [86]. These tissue responses suggested that OCP enhances new bone formation during the hydrolysis from OCP to CDHA [10,40]. Later studies confirmed that the stimulatory capacity of OCP to bone tissue-related cells is induced while OCP is hydrolyzed into CDHA: the hydrolysis evokes inorganic ions exchange, that is, $Ca^{2+}$ incorporation into OCP and Pi ions release from OCP over time under physiological conditions [10]. These comparative performances of OCP, $\beta$-TCP, and HA were summarized (Table 1) [84,87].

## The mechanism of OCP in new bone formation

*In vitro* studies using several culture techniques found the following cellular responses to OCP in relation to new bone formation: (1) OCP enhances the early stage of differentiation of mouse bone marrow stromal ST-2 cells to osteoblast in a dose-dependent manner [41]; (2) Osteoclast formation is increased from bone marrow macrophages without the addition of the osteoclast differentiation factor receptor activator of NF-kappaB ligand (RANKL) in a co-culture with osteoblasts, with enhanced expression of RANKL in osteoblasts [10]; (3) OCP enhances differentiation of mesenchymal stem cells (MSCs) toward osteocytes [86] when using the IDG-SW3 cell-line, which exhibits a differentiation phenotype toward osteocytes [88, 89]; (4) OCP enhances osteoblast differentiation more than HA and $\beta$-TCP in a MSCs 3D spheroid culture incorporating these calcium phosphate particles [84]; (5) OCP enhances macrophage migration toward own surfaces through moderate dissolution of $Ca^{2+}$ [40]. OCP has also shown the stimulatory or regulatory capacity to bone tissue-related cells, including chondrogenic cells [90], tendon stem/progenitor cells [91], human umbilical vein endothelial cells (HUVECs) [10], and C2C12 myoblast differentiation [92]. However, further investigations are needed to elucidate the molecular mechanisms by which OCP modulates the activities of these bone tissue-related cell types.

## Preparation of OCP/Gel composite

Because OCP cannot be sintered while maintaining its single crystal phase [93,94] and lacks sufficient mechanical strength, unlike HA and $\beta$-TCP, the handling performance can be significantly improved by combining with polymeric materials [40]. OCP composite materials with tissue-derived macromolecules such as collagen, gelatin, and hyaluronic acid, or with synthetic polymers, including PLGA, have been investigated [40]. Among these polymeric materials, collagen and gelatin possess cell-adhesive sites, high biocompatibility, and have been widely applied as biomedical materials [95,96]. Furthermore, gelatin exhibits a sol-gel transition at low temperatures and is advantageous for material fabrication [97]. OCP/Gel composites with porous structure have been prepared to investigate their bone generative capacity in various animal bone defect models [43,46,98,99]. Gelatin, a natural biodegradable polymer, is extracted from animal tissue, such as bovine bone and porcine skin, under acidic or alkaline conditions [100]. Gelatin is a random-coiled protein obtained from the thermal denaturation of collagen molecules [97]. Gelatin forms a hydrogel by forming a collagen-like triple helix structure from a part of random coil protein when the hot gelatin solution is cooled [101–104]. In contrast, collagen solution forms a hydrogel at physiological temperature [105–107]. The porous gelatin form is obtained through the lyophilization of gelatin hydrogel and applied as a scaffold for tissue regeneration, including bone tissue. For the preparation of OCP/Gel composites, (1) the mixing method [43,99] and (2) the co-precipitation method [98] have been reported. In the mixing methods, the OCP granules synthesized by direct precipitation methods are soaked in the gelatin solution and stirred under a low temperature, such as 4ºC [99]. The hydrogel of gelatin with OCP granule is lyophilized and then heated under vacuum conditions (dehydrothermal treatment) to form a porous composite. The dehydrothermal treatment is performed for the crosslinking of gelatin molecules, and the OCP maintains its crystal structure, which is confirmed by X-ray diffraction [108]. The content and granular size of OCP in the porous composite can be controlled easily using this method [43,99]. The synthesis and preparation of the OCP/Gel composites were summarized (Fig. 2).

The microstructure of OCP/Gel composite prepared

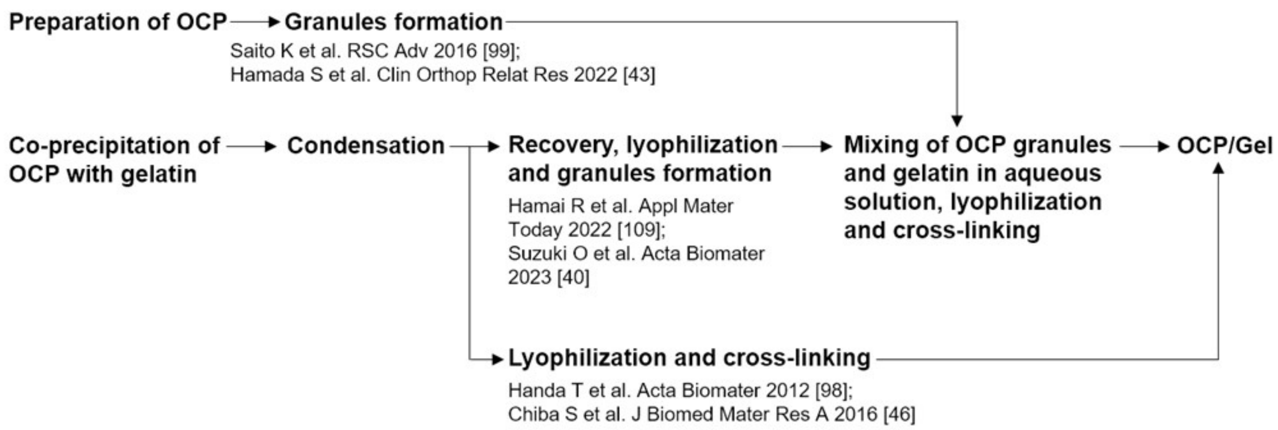


**Fig. 2. Flow charts for the synthesis and preparation of OCP/Gel composites.** Created by Microsoft PowerPoint.

by the mixing method was observed using a scanning electron microscope (Fig. 3). The average pore size of composites is approximately 200 $\mu$m, and porosity is around 90% when 17−44 wt.% of OCP granule with a diameter of 300−500 $\mu$m is mixed with gelatin [108]. In the co-precipitation method, OCP crystals are precipitated under supersaturation with respect to OCP and HA at higher temperatures by mixing a calcium salt solution with a phosphate solution containing gelatin molecules [98]. The precipitation obtained by the co-precipitation method was a single phase of OCP in the range from 0.1 to 2.0% of gelatin [98]. However, the synthesis condition of OCP co-precipitated with collagen is limited: the synthesis in the presence of collagen molecules was carried out at 25ºC to avoid the molecules denaturing [40]. The slurry of OCP with gelatin is lyophilized in the mold, and then dehydrothermal treatment is performed. The OCP/Gel composites prepared by the co-precipitation method have large pore size distributions ranging from 10 to 500 $\mu$m and high porosity of more than 90% [98]. Each OCP crystal with plate-like morphology oriented toward the long axis is dispersed homogeneously in the gelatin matrix [98]. The OCP/Gel composites prepared by mixing and co-precipitation methods can be molded into optimal shapes and sizes for filling the bone defects.

## Comparison of OCP with other calcium phosphate bone substitute materials

The solubility of calcium phosphate-based bone substitute materials affects the resorptive activity of osteoclasts, with more soluble calcium phosphate materials exhibiting superior biodegradability and bone formation effects [110–112]. The details of OCP solubility and hydrolysis under different conditions have been reported by Tung *et al*. [71]. A comparative study on the resorbability and bone formation-promoting effects of $\beta$-TCP, non-sintered stoichiometric HA, and OCP has been conducted [87], where the particle sizes of OCP and HA ranged from 300 to 500 µm, while those of $\beta$-TCP ranged from 250 to 500 µm. The new bone formation and remaining implants in the critical-sized mouse calvarial bone defect treated with OCP, sintered $\beta$-TCP, and sintered HA granules with a diameter of 300 to 500 µm were also evaluated [84]. The results of histomorphometric analysis of newly formed bone and remaining implants in the rat and mouse calvarial defect treated with calcium phosphates are shown in Table 1. These results indicate that OCP exhibited the highest resorbability and promoted bone formation in calvarial defects more effectively than other calcium phosphate bone substitute materials. Among calcium phosphate-based bone substitute materials, OCP exhibited the highest capacity to enhance alkaline phosphatase activity [17]. Compared to HA, the implanted OCP promoted greater bone formation [10,84]. In another study comparing the tissue response and osteoconductivity of OCP and sintered HA blocks, the OCP block exhibited approximately 30% bone replacement at four weeks post-implantation, whereas no bone replacement was observed in the sintered HA block during the same period [28]. When OCP was implanted into the tibial bone marrow of rats and compared to $\beta$-TCP, OCP demonstrated a similar bone formation outcome to $\beta$-TCP. However, while $\beta$-TCP showed the highest bone formation rate at day 14, OCP exhibited a gradual increase in bone formation up to day 56 [47]. Furthermore, at 56 days post-implantation, osteocalcin staining showed positive cells around both $\beta$-TCP and OCP, whereas osteopontin staining showed positive cells around OCP but not around $\beta$-TCP [47]. In a study comparing the osteoconductivity of OCP and ACP [113], implantation into rat calvarial defects for eight weeks resulted in 16.9 $\pm$ 2.9% new bone formation in the ACP group, whereas the OCP group exhibited 38.2 $\pm$ 4.3%, demonstrating the superior bone-forming ability of OCP. In addition to the superior biodegradability and osteogenic capacity of OCP-based composites, it has been increasingly recognized that appropriately regulated inflammation plays a critical role in promoting bone regeneration [47,114–116]. Specifically, such inflammation fa-

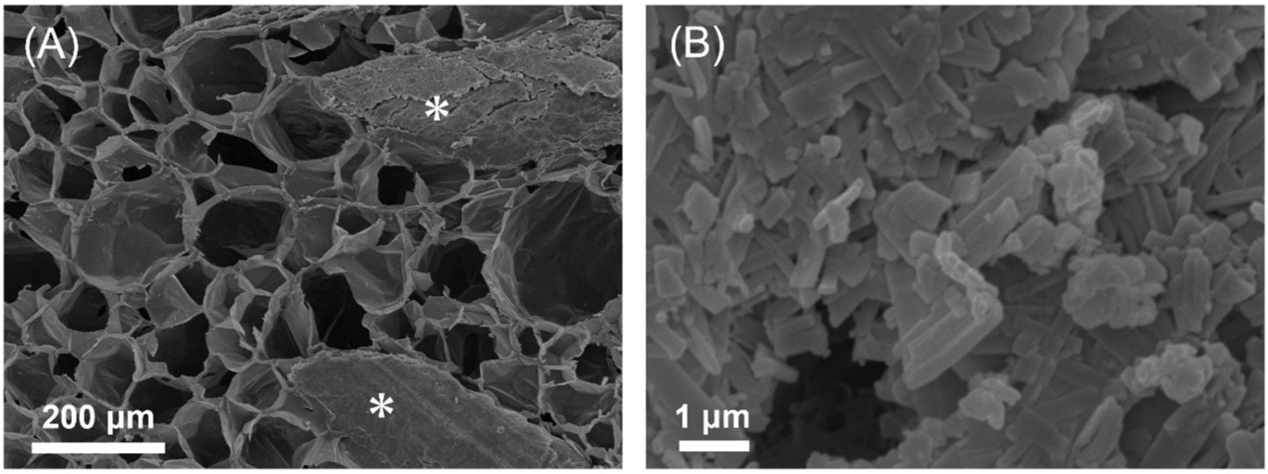


**Fig. 3. Scanning electron microscope images of OCP/Gel composite prepared by the mixing method at lower (A) and higher magnifications (B).** The magnified image corresponds to the granule consisting of aggregated OCP plate-like crystals in the composite. Asterisks indicate OCP granules. Bars = 200 $\mu$m (A) and 1 $\mu$m (B). Reproduced from Hamai R *et al*. [109], Appl Mater Today 2022; 26: 101279 with permission.

cilitates the recruitment and differentiation of mesenchymal stem cells into osteoblasts, thereby contributing to effective bone repair [117,118]. The favorable tissue response induced by OCP/Gel, as evidenced by early bone formation and resorption, may in part reflect its ability to create a local environment that supports this inflammation-mediated regenerative process. The composites of OCP have also been reported to exhibit excellent biodegradability and bone formation capability. OCP/Col has been shown to undergo early biodegradation and promote bone formation more effectively than $\beta$-TCP [119]. Similarly, OCP/alginate (Alg) has demonstrated superior early biodegradability and bone formation compared to $\beta$-TCP [18]. Additionally, OCP/Gel and OCP/PLGA have been reported to promote earlier bone formation than Gel and exhibit superior early biodegradability and bone formation compared to PLGA, respectively [43,44,108]. The biodegradability and bone formation of gelatin, OCP/Gel, and $\beta$-TCPs with porosities of 60% and 71–80% were evaluated by a histomorphometric analysis (Fig. 4, reproduced from reference [46]). The implanted OCP/Gel was prepared through the co-precipitation method described above [98]. At two and four weeks post-implantation, hematoxylin and eosin staining of decalcified specimens indicated that OCP/Gel promoted more extensive new bone formation around OCP compared to gelatin and $\beta$-TCPs. Concurrently, OCP/Gel demonstrated earlier in vivo degradation and resorption than both $\beta$-TCPs with 60% porosity and those with high porosity (71–80%). Quantitative analysis of newly formed bone showed that OCP/Gel significantly enhanced bone formation compared to $\beta$-TCPs. At eight weeks in the cortical area, the percentage of newly formed bone (mean $\pm$ SD) was 16.9 $\pm$ 6.2% for gelatin, 25.6 $\pm$ 3.6% for OCP/Gel, 9.1 $\pm$ 5.2% for $\beta$-TCP (71–80%), and 8.7 $\pm$ 3.5% for $\beta$-TCP (60%). The OCP/Gel group exhibited a significantly higher amount of new bone formation than both $\beta$-TCP groups ($p < 0.05$).

The bone regeneration of the rabbit tibia bone defect was analyzed by representative computed tomography (Fig. 5, reproduced from reference [46]). The $\beta$-TCP groups with porosities of 60% and 71–80% tended to exhibit greater residual material and reduced bone formation, whereas the OCP/Gel group showed less residual material and a greater extent of bone formation. These observations suggest that OCP/Gel has superior biodegradability and osteogenic potential compared to $\beta$-TCP.

The synthesis and performance of composites of gelatin with HA [120,121], $\beta$-TCP [122,123], and OCP [98,99] collected from published papers were summarized (Table 2). These composites were obtained either by mixing calcium phosphate granules with gelatin or by a co-precipitation method prepared in an aqueous solution in the presence of gelatin. The composite materials possess porous spongy forms and exhibit osteogenic *in vitro*, osteoconductive, and resorbable properties *in vivo* conditions.

## Increasing the osteoconductive property of OCP by introducing structural defects

In the rat calvarial bone defects, OCP/Gel composites exhibit superior bone formation and biodegradation capacities [98]. The crystallinity of OCP separated from the slurry of wet synthesis in the presence of gelatin (c-OCP) was different [109] from OCP synthesized in the absence of gelatin (w-OCP) [20]. Crystallinity is regulated by the presence of lattice defects, such as point defect, line defect, and plane defect. The deficiency of $Ca^{2+}$ in OCP is a point defect. The Ca/P molar ratio of c-OCP and w-OCP was similar, which indicates that these OCPs include a similar density of point defects in the lattices [109]. Therefore, the lattice structure of OCPs was analyzed by a HRTEM. The

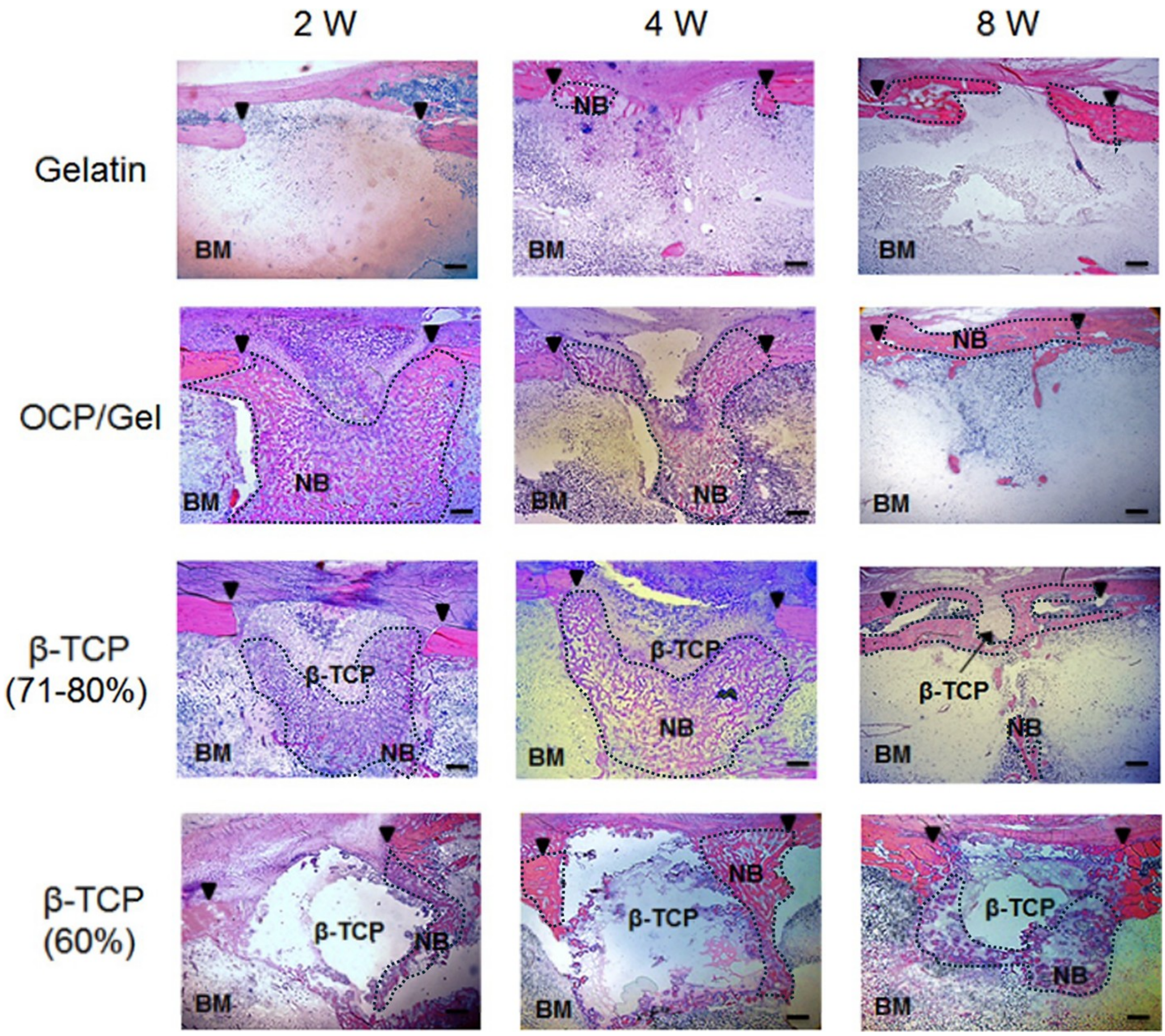


**Fig. 4. Overview of hematoxylin and eosin–stained sections showing rabbit tibia bone defect regions following treatment with gelatin, OCP/Gel, $\beta$-TCP (71–80%), and $\beta$-TCP (60%) at 2, 4, and 8 weeks.** The newly formed bone area is enclosed with a dashed line. Scale bars = 1 mm. ▼, Defect margin; NB, newly formed bone; BM, bone marrow; $\beta$-TCP, $\beta$-tricalcium phosphate. Reproduced from Chiba S *et al*. [46], J Biomed Mater Res A 2016; 104: 2833–2842 with permission. The figure is being revised to outline the newly formed bone with dashed lines.

extra half-planes inserted in (002) and (030) were observed in HRTEM images of both OCPs, which means that this OCP contains a line defect of edge dislocation having the Burgers vector of $\vec{b}$ = 1/2 [001] and 1/3 [010] [109]. The fast Fourier transform (FFT)-inverse FFT analysis clearly visualized the distribution of dislocations in the OCP. The total density of dislocation was higher in the c-OCP than in the w-OCP, and the number of dislocations having $\vec{b}$ = 1/2 [001] was larger than that having $\vec{b}$ = 1/3 [010] [109]. In addition, the morphology of the c-OCP plate crystal grew well oriented toward the *c*-axis compared to w-OCP, which suggests that the adsorption of gelatin molecules onto the *b*-plane controls the crystal growth direction [109]. Thus, the lower crystallinity of c-OCP is owing to the high dislocation density, and the incorporation mechanism of dislocation could be involved with the oriented crystal growth of c-OCP [109]. Previous literature reported that the adsorption of silk fibroin molecules onto the OCP surface during the crystal growth is associated with the incorporation of dislocations [124]. The higher solubility of OCP than HA in physiological conditions induces the hydrolysis reaction of OCP [7]. The chemical environment changed by OCP hydrolysis promotes osteoblastic differentiation of MSC [82]. The dissociation rate constant normalized by surface area was higher in c-OCP than in w-OCP in 150 mM tris (hydroxymethyl) aminomethane

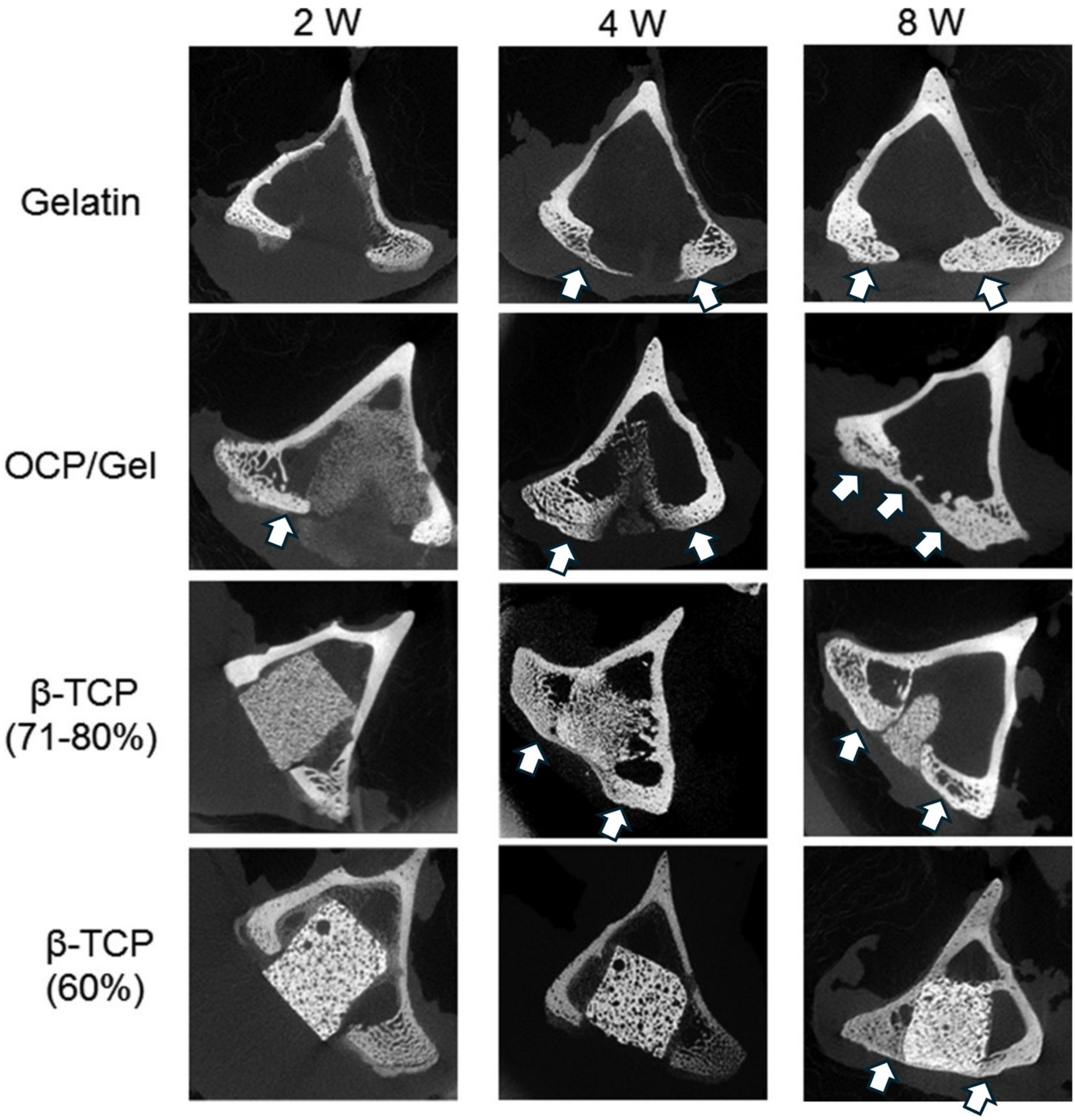


**Fig. 5. Representative micro-CT images of rabbit tibia bone defect regions following treatment with gelatin, OCP/Gel, $\beta$-TCP (71–80%), and $\beta$-TCP (60%) at 2, 4, and 8 weeks.** The arrows indicate the newly formed cortical bone. Reproduced from Chiba S *et al*. [46], J Biomed Mater Res A, 2016; 104: 2833–2842 with permission. A modification is being made to point out the newly formed bone with arrows.

(Tris)-HCl buffer. The rate of $PO_4$/$HPO_4$ on the surface of c-OCP increased, whereas the rate on the surface of w-OCP was maintained after the incubations [109]. These findings suggest that c-OCP undergoes faster hydrolysis than w-OCP under physiological conditions. The lattice strain is induced around the edge dislocation, and its density increases the internal energy of the crystal [125,126]. The increase in internal energy by edge dislocation density (ρ) of crystals is explained by equation (1) [126]:

$$\Delta E_{dis} = \frac{\mu|\vec{b}|^2}{4\pi(1-\nu)} ln\left(\frac{r}{r_0}\right) \times \rho \times V_m \quad (1)$$

where $\overrightarrow{b}$, r, and $r_0$ are the magnitude of the Burgers vector, the average mid-distance between dislocations, and the radius of the dislocation core, respectively. The values of $\mu$, ν, and $V_m$ are the elastic modulus, Poisson's ratio,

**Table 2. Synthesis and performance of HA/Gel, $\beta$-TCP/Gel, and OCP/Gel composites.**

| Materials | Synthesis | Bulk form | Tissue/cell responses | References |
|---|---|---|---|---|
| HA/gelatin | Granules mixing | Porous sponge | Osteogenic differentiation (*in vitro* cell culture) | Kim HW *et al.* 2005 [120] |
| HA/gelatin | Co-precipitation | Porous sponge | Osteogenic differentiation (*in vitro* cell culture) | Kim HW *et al.* 2005 [120] |
| OCP/gelatin | Granules mixing | Porous sponge | Osteoconductive/ resorbable | Saito K *et al.* 2016 [99] |
| OCP/gelatin | Co-precipitation | Porous sponge | Osteoconductive/ resorbable | Handa T *et al.* 2012 [98] |
| $\beta$-TCP/gelatin | Granules mixing | Porous sponge | Osteogenic differentiation (*in vitro* cell culture) | Takahashi Y *et al.* 2005 [122] |

HA, hydroxyapatite; OCP, octacalcium phosphate; $\beta$-TCP, $\beta$-tricalcium phosphate.

and molar volume of OCP, respectively. Although the activation energy of chemical reactions, such as dissolution and hydrolysis reactions, is the same between w-OCP and c-OCP, the increase in internal energy by high edge dislocation density decreases relatively the activation energy of reactions [109]. In the 3D cell culture of MSC using the oxygen-permeable culture chip, the spectroscopic analysis indicated that the hydrolysis of c-OCP aggregated with MSC spheroids was promoted compared to that of w-OCP [109]. Furthermore, the cells aggregated with c-OCP expressed higher alkaline phosphatase activity, an early and middle-stage osteoblastic differentiation marker [109].

The bone regenerative capacities of c-OCP and w-OCP were compared by implanting them in critical-sized rat calvarial defects [40,109]. The porous gelatin composite forms prepared by the mixing method were used for the implantation of these OCP granules [99] to exclude the effect of adsorbed gelatin onto c-OCP [40,109]. These w-OCP/Gel and c-OCP/Gel composites had similar porous structures, and these OCP granules were dispersed homogeneously in the porous gelatin structure. The histological analysis indicated that the amount of new bone was increased, and the remaining granule amount was decreased in the defect treated with c-OCP/Gel composites compared to w-OCP/composite [40,109]. The increased dissolution and hydrolysis rates due to dislocations with high density in c-OCP could contribute to the formation of new bone tissue by promoting osteoblast differentiation of MSCs through increased ion dissolution [40,109]. OCP is gradually resorbed by osteoclasts formed from macrophages [41,47,85]. The solubility of c-OCP was enhanced by the dislocation with high density, and the OCP crystals could be dissolved and resorbed easily in the bone defect [40,109]. Therefore, the incorporation of edge dislocation is expected to be a parameter in designing the OCP bone substitute with enhanced osteoconductivity and biodegradability.

## Effect of gelatin biodegradation on bone regeneration

Gelatin is resorbed by matrix metalloproteinases (MMPs), such as MMP-2 (gelatinase A) and MMP-9 (gelatinase B) [127,128]. Collagen is also decomposed by these MMPs and their living bodies, and their degradation products regulate the healing of tissue [129]. The generation of structure degradation products is regulated by the type of collagen and MMP. In the bone tissue, C-propeptide (120 kDa), a type I collagen degradation product generated by MMP-9, stimulates endothelial chemoattractant and induces vascularization [130]. The type-I collagen degradation products of proline-glycine-proline (PGP) also promote angiogenesis through the migration, proliferation, and lumen formation of endothelial cells [129,131]. The PGP is generated by MMP-8/MMP-9 and prolyl endopeptidase [131]. The collagen sponge loaded with N-acetylated PGP promotes mouse wound healing by increasing the newly formed vessels [132]. The administration of N-acetylated PGP induces the circulating angiogenic cells via activation of CXCR2 and reduces tissue necrosis in mice [133]. The N-acetylated PGP binds the receptor of CXCR2 on the endothelial progenitor cells to induce angiogenesis [132]. PGP has a similar role to the SGP motif in interleukin-8 (IL-8) [132,134]. IL-8 binds CXCR1/CXCR2 and contributes to the tube formation of HUVECs [135,136]. In addition, MMPs regulate the bone regeneration [137–139]. Previous literature reported that the MMP-9-deficient mouse suppresses osteoblastic differentiation by reducing the bioavailability of vascular endothelial growth factor in the process of fracture healing [140]. Vascularization is well-known to be an important factor in bone regeneration [141–144]. The newly formed vessels supply the inorganic ions for calcification, nutrients, and support stem cell migration [141,142]. Recently, H-type blood vessels have attracted attention to promote the bone regeneration [145–148]. Based on the collagen degradation involved in tissue regeneration, degradation of porous gelatin by MMP may also affect bone regeneration because gelatin is a random-coiled protein obtained from collagen. In the previous literature, the effects of gelatin degradation on bone regeneration were investigated by the implantation of porous gelatin scaffolds with different gelatin content (1, 3, 5, and 7 w/v%) [149]. Type A porcine skin-derived gelatin with 50–100 kDa was used for the preparation of sponges [149]. The bone volume and newly formed vessels were increased in the defect treated with a sponge at higher gelatin content compared to lower gelatin content [149]. The accumulated MMP-9-positive cells were observed in the sponge at higher gelatin content, which may provide a large amount of gelatin degradation products [149]. The molecular weight of gelatin is lower than that of C-propeptide. Therefore, the degradation products containing PGP could be generated by accumulated MMP-9-positive cells. The previous literature also examined whether the degradation products of the gelatin sponge generated by MMP-9/PE affect angiogenesis *in vitro* [149]. The addition of sponge degradation products into the culture media promoted the capillary-like structure of HUVECs compared to gelatin [149]. These results suggest that the degradation of the gelatin sponge could contribute to bone regeneration through the induction of angiogenesis [149]. MMP-9 is produced by bone marrow-derived cells, such as macrophages, neutrophils, and osteoclasts [140,150]. The low $Ca^{2+}$ concentration around OCP stimulates the migration of macrophages and osteoclast formation [40]. It suggests that the macrophage or osteoclast accumulation induced by the chemical environment around OCP could contribute to promoting the generation of PGP delivered from gelatin in the OCP/Gel composites. The degradation of gelatin in the bone defect treated by OCP/Gel composites was observed by histological analysis [43]. However, TRAP-positive cells are localized on the OCP but not around the gelatin matrix in the OCP/Gel composites implanted in the bone defect models [43]. Therefore, the degradation of the gelatin matrix in OCP/Gel composites could support the angio-osteogenesis induced by OCP [10] in the bone defect. The relationship between the biomaterial characteristics of the OCP and OCP/Gel composite and the vascularization-supported bone formation coupled with the material resorption are illustrated conceptually (Fig. 6).

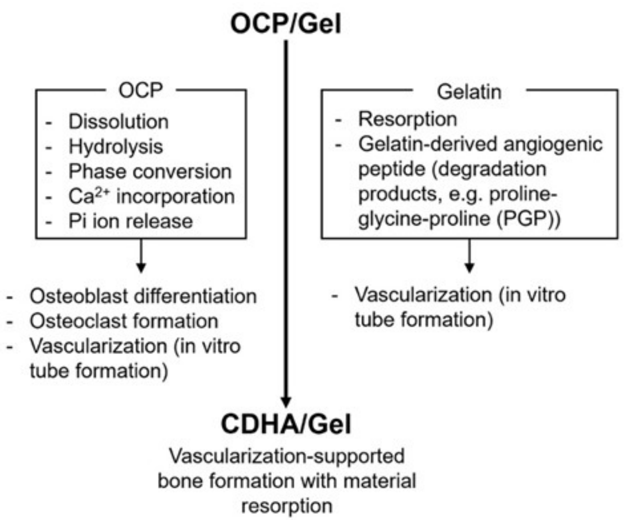


**Fig. 6. A conceptual diagram illustrating the relationship between the biomaterial characteristics of the OCP and the OCP/Gel composite, and vascularization-supported bone formation coupled with material resorption.** Created by Microsoft PowerPoint.

## Perspective on orthopedic application of OCP/Gel

Extensive bone loss resulting from trauma or disease is a major contributor to musculoskeletal disorders, often leading to disability, frailty, and a diminished quality of life. One of the persistent challenges in clinical practice is achieving effective healing of large bone defects, particularly in load-bearing regions. Critical-sized defects, generally defined as gaps of 3 cm or more in humans, are especially difficult to repair due to the compromised regenerative capacity of bone tissue [108,151]. Despite surgical intervention, delayed union or nonunion occurs in approximately 5% to 10% of fractures. In segmental or critical-sized bone defects, the failure rate of conventional reconstruction techniques remains high, often exceeding 20% to 30%, and successful union frequently requires complex, staged procedures [152,153]. Autologous bone grafts represent the current clinical gold standard for the treatment of large bone defects, offering a combination of high biological activity and mechanical strength necessary for functional bone regeneration. However, their use is constrained by notable limitations, including donor site morbidity, limited graft availability, and high rates of graft resorption [154,155]. The search for an ideal bone substitute material capable of replicating the properties of autologous bone has garnered considerable attention; however, a definitive and fully satisfactory solution remains elusive. The evaluation of synthetic bone substitute materials for clinical use in orthopedic surgery is principally guided by the biological and mechanical benchmarks established by autologous bone grafts [156,157]. Bone substitute materials must be highly bioactive, with osteoconductive and osteoinductive properties, to promote bone formation without the need for additional cells or growth factors. The OCP/Gel composite demonstrated earlier bone remodeling and cortical bone repair in a shorter time in critical-sized femoral cortical defects in rats and rabbits, suggesting that it could be used as a bone replacement material to treat severe bone defects [43,46]. In addition, the graft must possess sufficient mechanical strength to support long-term tissue regeneration when implanted into load-bearing defects and must be capable of withstanding physiological loading conditions [158–160]. Synthetic bone substitute materials must be fabricated through a controlled manufacturing process to achieve a reproducible structure characterized by high porosity and interconnectivity, thereby facilitating sufficient nutrient exchange and angiogenesis essential for supporting bone formation and continuous remodeling [108,156]. Current synthetic bone substitute materials, primarily composed of calcium phosphate or bioactive glass, are widely used in clinical practice. HA offers excellent mechanical strength and osteoconductivity but lacks sufficient osteoinductivity and biodegradability. Conversely, $\beta$-TCP possesses appropriate porosity for bone regeneration but exhibits inadequate mechanical strength [161–163]. As a result, existing synthetic bone substitute materials are largely limited to the repair of small, non-load-bearing bone defects, emphasizing the need for novel materials suitable for larger, load-bearing applications. To maintain sufficient strength for such applications, bone substitute materials typically require a lower porosity of approximately 50% [164–166]. However, limited bioactivity remains a major challenge, as current synthetic materials often fail to promote adequate bone formation in large defects. While OCP/Gel demonstrated favorable osteogenic potential, it similarly lacks the mechanical strength necessary for structural grafting [167]. OCP/Gel has attracted significant attention as a biocompatible material with high osteoinductive potential. However, because gelatin is a naturally derived polymer, the mechanical strength of the composite remains limited, making it unsuitable for application in load-bearing sites. This limitation becomes particularly critical in clinical scenarios such as fracture repair or reconstruction of large segmental defects, where mechanical stability is essential. Therefore, one of the major future challenges is to improve the mechanical performance of the composite without compromising its biocompatibility and biodegradability. Strategies under investigation include compositing OCP with synthetic polymers such as PLGA, which can enhance structural integrity while maintaining controlled degradation kinetics and biological activity [44]. Indeed, several reports have described mechanically reinforced bone graft substitutes using synthetic polymers or crosslinking techniques, which demonstrate improved strength while retaining osteoconductivity [168–171]. Optimization of crosslinking density and scaffold architecture is also an important area for further research to address the demands of load-bearing bone regeneration. Further studies are required to optimize composite formulations, assess defect size limitations, and evaluate the mechanical properties of regenerated bone relative to host bone. Despite considerable progress, an ideal synthetic bone substitute material has yet to be established. Given the promising preclinical outcomes of OCP/Gel composites [43] and their successful application in dental reconstructive procedures by OCP/Col [42], a careful, stepwise clinical evaluation is warranted. In Japan, the use of OCP/Gel in the field of orthopedic surgery has been approved since October 2024. Its approved indications include stable bone defects associated with benign bone tumors of the extremities, trauma, and periarticular osteotomies, as well as donor sites following iliac bone harvesting. OCP/Gel demonstrates superior shape-conforming capabilities and is expected to achieve complete filling of bone defects without leaving voids upon implantation. Owing to these properties, it holds the potential for effectively promoting bone regeneration when applied to defect sites following the resection of benign bone tumors, as well as in tibial and pelvic osteotomy procedures. Although OCP/Gel has been approved for orthopedic use in Japan, no clinical reports have yet been published demonstrating its safety and efficacy in actual human applications. This represents a critical evidence gap that needs to be addressed to translate its promising preclinical performance into routine clinical practice. Currently, a prospective multicenter clinical study is ongoing in Japan to evaluate the clinical utility of OCP/Gel in the treatment of benign bone tumors and periarticular osteotomies, comparing its performance with that of FDA-approved synthetic bone graft substitutes. Future publication of the study results is expected to clarify its clinical potential and support its wider adoption. Future expansion of its application to the spinal surgery field is anticipated. In this context, the clinical relevance of OCP/Gel for treating critical-sized bone defects deserves further exploration. While its approved indications are currently limited to stable, non-load-bearing defects, the material's favorable osteoinductive properties—demonstrated in preclinical models of large cortical defects [43,46]—suggest a potential translational application in larger or structurally compromised defects. The ongoing multicenter trial will provide valuable insight into this possibility. Although direct comparative clinical trials between OCP/Gel and autologous bone grafts are not yet available, preliminary observations suggest favorable handling characteristics, biocompatibility, and early radiographic bone healing. In particular, OCP/Gel may offer reduced donor site morbidity, shorter operative times, and easier defect filling compared to autografts. These practical advantages may contribute to faster recovery and fewer complications in selected patients. To address the mechanical limitations of OCP/Gel, several strategies are being explored for future clinical translation. These include compositing with synthetic polymers like PLGA [44] and fabrication of 3D-printed scaffolds tailored to match defect geometry and mechanical requirements [168,169]. Additionally, in non-load-bearing anatomical sites, such as post-resection voids from benign bone tumors, the inherent mechanical weakness of gelatin-based scaffolds may be of lesser clinical concern. The clinical scope of OCP/Gel could be significantly expanded if such adaptations prove successful in future trials. In addition, the potential application of OCP/Gel composites in spinal fusion procedures requires thorough investigation to establish their efficacy and mechanical reliability in load-bearing environments. Notably, favorable outcomes of OCP/Gel in a miniature swine spinal fusion model have been reported [172]. In this study, OCP/Gel and autologous bone were transplanted into spinal cages and compared, demonstrating comparable performance between the two materials. These findings further support the rationale for pursuing clinical studies in orthopedic spinal applications. Positive results from these preliminary investigations could justify expansion to larger defects commonly encountered in trauma and reconstructive orthopedic surgery. Despite its promising biological activity, several challenges must be addressed before OCP/Gel can be broadly adopted in clinical practice. From a translational standpoint, the scalability and consistency of composite manufacturing, regulatory approval processes for new formulations, and cost-effectiveness relative to existing materials represent practical hurdles. Furthermore, its current mechanical limitations restrict use in load-bearing applications, requiring strategic modifications to broaden its surgical indications. From a safety perspective, the potential risks associated with OCP/Gel should also be considered. Gelatin, being derived from animal sources, may pose a risk of immunogenicity, particularly if not thoroughly purified or if sourced from non-human species. However, it is noteworthy that in clinical studies using OCP/Col composites, no allergic reactions or immune-related complications have been reported, suggesting that the risk of immunogenicity may be low under appropriate processing conditions [42]. Nevertheless, comprehensive histological and immunological evaluations are warranted in future trials. Additionally, the rapid degradation of gelatin may lead to localized changes in pH, which could potentially affect cell viability or local bone microenvironment stability. These aspects underline the importance of establishing long-term biocompatibility and degradation profiles through rigorous in vivo testing. Overall, while OCP/Gel holds significant clinical promise, its successful translation will depend not only on demonstrating efficacy but also on ensuring safety, reproducibility, and mechanical optimization tailored to surgical demands.

## Conclusions

This review summarizes the bone regenerative potential of OCP/Gel composite in an orthopedic rat femur defect model and its general properties as a bone substitute material in comparison with $\beta$-TCP. OCP/Gel was shown to exhibit greater biodegradability than $\beta$-TCP, accompanied by higher bone regenerative properties. These characteristics are considered due to the intrinsic material properties of OCP itself and the associated degradability of its composite with gelatin matrix to follow the rate of new bone formation simultaneously. The studies suggested that OCP/Gel composites could be utilized in vascularized bone regeneration, representing a potential breakthrough direction. Further experimental studies using various bone defect models are warranted to provide useful information on the effectiveness of OCP/Gel for its clinical application for various bone defects, not only in the field of orthopedic surgery but also in the field of oral surgery.

## List of Abbreviations

ACP, amorphous calcium phosphate; CDHA, Ca-deficient hydroxyapatite; $CO_3Ap$, carbonate apatite; DCPA, dicalcium phosphate anhydrous; FFT, fast Fourier transform; HA, hydroxyapatite; HRTEM, high-resolution transmission electron microscope/microscopy; HUVECs, human umbilical vein endothelial cells; MMPs, matrix metalloproteinases; MSCs, mesenchymal stem cells; micro-CT, micro–computed tomography; OCP, octacalcium phosphate; OCP/Col, octacalcium phosphate/collagen; OCP/Gel, octacalcium phosphate/gelatin; PCL, poly(ε-caprolactone); Pi, inorganic phosphate; PLGA, poly(lactide-*co*-glycolide); RANKL, receptor activator of NF-kappaB ligand; SBF, simulated body fluid; TRAP, tartrate-resistant acid phosphatase; VESTA, visualization for electronic and structural analysis; $\beta$-TCP, $\beta$-tricalcium phosphate.

## Availability of Data and Materials

This article is a review and does not include any newly generated or analyzed datasets. All supporting information can be found in the referenced publications.

## Author Contributions

OS contributed to the conception and design of the work and to the methodology. YM and RH contributed to the methodology and analysis and interpretation of data. RK contributed to data interpretation. OS, YM, and RH drafted the manuscript. TA critically revised the manuscript for important intellectual content. All authors contributed to editorial revisions, read and approved the final manuscript, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

## Ethics Approval and Consent to Participate

Not applicable.

## Acknowledgments

The authors sincerely thank Prof. Eiji Itoi for his expert supervision, constructive suggestions, and continuous encouragement, which were essential for the completion of this study.

## Funding

This study was supported in part by the MEXT/JSPS KAKENHI, Grant Numbers JP23106010, JP21H03121, JP21K19586, and JP24K02624.

## Conflict of Interest

The authors declare no conflict of interest.

## References

[1] Oyane A, Wang X, Sogo Y, Ito A, Tsurushima H. Calcium phosphate composite layers for surface-mediated gene transfer. Acta Biomaterialia. 2012; 8: 2034–2046. https://doi.org/10.1016/j.actbio.2012.02.003.

[2] LeGeros RZ. Calcium phosphate-based osteoinductive materials. Chemical Reviews. 2008; 108: 4742–4753. https://doi.org/10.1021/cr800427g.

[3] Bohner M, Santoni BLG, Döbelin N. $\beta$-tricalcium phosphate for bone substitution: Synthesis and properties. Acta Biomaterialia. 2020; 113: 23–41. https://doi.org/10.1016/j.actbio.2020.06.022.

[4] Sweedy A, Bohner M, van Lenthe GH, Baroud G. A novel method for segmenting and aligning the pre- and post-implantation scaffolds of resorbable calcium-phosphate bone substitutes. Acta Biomaterialia. 2017; 54: 441–453. https://doi.org/10.1016/j.actbio.2017.03.001.

[5] Fukuda N, Takamaru N, Akita K, Kudoh K, Ishikawa K, Miyamoto Y. Application and evaluation of carbonate apatite granules for mandibular bone defect reconstruction after jawbone cyst enucleation-a retrospective case series. Oral and Maxillofacial Surgery. 2024; 29: 14. https://doi.org/10.1007/s10006-024-01306-2.

[6] Ishikawa K, Hayashi K. Carbonate apatite artificial bone. Science and Technology of Advanced Materials. 2021; 22: 683–694. https://doi.org/10.1080/14686996.2021.1947120.

[7] Brown WE, Mathew M, Tung MS. Crystal chemistry of octacalcium phosphate. Progress in Crystal Growth and Characterization. 1981; 4: 59–87. https://doi.org/10.1016/0146-3535(81)90048-4.

[8] Chow LC. Next generation calcium phosphate-based biomaterials. Dental Materials Journal. 2009; 28: 1–10. https://doi.org/10.4012/dmj.28.1.

[9] Driessens FCM. Physiology of hard tissues in comparison with the solubility of synthetic calcium phosphates. Annals of the New York Academy of Sciences. 1988; 523: 131–136. https://doi.org/10.1111/j.1749-6632.1988.tb38507.x.

[10] Suzuki O, Shiwaku Y, Hamai R. Octacalcium phosphate bone substitute materials: Comparison between properties of biomaterials and other calcium phosphate materials. Dental Materials Journal. 2020; 39: 187–199. https://doi.org/10.4012/dmj.2020-001.

[11] Brown WE, Smith JP, Lehr JR, Frazier AW. Octacalcium phosphate and hydroxyapatite: Crystallographic and chemical relations between octacalcium phosphate and hydroxyapatite. Nature. 1962; 196: 1050–1055. https://doi.org/10.1038/1961050a0.

[12] Mathew M, Brown W, Schroeder L, Dickens B. Crystal structure of octacalcium bis (hydrogenphosphate) tetrakis (phosphate) pentahydrate, $Ca_8(HPO_4)_2(PO_4)_4 \cdot 5H_2O$. Journal of Crystallographic and Spectroscopic Research. 1988; 18: 235–250. https://doi.org/10.1007/BF01194315.

[13] Brown WE. Crystal growth of bone mineral. Clinical Orthopaedics and Related Research. 1966; 44: 205–220.

[14] Eanes ED, Meyer JL. The maturation of crystalline calcium phosphates in aqueous suspensions at physiologic pH. Calcified Tissue Research. 1977; 23: 259–269. https://doi.org/10.1007/bf02012795.

[15] Simon P, Grüner D, Worch H, Pompe W, Lichte H, El Khassawna T, *et al*. First evidence of octacalcium phosphate@osteocalcin nanocomplex as skeletal bone component directing collagen triple-helix nanofibril mineralization. Scientific Reports. 2018; 8: 13696. https://doi.org/10.1038/s41598-018-31983-5.

[16] Döbelin N, Suzuki O, Drouet C, Ločs J, Insley G, Procter P. Workshop on the latest advances in biomedical applications of octacalcium phosphate. Journal of Biomedical Materials Research Part B: Applied Biomaterials. 2025; 113: e35500. https://doi.org/10.1002/jbm.b.35500.

[17] Kovrlija I, Locs J, Loca D. Octacalcium phosphate: Innovative vehicle for the local biologically active substance delivery in bone regeneration. Acta Biomaterialia. 2021; 135: 27–47. https://doi.org/10.1016/j.actbio.2021.08.021.

[18] Kovrlija I, Pańczyszyn E, Demir O, Laizane M, Corazzari M, Locs J, *et al*. Doxorubicin loaded octacalcium phosphate particles as controlled release drug delivery systems: Physico-chemical characterization, in vitro drug release and evaluation of cell death pathway. International Journal of Pharmaceutics. 2024; 653: 123932. https://doi.org/10.1016/j.ijpharm.2024.123932.

[19] Komlev VS, Barinov SM, Bozo II, Deev RV, Eremin II, Fedotov AY, *et al*. Bioceramics composed of octacalcium phosphate demonstrate enhanced biological behavior. ACS Applied Materials & Interfaces. 2014; 6: 16610–16620. https://doi.org/10.1021/am502583p.

[20] Suzuki O, Nakamura M, Miyasaka Y, Kagayama M, Sakurai M. Bone formation on synthetic precursors of hydroxyapatite. The Tohoku Journal of Experimental Medicine. 1991; 164: 37–50. https://doi.org/10.1620/tjem.164.37.

[21] Lin S, LeGeros RZ, LeGeros JP. Adherent octacalciumphosphate coating on titanium alloy using modulated electrochemical deposition method. Journal of Biomedical Materials Research Part A. 2003; 66A: 819–828. https://doi.org/10.1002/jbm.a.10072.

[22] Barrère F, van der Valk CM, Dalmeijer RA, van Blitterswijk CA, de Groot K, Layrolle P. In vitro and in vivo degradation of biomimetic octacalcium phosphate and carbonate apatite coatings on titanium implants. Journal of Biomedical Materials Research Part A. 2003; 64: 378–387. https://doi.org/10.1002/jbm.a.10291.

[23] Bigi A, Bracci B, Cuisinier F, Elkaim R, Fini M, Mayer I, *et al*. Human osteoblast response to pulsed laser deposited calcium phosphate coatings. Biomaterials. 2005; 26: 2381–2389. https://doi.org/10.1016/j.biomaterials.2004.07.057.

[24] Barrère F, van der Valk CM, Dalmeijer RAJ, Meijer G, van Blitterswijk CA, de Groot K, *et al*. Osteogenecity of octacalcium phosphate coatings applied on porous metal implants. Journal of Biomedical Materials Research Part A. 2003; 66A: 779–788. https://doi.org/10.1002/jbm.a.10454.

[25] Dekker RJ, de Bruijn JD, Stigter M, Barrere F, Layrolle P, van Blitterswijk CA. Bone tissue engineering on amorphous carbonated apatite and crystalline octacalcium phosphate-coated titanium discs. Biomaterials. 2005; 26: 5231–5239. https://doi.org/10.1016/j.biomaterials.2005.01.057.

[26] Zhou Y, Liu Z, Noda D, Yamada I, Tagaya M. Preparation of octacalcium phosphate thin film with exposing reactive crystalline plane in biological fluid. ACS Biomaterials Science & Engineering. 2025; 11: 1150–1160. https://doi.org/10.1021/acsbiomaterials.4c02011.

[27] Komlev VS, Popov VK, Mironov AV, Fedotov AY, Teterina AY, Smirnov IV, *et al*. 3D printing of octacalcium phosphate bone substitutes. Frontiers in Bioengineering and Biotechnology. 2015; 3: 81. https://doi.org/10.3389/fbioe.2015.00081.

[28] Sugiura Y, Munar ML, Ishikawa K. Fabrication of octacalcium phosphate block through a dissolution-precipitation reaction using a calcium sulphate hemihydrate block as a precursor. Journal of Materials Science: Materials in Medicine. 2018; 29: 151. https://doi.org/10.1007/s10856-018-6162-1.

[29] Sugiura Y, Ono F, Nohara M, Funabiki M, Kutara K, Kanda T, *et al*. Enhanced cortical bone formation ability and antibacterial properties of silica-substituted octacalcium phosphate blocks through silver loading. Materials Chemistry and Physics. 2026; 348: 131575. https://doi.org/10.1016/j.matchemphys.2025.131575.

[30] Combes C, Rey C. Amorphous calcium phosphates: Synthesis, properties and uses in biomaterials. Acta Biomaterialia. 2010; 6: 3362–3378. https://doi.org/10.1016/j.actbio.2010.02.017.

[31] Habraken WJ, Tao J, Brylka LJ, Friedrich H, Bertinetti L, Schenk AS, *et al*. Ion-association complexes unite classical and non-classical theories for the biomimetic nucleation of calcium phosphate. Nature Communications. 2013; 4: 1507. https://doi.org/10.1038/ncomms2490.

[32] Moradian-Oldak J, Iijima M, Bouropoulos N, Wen HB. Assembly of amelogenin proteolytic products and control of octacalcium phosphate crystal morphology. Connective Tissue Research. 2003; 44: 58–64. https://doi.org/10.1080/03008200390152106.

[33] Furedi-Milhofer H, Moradian-Oldak J, Weiner S, Veis A, Mintz KP, Addadf L. Interactions of matrix proteins from mineralized tissues with octacalcium phosphate. Connective Tissue Research. 1994; 30: 251–264. https://doi.org/10.3109/03008209409015041.

[34] Addadi L, Weiner S. Control and design principles in biological mineralization. Angewandte Chemie International Edition in English. 1992; 31: 153–169. https://doi.org/10.1002/anie.199201531.

[35] Tseng Y, Zhan J, Lin KSK, Mou C, Chan JCC. High resolution 31P NMR study of octacalcium phosphate. Solid State Nuclear Magnetic

Resonance. 2004; 26: 99–104. https://doi.org/10.1016/j.ssnmr.2004.06.002.

[36] Tseng Y, Mou C, Chan JCC. Solid-state NMR study of the transformation of octacalcium phosphate to hydroxyapatite: A mechanistic model for central dark line formation. Journal of the American Chemical Society. 2006; 128: 6909–6918. https://doi.org/10.1021/ja060336u.

[37] Chickerur NS, Tung MS, Brown WE. A mechanism for incorporation of carbonate into apatite. Calcified Tissue International. 1980; 32: 55–62. https://doi.org/10.1007/BF02408521.

[38] Davies E, Müller KH, Wong WC, Pickard CJ, Reid DG, Skepper JN, *et al*. Citrate bridges between mineral platelets in bone. Proceedings of the National Academy of Sciences. 2014; 111: E1354–1363. https://doi.org/10.1073/pnas.1315080111.

[39] Eidelman N, Chow LC, Brown WE. Calcium phosphate phase transformations in serum. Calcified Tissue International. 1987; 41: 18–26. https://doi.org/10.1007/BF02555126.

[40] Suzuki O, Hamai R, Sakai S. The material design of octacalcium phosphate bone substitute: increased dissolution and osteogenecity. Acta Biomaterialia. 2023; 158: 1–11. https://doi.org/10.1016/j.actbio.2022.12.046.

[41] Suzuki O. Octacalcium phosphate: Osteoconductivity and crystal chemistry. Acta Biomaterialia. 2010; 6: 3379–3387. https://doi.org/10.1016/j.actbio.2010.04.002.

[42] Kawai T, Kamakura S, Matsui K, Fukuda M, Takano H, Iino M, *et al*. Clinical study of octacalcium phosphate and collagen composite in oral and maxillofacial surgery. Journal of Tissue Engineering. 2020; 11: 2041731419896449. https://doi.org/10.1177/2041731419896449.

[43] Hamada S, Mori Y, Shiwaku Y, Hamai R, Tsuchiya K, Baba K, *et al*. Octacalcium phosphate/gelatin composite (OCP/Gel) enhances bone repair in a critical-sized transcortical femoral defect rat model. Clinical Orthopaedics & Related Research. 2022; 480: 2043–2055. https://doi.org/10.1097/CORR.0000000000002257.

[44] Oizumi I, Hamai R, Shiwaku Y, Mori Y, Anada T, Baba K, *et al*. Impact of simultaneous hydrolysis of OCP and PLGA on bone induction of a PLGA-OCP composite scaffold in a rat femoral defect. Acta Biomaterialia. 2021; 124: 358–373. https://doi.org/10.1016/j.actbio.2021.01.048.

[45] Heydari Z, Mohebbi-Kalhori D, Afarani MS. Engineered electrospun polycaprolactone (PCL)/octacalcium phosphate (OCP) scaffold for bone tissue engineering. Materials Science and Engineering: C. 2017; 81: 127–132. https://doi.org/10.1016/j.msec.2017.07.041.

[46] Chiba S, Anada T, Suzuki K, Saito K, Shiwaku Y, Miyatake N, *et al*. Effect of resorption rate and osteoconductivity of biodegradable calcium phosphate materials on the acquisition of natural bone strength in the repaired bone. Journal of Biomedical Materials Research Part A. 2016; 104: 2833–2842. https://doi.org/10.1002/jbm.a.35828.

[47] Miyatake N, Kishimoto KN, Anada T, Imaizumi H, Itoi E, Suzuki O. Effect of partial hydrolysis of octacalcium phosphate on its osteoconductive characteristics. Biomaterials. 2009; 30: 1005–1014. https://doi.org/10.1016/j.biomaterials.2008.10.058.

[48] Kay MI, Young RA, Posner AS. Crystal structure of hydroxyapatite. Nature. 1964; 204: 1050–1052. https://doi.org/10.1038/2041050a0.

[49] Yashima M, Sakai A, Kamiyama T, Hoshikawa A. Crystal structure analysis of $\beta$-tricalcium phosphate $Ca_3(PO_4)_2$ by neutron powder diffraction. Journal of Solid State Chemistry. 2003; 175: 272–277. https://doi.org/10.1016/S0022-4596(03)00279-2.

[50] Arcos D, Vallet-Regí M. Substituted hydroxyapatite coatings of bone implants. Journal of Materials Chemistry B. 2020; 8: 1781–1800. https://doi.org/10.1039/C9TB02710F.

[51] Jiang Y, Yuan Z, Huang J. Substituted hydroxyapatite: A recent development. Materials Technology. 2020; 35: 785–796. https://doi.org/10.1080/10667857.2019.1664096.

[52] Wang X, Huang S, Peng Q. Metal ion-doped hydroxyapatite-based materials for bone defect restoration. Bioengineering. 2023; 10: 1367. https://doi.org/10.3390/bioengineering10121367.

[53] Radulescu DE, Vasile OR, Andronescu E, Ficai A. Latest research of doped hydroxyapatite for bone tissue engineering. International Journal of Molecular Sciences. 2023; 24: 13157. https://doi.org/10.3390/ijms241713157.

[54] Hayakawa S, Okada Y, Yoshioka T. Comparative study of the effects of fluoride treatment with cyclic variations in pH on the structures of stoichiometric, calcium-deficient, and carbonated hydroxyapatites. Journal of the Ceramic Society of Japan. 2025; 133: 15–24. https://doi.org/10.2109/jcersj2.24094.

[55] Klecandová L, Slíva A, Šindler M, Šupová M, Kumar Sathish S, Kupková J, *et al*. Effect of processing on structural and morphological changes of hydroxyapatite-based materials. ChemistrySelect. 2025; 10: e202405109. https://doi.org/10.1002/slct.202405109.

[56] Pazourková L, Peikertová P, Hundáková M, Simha Martynková G. Preparation of calcium deficient hydroxyapatite on the montmorillonite substrate: Structure and morphology. Materials Today: Proceedings. 2021; 37: 35–41. https://doi.org/10.1016/j.matpr.2020.02.927.

[57] Zhang H, Zhang M. Characterization and thermal behavior of calcium deficient hydroxyapatite whiskers with various Ca/P ratios. Materials Chemistry and Physics. 2011; 126: 642–648. https://doi.org/10.1016/j.matchemphys.2010.12.067.

[58] Shi H, He F, Ye J. Synthesis and structure of iron- and strontium-substituted octacalcium phosphate: Effects of ionic charge and radius. Journal of Materials Chemistry B. 2016; 4: 1712–1719. https://doi.org/10.1039/c5tb02247a.

[59] Yamada I, Tagaya M. Immobilization of 2,2′-bipyridine-5,5′-dicarboxylic acid in layered octacalcium phosphate. Colloid and Interface Science Communications. 2019; 30: 100182. https://doi.org/10.1016/j.colcom.2019.100182.

[60] Yokoi T, Goto T, Hara M, Sekino T, Seki T, Kamitakahara M, *et al*. Incorporation of tetracarboxylate ions into octacalcium phosphate for the development of next-generation biofriendly materials. Communications Chemistry. 2021; 4: 4. https://doi.org/10.1038/s42004-020-00443-5.

[61] Yokoi T, Shimabukuro M, Kawashita M. Octacalcium phosphate with incorporated carboxylate ions: A review. Science and Technology of Advanced Materials. 2022; 23: 434–445. https://doi.org/10.1080/14686996.2022.2094728.

[62] Boanini E, Gazzano M, Rubini K, Bigi A. Collapsed octacalcium phosphate stabilized by ionic substitutions. Crystal Growth & Design. 2010; 10: 3612–3617. https://doi.org/10.1021/cg100494f.

[63] Onuma K, Ito A. Cluster growth model for hydroxyapatite. Chemistry of Materials. 1998; 10: 3346–3351. https://doi.org/10.1021/cm980062c.

[64] Posner AS, Betts F. Synthetic amorphous calcium phosphate and its relation to bone mineral structure. Accounts of Chemical Research. 1975; 8: 273–281. https://doi.org/10.1021/ar50092a003.

[65] Wang L, Li S, Ruiz-Agudo E, Putnis CV, Putnis A. Posner's cluster revisited: direct imaging of nucleation and growth of nanoscale calcium phosphate clusters at the calcite-water interface. CrystEngComm. 2012; 14: 6252. https://doi.org/10.1039/C2CE25669J.

[66] Mancardi G, Hernandez Tamargo CE, Di Tommaso D, de Leeuw NH. Detection of Posner's clusters during calcium phosphate nucleation: A molecular dynamics study. Journal of Materials Chemistry B. 2017; 5: 7274–7284. https://doi.org/10.1039/c7tb01199g.

[67] Song C, Ding Z, Song Q, Chen J, Fan Y, Han Y. In situ fluorescence probing of the formation of calcium phosphate prenucleation clusters. The Journal of Physical Chemistry B. 2022; 126: 9850–9859. https://doi.org/10.1021/acs.jpcb.2c05311.

[68] Carrodeguas RG, De Aza S. $\alpha$-tricalcium phosphate: Synthesis, properties and biomedical applications. Acta Biomaterialia. 2011; 7: 3536–3546. https://doi.org/10.1016/j.actbio.2011.06.019.

[69] Momma K, Izumi F. VESTA 3 for three-dimensional visualization of crystal, volumetric and morphology data. Journal of Ap-

plied Crystallography. 2011; 44: 1272–1276. https://doi.org/10.1107/S0021889811038970.

[70] Moreno EC, Kresak M, Zahradnik RT. Fluoridated hydroxyapatite solubility and caries formation. Nature. 1974; 247: 64–65. https://doi.org/10.1038/247064a0.

[71] Tung MS, Eidelman N, Sieck B, Brown WE. Octacalcium phosphate solubility product from 4 to 37°C. Journal of Research of the National Bureau of Standards. 1988; 93: 613–624. https://doi.org/10.6028/jres.093.153.

[72] Gregory TM, Moreno EC, Patel JM, Brown WE. Solubility of $\beta$-$Ca_3(PO_4)_2$ in the system $Ca(OH)_2$-$H_3PO_4$-$H_2O$ at 5, 15, 25, and 37°C. Journal of Research of the National Bureau of Standards Section A, Physics and Chemistry. 1974; 78A(6): 667–674. https://doi.org/10.6028/jres.078A.042.

[73] Kokubo T, Takadama H. How useful is SBF in predicting in vivo bone bioactivity? Biomaterials. 2006; 27: 2907–2915. https://doi.org/10.1016/j.biomaterials.2006.01.017.

[74] Cho S, Nakanishi K, Kokubo T, Soga N, Ohtsuki C, Nakamura T, *et al*. Dependence of apatite formation on silica gel on its structure: Effect of heat treatment. Journal of the American Ceramic Society. 1995; 78: 1769–1774. https://doi.org/10.1111/j.1151-2916.1995.tb08887.x.

[75] Iijima M, Kamemizu H, Wakamatsu N, Goto T, Doi Y, Moriwaki Y. Transition of octacalcium phosphate to hydroxyapatite in solution at pH 7.4 and 37°C. Journal of Crystal Growth. 1997; 181: 70–78. https://doi.org/10.1016/S0022-0248(97)00230-3.

[76] Tung MS, Tomazic B, Brown WE. The effects of magnesium and fluoride on the hydrolysis of octacalcium phosphate. Archives of Oral Biology. 1992; 37: 585–591. https://doi.org/10.1016/0003-9969(92)90142-u.

[77] LeGeros RZ, Daculsi G, Orly I, Abergas T, Torres W. Solution-mediated transformation of octacalcium phosphate (OCP) to apatite. Scanning Microscopy. 1989; 3: 129–137; discussion 137–138. https://digitalcommons.usu.edu/microscopy/vol3/iss1/16.

[78] LeGeros RZ, Kijkowska R, LeGeros JP. Formation and transformation of octacalcium phosphate, OCP: A preliminary report. Scanning Electron Microscopy. 1984; Pt 4: 1771–1777.

[79] Bigi A, Boanini E, Cojazzi G, Falini G, Panzavolta S. Morphological and structural investigation of octacalcium phosphate hydrolysis in the presence of polyacrylic acids: effect of relative molecular weights. Crystal Growth & Design. 2001; 1: 239–244. https://doi.org/10.1021/cg005551f.

[80] Hamai R, Tsuchiya K, Suzuki O. Adsorption of serum albumin onto octacalcium phosphate in supersaturated solutions regarding calcium phosphate phases. Materials. 2019; 12: 2333. https://doi.org/10.3390/ma12142333.

[81] Tsuboi Y, Hamai R, Okuyama K, Tsuchiya K, Shiwaku Y, Yamauchi K, *et al*. Adsorption of serum fetuin onto octacalcium phosphate and its relation to osteogenic property. International Journal of Molecular Sciences. 2025; 26: 1391. https://doi.org/10.3390/ijms26031391.

[82] Okuyama K, Shiwaku Y, Hamai R, Mizoguchi T, Tsuchiya K, Takahashi T, *et al*. Differentiation of committed osteoblast progenitors by octacalcium phosphate compared to calcium-deficient hydroxyapatite in Lepr-cre/Tomato mouse tibia. Acta Biomaterialia. 2022; 142: 332–344. https://doi.org/10.1016/j.actbio.2022.02.016.

[83] Guo H, Su J, Wei J, Kong H, Liu C. Biocompatibility and osteogenicity of degradable Ca-deficient hydroxyapatite scaffolds from calcium phosphate cement for bone tissue engineering. Acta Biomaterialia. 2009; 5: 268–278. https://doi.org/10.1016/j.actbio.2008.07.018.

[84] Sato T, Anada T, Hamai R, Shiwaku Y, Tsuchiya K, Sakai S, *et al*. Culture of hybrid spheroids composed of calcium phosphate materials and mesenchymal stem cells on an oxygen-permeable culture device to predict in vivo bone forming capability. Acta Biomaterialia. 2019; 88: 477–490. https://doi.org/10.1016/j.actbio.2019.03.001.

[85] Kikawa T, Kashimoto O, Imaizumi H, Kokubun S, Suzuki O. Intramembranous bone tissue response to biodegradable octacalcium phosphate implant. Acta Biomaterialia. 2009; 5: 1756–1766. https://doi.org/10.1016/j.actbio.2008.12.008.

[86] Saito S, Hamai R, Shiwaku Y, Hasegawa T, Sakai S, Tsuchiya K, *et al*. Involvement of distant octacalcium phosphate scaffolds in enhancing early differentiation of osteocytes during bone regeneration. Acta Biomaterialia. 2021; 129: 309–322. https://doi.org/10.1016/j.actbio.2021.05.017.

[87] Kamakura S, Sasano Y, Shimizu T, Hatori K, Suzuki O, Kagayama M, *et al*. Implanted octacalcium phosphate is more resorbable than beta-tricalcium phosphate and hydroxyapatite. Journal of Biomedical Materials Research. 2002; 59: 29–34. https://doi.org/10.1002/jbm.1213.

[88] Woo SM, Rosser J, Dusevich V, Kalajzic I, Bonewald LF. Cell line IDG-SW3 replicates osteoblast-to-late-osteocyte differentiation in vitro and accelerates bone formation in vivo. Journal of Bone and Mineral Research. 2011; 26: 2634–2646. https://doi.org/10.1002/jbmr.465.

[89] Owen R, Wittkowske C, Lacroix D, Perrault CM, Reilly GC. $\beta$-glycerophosphate, not low magnitude fluid shear stress, increases osteocytogenesis in the osteoblast-to-osteocyte cell line IDG-SW3. Connective Tissue Research. 2024; 65: 313–329. https://doi.org/10.1080/03008207.2024.2375065.

[90] Shibuya I, Yoshimura K, Miyamoto Y, Yamada A, Takami M, Suzawa T, *et al*. Octacalcium phosphate suppresses chondrogenic differentiation of ATDC5 cells. Cell and Tissue Research. 2013; 352: 401–412. https://doi.org/10.1007/s00441-012-1548-8.

[91] Liu X, Shiwaku Y, Hamai R, Tsuchiya K, Takahashi T, Suzuki O. Effect of octacalcium phosphate crystals on the osteogenic differentiation of tendon stem/progenitor cells in vitro. International Journal of Molecular Sciences. 2023; 24: 1235. https://doi.org/10.3390/ijms24021235.

[92] Chen HT, Shiwaku Y, Hamai R, Tsuchiya K, Yamauchi K, Takahashi T, *et al*. C2C12 myoblast differentiation stimulated by octacalcium phosphate material. Dental Materials Journal. 2025; 44: 446–455. https://doi.org/10.4012/dmj.2024-382.

[93] Fowler BO, Moreno EC, Brown WE. Infra-red spectra of hydroxyapatite, octacalcium phosphate and pyrolysed octacalcium phosphate. Archives of Oral Biology. 1966; 11: 477–492. https://doi.org/10.1016/0003-9969(66)90154-3.

[94] Nelson DGA, McLean JD. High-resolution electron microscopy of octacalcium phosphate and its hydrolysis products. Calcified Tissue International. 1984; 36: 219–232. https://doi.org/10.1007/bf02405321.

[95] Busra MFM, Lokanathan Y. Recent development in the fabrication of collagen scaffolds for tissue engineering applications: A review. Current Pharmaceutical Biotechnology. 2019; 20: 992–1003. https://doi.org/10.2174/1389201020666190731121016.

[96] Sonwane S, Bonde S, Bonde C, Chandarana C. Advances in gelatin-based scaffolds for tissue engineering applications: A review. Journal of Drug Delivery Science and Technology. 2025; 107: 106789. https://doi.org/10.1016/j.jddst.2025.106789.

[97] Veis A, Cohen J. Reversible transformation of gelatin to the collagen structure. Nature. 1960; 186: 720–721. https://doi.org/10.1038/186720a0.

[98] Handa T, Anada T, Honda Y, Yamazaki H, Kobayashi K, Kanda N, *et al*. The effect of an octacalcium phosphate co-precipitated gelatin composite on the repair of critical-sized rat calvarial defects. Acta Biomaterialia. 2012; 8: 1190–1200. https://doi.org/10.1016/j.actbio.2011.12.002.

[99] Saito K, Anada T, Shiwaku Y, Chiba S, Miyatake N, Suzuki K, *et al*. Dose-dependent enhancement of octacalcium phosphate biodegradation with a gelatin matrix during bone regeneration in a rabbit tibial defect model. RSC Advances. 2016; 6: 64165–64174. https://doi.org/10.1039/C6RA07602E.

[100] Gorgieva S, Kokol V. Collagen- vs. gelatine-based biomaterials

and their biocompatibility: Review and perspectives. Biomaterials Applications for Nanomedicine. 2011; 2: 17–52. https://doi.org/10.5772/24118.

[101] Ross-Murphy SB. Structure and rheology of gelatin gels: Recent progress. Polymer. 1992; 33: 2622–2627. https://doi.org/10.1016/0032-3861(92)91146-S.

[102] Guo L, Colby RH, Lusignan CP, Whitesides TH. Kinetics of triple helix formation in semidilute gelatin solutions. Macromolecules. 2003; 36: 9999–10008. https://doi.org/10.1021/ma034264s.

[103] Choi W, Hong J. Regulation of the gelatin helix-to-coil transition through chain confinements at the polymer-protein interface and protein-protein interface. Acta Biomaterialia. 2025; 195: 216–224. https://doi.org/10.1016/j.actbio.2025.02.003.

[104] Qiao C, Zhang J, Ma X, Liu W, Liu Q. Effect of salt on the coil-helix transition of gelatin at early stages: Optical rotation, rheology and DSC studies. International Journal of Biological Macromolecules. 2018; 107: 1074–1079. https://doi.org/10.1016/j.ijbiomac.2017.09.079.

[105] Holder AJ, Badiei N, Hawkins K, Wright C, Williams PR, Curtis DJ. Control of collagen gel mechanical properties through manipulation of gelation conditions near the sol–gel transition. Soft Matter. 2018; 14: 574–580. https://doi.org/10.1039/c7sm01933e.

[106] Antoine EE, Vlachos PP, Rylander MN. Review of collagen I hydrogels for bioengineered tissue microenvironments: Characterization of mechanics, structure, and transport. Tissue Engineering Part B: Reviews. 2014; 20: 683–696. https://doi.org/10.1089/ten.TEB.2014.0086.

[107] Huang D, Li Y, Ma Z, Lin H, Zhu X, Xiao Y, *et al*. Collagen hydrogel viscoelasticity regulates MSC chondrogenesis in a ROCK-dependent manner. Science Advances. 2023; 9: eade9497. https://doi.org/10.1126/sciadv.ade9497.

[108] Mori Y, Hamai R, Aizawa T, Suzuki O. Impact of octacalcium phosphate/gelatin (OCP/Gel) composite on bone repair in refractory bone defects. The Tohoku Journal of Experimental Medicine. 2023; 260: 245–252. https://doi.org/10.1620/tjem.2023.J040.

[109] Hamai R, Sakai S, Shiwaku Y, Anada T, Tsuchiya K, Ishimoto T, *et al*. Octacalcium phosphate crystals including a higher density dislocation improve its materials osteogenecity. Applied Materials Today. 2022; 26: 101279. https://doi.org/10.1016/j.apmt.2021.101279.

[110] Yamada S, Heymann D, Bouler JM, Daculsi G. Osteoclastic resorption of calcium phosphate ceramics with different hydroxyapatite/beta-tricalcium phosphate ratios. Biomaterials. 1997; 18: 1037–1041. https://doi.org/10.1016/s0142-9612(97)00036-7.

[111] Shiwaku Y, Neff L, Nagano K, Takeyama K, de Bruijn J, Dard M, *et al*. The crosstalk between osteoclasts and osteoblasts is dependent upon the composition and structure of biphasic calcium phosphates. PLoS One. 2015; 10: e0132903. https://doi.org/10.1371/journal.pone.0132903.

[112] Le Gars Santoni B, Niggli L, Dolder S, Loeffel O, Sblendorio GA, Heuberger R, *et al*. Effect of minor amounts of $\beta$-calcium pyrophosphate and hydroxyapatite on the physico-chemical properties and osteoclastic resorption of $\beta$-tricalcium phosphate cylinders. Bioactive Materials. 2022; 10: 222–235. https://doi.org/10.1016/j.bioactmat.2021.09.003.

[113] Kobayashi K, Anada T, Handa T, Kanda N, Yoshinari M, Takahashi T, *et al*. Osteoconductive property of a mechanical mixture of octacalcium phosphate and amorphous calcium phosphate. ACS Applied Materials & Interfaces. 2014; 6: 22602–22611. https://doi.org/10.1021/am5067139.

[114] Mountziaris PM, Mikos AG. Modulation of the inflammatory response for enhanced bone tissue regeneration. Tissue Engineering Part B: Reviews. 2008; 14: 179–186. https://doi.org/10.1089/ten.teb.2008.0038.

[115] Goodman SB, Pajarinen J, Yao Z, Lin T. Inflammation and bone repair: From particle disease to tissue regeneration. Frontiers in Bioengineering and Biotechnology. 2019; 7: 230. https://doi.org/10.3389/fbioe.2019.00230.

[116] Su N, Villicana C, Yang F. Immunomodulatory strategies for bone regeneration: A review from the perspective of disease types. Biomaterials. 2022; 286: 121604. https://doi.org/10.1016/j.biomaterials.2022.121604.

[117] Kamimura M, Mori Y, Sugahara-Tobinai A, Takai T, Itoi E. Impaired fracture healing caused by deficiency of the immunoreceptor adaptor protein DAP12. PLoS One. 2015; 10: e0128210. https://doi.org/10.1371/journal.pone.0128210.

[118] Mori Y, Adams D, Hagiwara Y, Yoshida R, Kamimura M, Itoi E, *et al*. Identification of a progenitor cell population destined to form fracture fibrocartilage callus in Dickkopf-related protein 3–green fluorescent protein reporter mice. Journal of Bone and Mineral Metabolism. 2016; 34: 606–614. https://doi.org/10.1007/s00774-015-0711-1.

[119] Kamakura S, Sasaki K, Homma T, Honda Y, Anada T, Echigo S, *et al*. The primacy of octacalcium phosphate collagen composites in bone regeneration. Journal of Biomedical Materials Research Part A. 2007; 83A: 725–733. https://doi.org/10.1002/jbm.a.31332.

[120] Kim H, Kim H, Salih V. Stimulation of osteoblast responses to biomimetic nanocomposites of gelatin–hydroxyapatite for tissue engineering scaffolds. Biomaterials. 2005; 26: 5221–5230. https://doi.org/10.1016/j.biomaterials.2005.01.047.

[121] Samadikuchaksaraei A, Gholipourmalekabadi M, Erfani Ezadyar E, Azami M, Mozafari M, Johari B, *et al*. Fabrication and in vivo evaluation of an osteoblast-conditioned nano-hydroxyapatite/gelatin composite scaffold for bone tissue regeneration. Journal of Biomedical Materials Research Part A. 2016; 104: 2001–2010. https://doi.org/10.1002/jbm.a.35731.

[122] Takahashi Y, Yamamoto M, Tabata Y. Osteogenic differentiation of mesenchymal stem cells in biodegradable sponges composed of gelatin and beta-tricalcium phosphate. Biomaterials. 2005; 26: 3587–3596. https://doi.org/10.1016/j.biomaterials.2004.09.046.

[123] Matsumoto G, Omi Y, Kubota E, Ozono S, Tsuzuki H, Kinoshita Y, *et al*. Enhanced regeneration of critical bone defects using a biodegradable gelatin sponge and $\beta$-tricalcium phosphate with bone morphogenetic protein-2. Journal of Biomaterials Applications. 2009; 24: 327–342. https://doi.org/10.1177/0885328208096523.

[124] Li L, Huang T, Mo X, Huang S, Deng S, Jiang Z, *et al*. In situ formation mechanism and osteogenic performance of dislocation-rich fibrous octacalcium phosphate mediated by silk fibroin. Small. 2025; 21: e08564. https://doi.org/10.1002/smll.202508564.

[125] Blum AE, Yund RA, Lasaga AC. The effect of dislocation density on the dissolution rate of quartz. Geochimica Et Cosmochimica Acta. 1990; 54: 283–297. https://doi.org/10.1016/0016-7037(90)90318-F.

[126] Hull D, Bacon DJ. Elastic properties of dislocations. In Hull D, Bacon DJ (eds.) Introduction to dislocations p63–68. 4th ed. Elsevier: Oxford. 2011.

[127] Kurzepa J, Baran M, Watroba S, Barud M, Babula D. Collagenases and gelatinases in bone healing. The focus on mandibular fractures. Current Issues in Pharmacy and Medical Sciences. 2014; 27: 121–126. https://doi.org/10.2478/cipms-2014-0029.

[128] Huang A, Honda Y, Li P, Tanaka T, Baba S. Integration of epigallocatechin gallate in gelatin sponges attenuates matrix metalloproteinase-dependent degradation and increases bone formation. International Journal of Molecular Sciences. 2019; 20: 6042. https://doi.org/10.3390/ijms20236042.

[129] Kisling A, Lust RM, Katwa LC. What is the role of peptide fragments of collagen I and IV in health and disease? Life Sciences. 2019; 228: 30–34. https://doi.org/10.1016/j.lfs.2019.04.042.

[130] Palmieri D, Camardella L, Ulivi V, Guasco G, Manduca P. Trimer carboxyl propeptide of collagen I produced by mature osteoblasts is chemotactic for endothelial cells. Journal of Biological Chemistry. 2000; 275: 32658–32663. https://doi.org/10.1074/jbc.M002698200.

[131] Gaggar A, Jackson PL, Noerager BD, O'Reilly PJ, McQuaid DB, Rowe SM, *et al*. A novel proteolytic cascade generates an extracellular matrix-derived chemoattractant in chronic neutrophilic inflammation. The Journal of Immunology. 2008; 180: 5662–5669. https://doi.org/10.4049/jimmunol.180.8.5662.

[132] Kwon YW, Heo SC, Lee TW, Park GT, Yoon JW, Jang IH, *et al*. N-acetylated proline-glycine-proline accelerates cutaneous wound healing and neovascularization by human endothelial progenitor cells. Scientific Reports. 2017; 7: 43057. https://doi.org/10.1038/srep43057.

[133] Kwon YW, Lee SJ, Heo SC, Lee TW, Park GT, Yoon JW, *et al*. Role of CXCR2 in the Ac-PGP-induced mobilization of circulating angiogenic cells and its therapeutic implications. Stem Cells Translational Medicine. 2019; 8: 236–246. https://doi.org/10.1002/sctm.18-0035.

[134] Weathington NM, van Houwelingen AH, Noerager BD, Jackson PL, Kraneveld AD, Galin FS, *et al*. A novel peptide CXCR ligand derived from extracellular matrix degradation during airway inflammation. Nature Medicine. 2006; 12: 317–323. https://doi.org/10.1038/nm1361.

[135] Li A, Dubey S, Varney ML, Singh RK. Interleukin-8-induced proliferation, survival, and MMP production in CXCR1 and CXCR2 expressing human umbilical vein endothelial cells. Microvascular Research. 2002; 64: 476–481. https://doi.org/10.1006/mvre.2002.2442.

[136] Li A, Dubey S, Varney ML, Dave BJ, Singh RK. IL-8 directly enhanced endothelial cell survival, proliferation, and matrix metalloproteinases production and regulated angiogenesis. The Journal of Immunology. 2003; 170: 3369–3376. https://doi.org/10.4049/jimmunol.170.6.3369.

[137] Khoswanto C. Role of matrix metalloproteinases in bone regeneration: Narrative review. Journal of Oral Biology and Craniofacial Research. 2023; 13: 539–543. https://doi.org/10.1016/j.jobcr.2023.06.002.

[138] Paiva KBS, Granjeiro JM. Matrix metalloproteinases in bone resorption, remodeling, and repair. Progress in Molecular Biology and Translational Science. 2017; 142: 203–303. https://doi.org/10.1016/bs.pmbts.2017.05.001.

[139] Chen K, Xu M, Lu F, He Y. Development of matrix metalloproteinases-mediated extracellular matrix remodeling in regenerative medicine: A mini review. Tissue Engineering and Regenerative Medicine. 2023; 20: 661–670. https://doi.org/10.1007/s13770-023-00536-x.

[140] Colnot C, Thompson Z, Miclau T, Werb Z, Helms JA. Altered fracture repair in the absence of MMP9. Development. 2003; 130: 4123–4133. https://doi.org/10.1242/dev.00559.

[141] Hankenson KD, Dishowitz M, Gray C, Schenker M. Angiogenesis in bone regeneration. Injury. 2011; 42: 556–561. https://doi.org/10.1016/j.injury.2011.03.035.

[142] Stegen S, van Gastel N, Carmeliet G. Bringing new life to damaged bone: the importance of angiogenesis in bone repair and regeneration. Bone. 2015; 70: 19–27. https://doi.org/10.1016/j.bone.2014.09.017.

[143] Grosso A, Burger MG, Lunger A, Schaefer DJ, Banfi A, Di Maggio N. It takes two to tango: Coupling of angiogenesis and osteogenesis for bone regeneration. Frontiers in Bioengineering and Biotechnology. 2017; 5: 68. https://doi.org/10.3389/fbioe.2017.00068.

[144] Diomede F, Marconi GD, Fonticoli L, Pizzicanella J, Merciaro I, Bramanti P, *et al*. Functional relationship between osteogenesis and angiogenesis in tissue regeneration. International Journal of Molecular Sciences. 2020; 21: 3242. https://doi.org/10.3390/ijms21093242.

[145] Xu Z, Kusumbe AP, Cai H, Wan Q, Chen J. Type H blood vessels in coupling angiogenesis-osteogenesis and its application in bone tissue engineering. Journal of Biomedical Materials Research Part B: Applied Biomaterials. 2023; 111: 1434–1446. https://doi.org/10.1002/jbm.b.35243.

[146] Liu X, Zhang P, Gu Y, Guo Q, Liu Y. Type H vessels: functions in bone development and diseases. Frontiers in Cell and Developmental Biology. 2023; 11: 1236545. https://doi.org/10.3389/fcell.2023.1236545.

[147] Kohara Y, Kitazawa R, Haraguchi R, Imai Y, Kitazawa S. Macrophages are requisite for angiogenesis of type H vessels during bone regeneration in mice. Bone. 2022; 154: 116200. https://doi.org/10.1016/j.bone.2021.116200.

[148] Zeng Y, Huang C, Duan D, Lou A, Guo Y, Xiao T, *et al*. Injectable temperature-sensitive hydrogel system incorporating deferoxamine-loaded microspheres promotes H-type blood vessel-related bone repair of a critical size femoral defect. Acta Biomaterialia. 2022; 153: 108–123. https://doi.org/10.1016/j.actbio.2022.09.018.

[149] Hatakeyama T, Hamai R, Shiwaku Y, Anada T, Sakai S, Sato T, *et al*. Effects of degradation products from gelatin spongy scaffolds on angio-osteogenic capacity. Science and Technology of Advanced Materials. 2023; 24: 2277675. https://doi.org/10.1080/14686996.2023.2277675.

[150] Cackowski FC, Anderson JL, Patrene KD, Choksi RJ, Shapiro SD, Windle JJ, *et al*. Osteoclasts are important for bone angiogenesis. Blood. 2010; 115: 140–149. https://doi.org/10.1182/blood-2009-08-237628.

[151] Garrido CA, Lobo SE, Turíbio FM, LeGeros RZ. Biphasic calcium phosphate bioceramics for orthopaedic reconstructions: Clinical outcomes. International Journal of Biomaterials. 2011; 2011: 1–9. https://doi.org/10.1155/2011/129727.

[152] Bowers KM, Anderson DE. Delayed union and nonunion: Current concepts, prevention, and correction: A review. Bioengineering. 2024; 11: 525. https://doi.org/10.3390/bioengineering11060525.

[153] Feltri P, Solaro L, Di Martino A, Candrian C, Errani C, Filardo G. Union, complication, reintervention and failure rates of surgical techniques for large diaphyseal defects: A systematic review and meta-analysis. Scientific Reports. 2022; 12: 9098. https://doi.org/10.1038/s41598-022-12140-5.

[154] Dimitriou R, Jones E, McGonagle D, Giannoudis PV. Bone regeneration: Current concepts and future directions. BMC Medicine. 2011; 9: 66. https://doi.org/10.1186/1741-7015-9-66.

[155] Arrington ED, Smith WJ, Chambers HG, Bucknell AL, Davino NA. Complications of iliac crest bone graft harvesting. Clinical Orthopaedics & Related Research. 1996; 329: 300–309. https://doi.org/10.1097/00003086-199608000-00037.

[156] Li JJ, Dunstan CR, Entezari A, Li Q, Steck R, Saifzadeh S, *et al*. A novel bone substitute with high bioactivity, strength, and porosity for repairing large and load-bearing bone defects. Advanced Healthcare Materials. 2019; 8: 1900641. https://doi.org/10.1002/adhm.201801298.

[157] Fernandez de Grado G, Keller L, Idoux-Gillet Y, Wagner Q, Musset AM, Benkirane-Jessel N, *et al*. Bone substitutes: A review of their characteristics, clinical use, and perspectives for large bone defects management. Journal of Tissue Engineering. 2018; 9: 2041731418776819. https://doi.org/10.1177/2041731418776819.

[158] Migliorini F, La Padula G, Torsiello E, Spiezia F, Oliva F, Maffulli N. Strategies for large bone defect reconstruction after trauma, infections or tumour excision: A comprehensive review of the literature. European Journal of Medical Research. 2021; 26: 118. https://doi.org/10.1186/s40001-021-00593-9.

[159] Kook I, You J, Kim DH, Park KC, Hwang KT. A retrospective cohort study of autogenous iliac strut bone grafting in large bone defects of the lower extremity. Scientific Reports. 2024; 14: 6059. https://doi.org/10.1038/s41598-024-56726-7.

[160] Oliva F, Migliorini F, Cuozzo F, Torsiello E, Hildebrand F, Maffulli N. Outcomes and complications of the reamer irrigator aspirator versus traditional iliac crest bone graft harvesting: A systematic review and meta-analysis. Journal of Orthopaedics and Traumatology. 2021; 22: 50. https://doi.org/10.1186/s10195-021-00612-9.

[161] Wagoner Johnson AJ, Herschler BA. A review of the mechanical behavior of CaP and CaP/polymer composites for applications in

bone replacement and repair. Acta Biomaterialia. 2011; 7: 16–30. https://doi.org/10.1016/j.actbio.2010.07.012.

[162] Dima O, Didilescu AC, Manole CC, Pameijer C, Călin C. Synthetic composites versus calcium phosphate cements in bone regeneration: A narrative review. Annals of Anatomy–Anatomischer Anzeiger. 2024; 255: 152273. https://doi.org/10.1016/j.aanat.2024.152273.

[163] Wang M, Xu Y, Cao L, Xiong L, Shang D, Cong Y, *et al*. Mechanical and biological properties of 3D printed bone tissue engineering scaffolds. Frontiers in Bioengineering and Biotechnology. 2025; 13: 1545693. https://doi.org/10.3389/fbioe.2025.1545693.

[164] Reitmaier S, Kovtun A, Schuelke J, Kanter B, Lemm M, Hoess A, *et al*. Strontium(II) and mechanical loading additively augment bone formation in calcium phosphate scaffolds. Journal of Orthopaedic Research. 2018; 36: 106–117. https://doi.org/10.1002/jor.23623.

[165] Han M, Zhang Z, Liu Z, Liu Y, Zhao H, Wang B, *et al*. Three-dimensional-cultured MSC-derived exosome with hydrogel for cerebral ischemia repair. Biomaterials Advances. 2023; 149: 213396. https://doi.org/10.1016/j.bioadv.2023.213396.

[166] Liu Q, Lu WF, Zhai W. Toward stronger robocast calcium phosphate scaffolds for bone tissue engineering: A mini-review and meta-analysis. Biomaterials Advances. 2022; 134: 112578. https://doi.org/10.1016/j.msec.2021.112578.

[167] Grecula MJ. CORR Insights(R): Octacalcium phosphate/gelatin composite (OCP/Gel) enhances bone repair in a critical-sized transcortical femoral defect rat model. Clinical Orthopaedics and Related Research. 2022; 480: 2056–2058. https://doi.org/10.1097/CORR.0000000000002370.

[168] Wang W, Zhang B, Li M, Li J, Zhang C, Han Y, *et al*. 3D printing of PLA/n-HA composite scaffolds with customized mechanical properties and biological functions for bone tissue engineering. Composites Part B: Engineering. 2021; 224: 109192. https://doi.org/10.1016/j.compositesb.2021.109192.

[169] Wang W, Wang L, Zhang B, Shang S, Zhao C, Zhang W, *et al*. 3D printing of personalized magnesium composite bone tissue engineering scaffold for bone and angiogenesis regeneration. Chemical Engineering Journal. 2024; 484: 149444. https://doi.org/10.1016/j.cej.2024.149444.

[170] Li S, Liu Z, Gao X, Cheng L, Xu Z, Li L, *et al*. Preparation and properties of a 3D printed nHA/PLA bone tissue engineering scaffold loaded with a $\beta$-CD–CHX combined dECM hydrogel. RSC Advances. 2024; 14: 9848–9859. https://doi.org/10.1039/d4ra00261j.

[171] Tan L, Ye Z, Zhuang W, Mao B, Li H, Li X, *et al*. 3D printed PLGA/MgO/PDA composite scaffold by low-temperature deposition manufacturing for bone tissue engineering applications. Regenerative Therapy. 2023; 24: 617–629. https://doi.org/10.1016/j.reth.2023.09.015.

[172] Mori Y, Hamai R, Kanabuchi R, Onoki T, Takahashi K, Hashimoto K, *et al*. Efficacy of octacalcium phosphate/gelatin (OCP/Gel) composite implantation for miniature swine lumbar interbody fusion. The Tohoku Journal of Experimental Medicine. 2025; 266: 135–144. https://doi.org/10.1620/tjem.2024.J138.

**Editor's note**: The Scientific Editor responsible for this paper was Moroni Lorenzo and Martin Stoddart.

**Received**: 3rd May 2025; **Accepted**: 22nd January 2026; **Published**: 30th April 2026