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Review

Impact of Octacalcium Phosphate/Gelatin (OCP/Gel) Composite on Bone Repair in Refractory Bone Defects

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In clinical settings, bone grafting is frequently used to treat bone defects. Therefore, the development of bone graft substitutes with superior bone formation ability is expected, instead of autogenous bone grafting. Octacalcium phosphate (OCP) has been developed as a bone graft substitute, and preclinical studies using OCP have reported superior bone formation ability compared with β-tricalcium phosphate. Furthermore, OCP has been used in composite forms with natural polymers such as collagen and gelatin to improve the usability of OCP, and OCP/collagen composite forms have been clinically applied in the dental field because of their excellent usability and osteogenic potential. This review describes the development and preclinical results of OCP and OCP/gelatin (OCP/Gel) composites and prospects for future applications in orthopedics. The development of bone graft substitutes that achieve a high degree of biodegradability and strength will be needed for the clinical application of OCP composites in orthopedics in the future.

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Introduction

In orthopedic surgery, bone grafting is becoming increasingly important for treating bone defects due to traumatic fractures, tumors, and revision orthopedic prosthesis surgeries as well as for bone grafting in spinal fusion surgery (Kinaci et al. 2014; Habraken et al. 2016). Autologous bone grafts were harvested from the patients' bodies; hence, there is no risk of transmitted infection, hepatitis, or other foreign-body reactions. Furthermore, they contain the osteoblasts and growth factors necessary for bone formation, allowing the repair of bone defects to be completed sooner (Giannoudis et al. 2005). However, autologous bone grafting alone cannot be used to treat large bone defects because of the possibility of fracture and residual pain at the bone grafting site, and the limited amount of bone that can be harvested.

Allogeneic bone grafting has limited availability regarding proper harvesting, preservation, and management of grafted bone fragments, and there are concerns regarding the risk of transmitted infection (Kinaci et al. 2014). Allografts should be sterilized to reduce the risk of transmitted infection, which may affect osteogenic characteristics (Singh et al. 2016). In contrast, bone graft substitutes are useful because they are synthetic, have no limitation on the amount used, have no possibility of infection, are minimally invasive, and can be used at any facility. Therefore, the development of bone graft substitutes with superior bioactivity continues (Giannoudis et al. 2005; Bohner et al. 2020).

Available bone graft substitutes are hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP), but they have not yet achieved the same osteogenic potential as autogenous bone. If the grafted bone remains and is not replaced by bone tissue, it may cause a foreign body reaction, infection, and destruction of the surrounding bone tissue, thus requiring synthetic bone graft substitutes with a better osteogenic potential and biodegradability.

Octacalcium phosphate (OCP) has been developed as a bone graft substitute (Suzuki 2010) and reported excellent osteogenic potential in nonclinical studies using OCP compared with β-TCP (Suzuki et al. 1991, 2006a, b; Imaizumi

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et al. 2006; Miyatake et al. 2009; Murakami et al. 2010). OCP takes up calcium ions and releases phosphate ions during conversion to HA (Suzuki et al. 2006a). Previous studies have shown that OCP induces the differentiation of bone marrow-derived mesenchymal cells into osteoblasts (Suzuki et al. 2006b; Anada et al. 2008; Sato et al. 2019) and that OCP promotes osteoclast differentiation (Takami et al. 2009). OCP is expected to show superior osteogenesis in bone defects compared with conventional bone graft substitutes. To improve the operability of OCP, a composite form with natural polymers such as collagen and gelatin was created (Handa et al. 2012; Chiba et al. 2016; Ishiko-Uzuka et al. 2017; Baba et al. 2020; Kawai et al. 2020; Oizumi et al. 2021). The OCP/collagen composite is now available for clinical practice after clinical trials in the dental field (Kawai et al. 2020). This review describes the development and preclinical results of the OCP/gelatin (OCP/Gel) composite as well as the prospects for its future application in orthopedic surgery.

Octacalcium Phosphate (OCP)

OCP is unique in its ability to convert HA *in vitro* and *in vivo* (Suzuki et al. 2006a, b). This conversion process increases the activities of various bone-related cells. Previous studies have shown that OCP promotes osteoblast differentiation from mesenchymal stem cells during OCP-HA conversion (Suzuki et al. 2006b; Anada et al. 2008) and enhances bone formation. Osteoblasts become embedded in the bone matrix and eventually differentiate into osteocytes, and OCP promotes osteoblastic differentiation *in vitro* and *in vivo* (Saito et al. 2021). In addition, amorphous calcium phosphate added to OCP has been shown to promote bone regeneration in cranial defects (Kobayashi et al. 2014).

OCP-HA conversion affects bone formation, inflammation, and resorption. OCP regulates macrophage migration by altering calcium ion concentrations (Hirayama et al. 2016) and promotes osteoclast formation via expression of the receptor activator of nuclear factor-kappa B ligand in osteoblasts (Takami et al. 2009). The different crystalline phases of OCP and HA have also been shown to promote osteoclast differentiation by altering the expression of the coupling factors secreted by osteoclasts (Shiwaku et al. 2019).

Various material properties of OCP and OCP-HA conversion have been shown to influence bone regeneration in cranial defects. For example, larger granule diameters (500-1,000 μm) of OCP promote bone formation and osteoclast-like cell formation in cranial defects (Murakami et al. 2010). In addition, the crystal length of OCP also affects the adhesion of mouse bone marrow-derived stromal cells and bone formation in mouse cranial defects (Honda et al. 2009).

OCP also promotes bone regeneration in long tubular bones. In rat tibia, slightly hydrolyzed OCP attenuated the initial inflammatory response and promoted bone formation compared with the original OCP (Miyatake et al. 2009). OCP can be composited with various polymers, such as gelatin and polylactic-co-glycolide acid (PLGA). Even in the composite form, the solubility and ionic behavior of OCP alter their cellular activity and osteoconductivity (Oizumi et al. 2021).

Among calcium phosphate bone graft substitutes, OCP showed the best ability to enhance alkaline phosphatase activity (Kovrlija et al. 2021); in comparison to HA, implanted OCPs promoted more bone formation (Suzuki et al. 2006b). In another study, the tissue response and osteoconductivity of OCP and sintered HA blocks were compared, and at 4 weeks after implantation, OCP blocks showed approximately 30% bone replacement, whereas sintered HA blocks showed no bone replacement in the same period (Sugiura et al. 2018). When OCP was implanted into the bone marrow of rat tibiae and compared to β-TCP, OCP showed almost the same bone formation results as β-TCP, although β-TCP showed the highest rate of bone formation at day 14, while OCP showed a gradual increase in bone formation until day 56 (Miyatake et al. 2009). Furthermore, at 56 days after implantation, osteocalcin staining showed positive cells around both β-TCP and OCP, while osteopontin staining showed positive cells in OCP but not in β-TCP (Miyatake et al. 2009). In a study comparing the osteoconductivity of OCP and amorphous calcium phosphate (Kobayashi et al. 2014), 8 weeks after implantation in the calvaria of rats, amorphous calcium phosphate showed 16.9 ± 2.9% (mean ± standard deviation) of new bone formation in the bone defect, while OCP showed 38.2 ± 4.3%, indicating a higher bone-forming capacity in OCP.

The solubility of calcium phosphate bone graft substitutes may affect the resorptive activity of osteoclasts, and calcium phosphate bone graft substitutes with higher solubility have better biodegradability and osteogenic effects (Yamada et al. 1997). Details of OCP solubility and hydrolysis under different conditions have been reported by Tung et al (1988). A comparative study has been conducted on the resorbability of β-TCP, HA, and OCP and their promotion of bone formation (Kamakura et al. 2002); the particle sizes of OCP and HA ranged from 300 to 500 μm, and those of β-TCP from 250 to 500 μm. The percentages of bone graft substitutes remaining in the rat cranial bone defects of OCP, β-TCP, and HA at 6 months after implantation were 6.58 ± 1.46, 23.4 ± 6.82, and 39.0 ± 6.86, respectively. The percentages of newly formed bone in OCP, β-TCP, and HA were 63.3 ± 7.52, 42.8 ± 13.6, and 24.2 ± 7.82, respectively. These results indicate that OCP was the most resorbable and promoted bone formation in the cranial bone defect better than other calcium phosphate bone graft substitutes.

α-TCP, monetite (dicalcium phosphate anhydrate, DCPA), and its hydrated counterpart, brushite (dicalcium phosphate dihydrate, DCPD), have higher *in vivo* reactivity than β-TCP and OCP, and are used as calcium phosphate cement paste that converts to HA and hardens *in vivo*, rather

than as a bone graft substitute that is replaced by physiological bone at an early stage (Lodoso-Torrecilla et al. 2021). Calcium phosphate cement has low strength even after hardening and low biodegradability due to its lack of porosity (Schroter et al. 2020), its main disadvantages compared to OCP bone graft substitutes. Developing bone graft substitutes as composites that can be used for vertebrae and load-bearing bones is mandatory.

Octacalcium Phosphate/Gelatin (OCP/Gel) Composite

OCP has the potential to be used as a bone replacement material, but its granular form requires improved manipulation. As OCP possesses water molecules in its structure, it may lose its characteristics during sintering, unlike HA and β-TCP. Therefore, the approaches are required to improve its operability by creating a composite with natural polymers, such as collagen and gelatin, and synthetic polymers, such as PLGA. Natural polymers are expected to improve cell adhesion and proliferation. Gelatin-based OCP composites have potential applications in the repair of severe bone defects. Gelatin is a denatured collagen molecule (Veis and Cohen 1960). Gelatin has high biocompatibility and retains the cell adhesion molecule RGD (Arg-Gly-Asp) motif (Ma et al. 2002). Therefore, gelatin is widely used as a matrix material in bone-tissue engineering (Tabata 2003).

A porous OCP/Gel composite material was developed and investigated its ability to repair critical-size cranial bone defects in rats (Handa et al. 2012; Ishiko-Uzuka et al. 2017), and standardized long bone defects in rabbits (Suzuki et al. 2012; Chiba et al. 2016) and ovariectomized rats (Baba et al. 2020). OCP/Gel has a porous structure, and even though gelatin is thermally cross-linked, the crystal structure of OCP is secured, and the characteristic structure is confirmed by X-ray diffraction analysis (Fig. 1) (Chiba et al. 2016; Baba et al. 2020). OCP/Gel complexes containing 17% or 44% OCP by weight are described as 17% OCP/Gel or 44% OCP/Gel. A previous study reported the average pore sizes of 182.4 ± 2.3 μm and 190.7 ± 5.9 μm and average porosities of 90.3 ± 1.5% and 92.2 ± 0.5% for the 17% OCP/Gel and 44% OCP/Gel composites, respectively (Baba et al. 2020). When the OCP/Gel was transplanted into the standardized defects, the composite was completely replaced by bone marrow tissue, followed by cortical bone regeneration (Handa et al. 2012; Suzuki et al. 2012; Chiba et al. 2016; Ishiko-Uzuka et al. 2017; Baba et al. 2020). These studies demonstrate the biodegradability and osteogenic potential of the OCP/Gel.

Development of a Drug Delivery System Using OCP

When new bone is formed, non-collagenous matrix proteins can accumulate around the OCP (Suzuki et al. 1991, 1993). Thus, OCP is capable of accumulating proteins around individual crystals owing to its specific adsorption affinity. Fig. 2 shows the presence of Maclura pomifera agglutinin lectin-binding glycoconjugates accumulated around the OCP implanted into the mouse calvaria. When OCP is hydrolyzed to HA under physiological conditions, the adsorption affinity can be changed progressively with the advancement of OCP-HA conversion (Suzuki et al. 1995). Furthermore, biologically important molecules can be suitably adsorbed onto OCP surfaces at physiological pH (Shiwaku et al. 2012). Therefore, it is interesting to consider the release of adsorbed proteins from OCP surfaces.

Bone regeneration is a complex physiological process

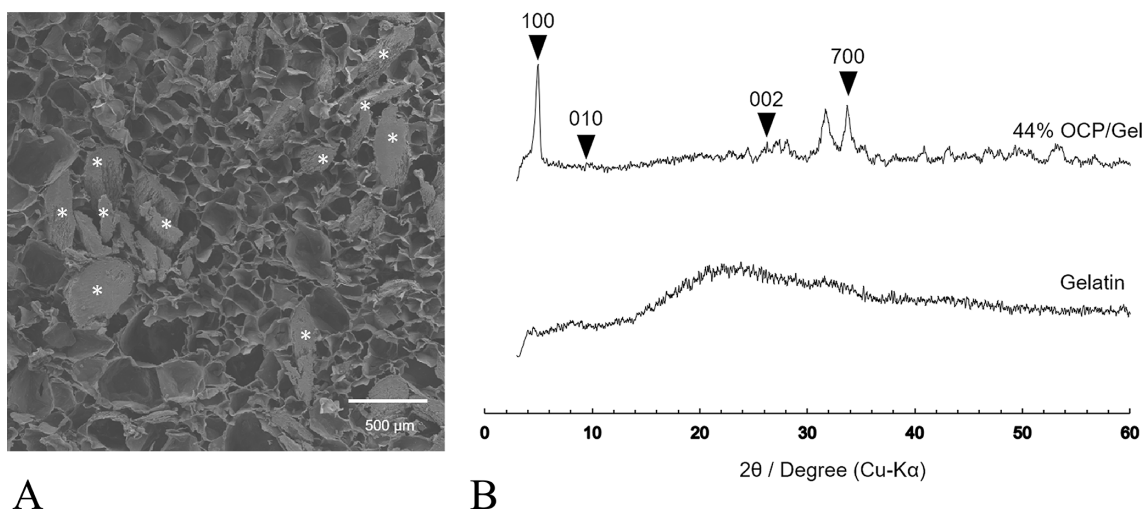


Fig. 1. Scanning electron microscope (SEM) image and X-ray diffraction of octacalcium phosphate (OCP) /gelatin (Gel) composite.
A. SEM image of OCP/Gel composite. B. X-ray diffraction patterns of OCP/Gel composite and gelatin. The asterisks indicate the OCP granules in the OCP/Gel composite pores. Bar = 500 μm. The arrowheads indicate the characteristic peaks for OCP corresponding to (100) at 2θ = 4.8°, (010) at 2θ = 9.8°, and (700) at 2θ = 33.6°. Reprinted from the reference (Baba et al. 2020) with permission.

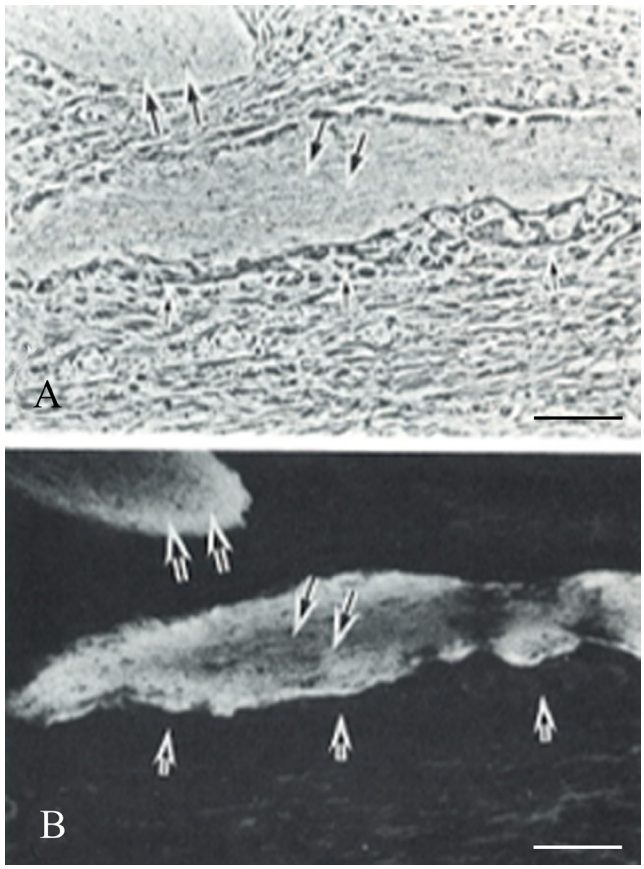


Fig. 2. Micrographs of the area of octacalcium phosphate (OCP) implantation in the murine calvaria.
A. Phase contrast micrograph of the area 13 days after implantation. B. Fluorescence micrograph stained with Maclura pomifera agglutinin (MPA)-fluorescein isothiocyanate (FITC) in the same field as A. Bars = 100 μm. Arrows indicated the presence of MPA lectin-binding glycoconjugates accumulation around the OCP. Reprinted from the reference (Suzuki et al. 1993) with permission.

that restores bone morphology and function. Calcium phosphate biomaterials may promote osteoconduction, osteoinduction, and the eventual replacement of newly formed tissue around the defect site (Henkel et al. 2013). Although research on bone replacement materials is evolving, there are studies involving the administration of bioactive substances to calcium phosphate to further promote bone formation.

The administration of bioactive substances is a strategy that can be implemented via systemic or local drug delivery routes. Traditional systemic drug delivery routes require large doses and may result in numerous side effects, organ complications, and systemic toxicity. The doses of bioactive substances may not be sufficient to achieve the expected results following systemic administration. However, local drug delivery seems to be effective in enhancing the distribution of drugs in the target organ (Kovrlija et al. 2021).

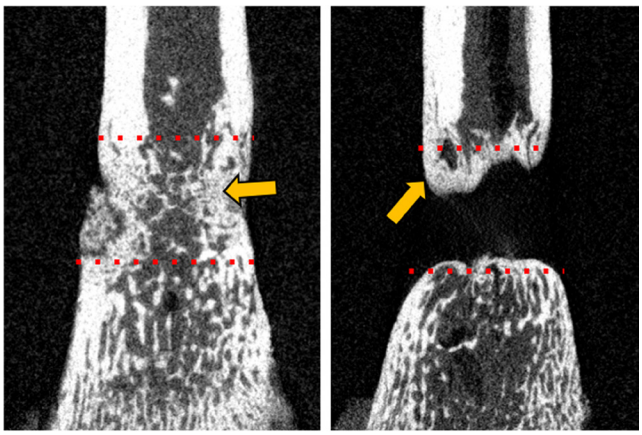
The practical application of OCP for drug delivery purposes is still a pioneering concept, and more *in vitro* and *in vivo* investigations are needed. OCP has been shown to interact with serum proteins of biological origin prior to the bone formation by osteoblasts (Suzuki et al. 1991, 1993, 1995). Since OCP has beneficial effects on bone formation, there are reports on the loading of carbonate (Chickerur et al. 1980; Tomazic et al. 1991), magnesium (Matsunaga 2008; Boanini et al. 2012a), strontium (Matsunaga and Murata 2009; Shi et al. 2016), iron (Shi et al. 2016, 2021), fluoride ions (Suzuki et al. 1995; Iijima et al. 1996; Shiwaku et al. 2012), copper gluconate (Koyama et al. 2022), and ascorbic acid (Kamitakahara et al. 2021) to further promote bone formation, bisphosphonates to inhibit osteoclast activation (Boanini et al. 2012b, 2015; Forte et al. 2017), and silver nanoparticles for antimicrobial purposes (Forte et al. 2018). Many studies on the combination of different therapeutic agents for OCP have achieved high values of drug loading capacity as their goals. This was achieved by hydrothermal (262.9 mg/g adsorbed ibuprofen) (Li et al. 2014), in situ drop precipitation (5.2 wt.% alendronic acid and 3.5 wt.% zoledronic acid) (Forte et al. 2017), laser deposition (5.5 at.% magnesium and 0.6 at.% strontium) (Boanini et al. 2012a), and electrochemical deposition (2.7-13.8% Sr^{2+} substitution) (Fan et al. 2020).

OCP has been shown to interact with serum proteins of biological origin prior to the bone formation by osteoblasts (Suzuki et al. 1991, 1993, 1995; Kaneko et al. 2011). Thus, OCP has been shown to have an affinity for protein molecules of biological origin. A recent study has reported that the *in vitro* adsorption of serum albumin on OCP was enhanced by the immersion of OCP in an aqueous solution containing calcium and phosphate ions, a certain degree of supersaturation with respect to calcium phosphate, and the presence of bovine serum albumin, an acidic protein (Hamai et al. 2019). The future development of technologies for the loading of bioactive substances, such as cytokines and growth factors, is expected.

Future Developments in OCP for Clinical Applications in Orthopedics

Extensive bone loss due to trauma or disease is the leading cause of musculoskeletal disorders, leading to disability, frailty, and reduced quality of life. A long-standing clinical problem is the proper healing of large bone defects or bone loss in load-bearing areas. Critical size gaps are typically 3 cm or larger in humans and are particularly difficult to repair because the ability of bones to repair is impaired (Garrido et al. 2011). Even with surgery, delayed healing or failure to heal occurs in 5%-10% of fractures, and failure to heal in segmental bone defects approaches nearly 100% (Dawson and Oreffo 2008).

Autologous bone grafts are the current clinical standard for treating large bone defects, which combines the essential criteria of high biological activity and strength to induce functional bone regeneration. However, despite these excellent results, the use of autologous bone grafts



A B

Fig. 3. Micro-computed tomography (CT) images of the area of octacalcium phosphate (OCP) /gelatin (Gel) and gelatin implantation in the rat femur.
A. Sagittal section of a defect in the rat femur four weeks after OCP/Gel composite implantation. B. Sagittal section of a defect in the rat femur four weeks after gelatin implantation. Yellow arrows indicate new bone formation with calcification. The red broken lines indicate the edges of bone defects. Reprinted from the reference (Hamada et al. 2022) with permission.

has been limited by significant limitations related to donor site morbidity, inadequate supply, and high graft resorption rates (Arrington et al. 1996).

The search for an ideal bone graft substitute material to replace autologous bone has generated interest, but a satisfactory solution is yet to be found. The criteria used to evaluate the properties of synthetic bone substitutes for clinical applications in the orthopedic field are based on those demonstrated by autogenous bone grafts (Li et al. 2019). Bone graft substitutes 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 transverse femoral cortical defects in rats, suggesting that it could be used as a bone replacement material to treat severe bone defects (Hamada et al. 2022) (Fig. 3).

In addition, the graft must be mechanically strong, able to support long-term tissue regeneration when implanted into a load-bearing defect, and capable of withstanding physiological loading. Synthetic bone graft substitutes must be manufactured through a controlled process and have a reproducible structure along with high porosity and interconnectivity to ensure adequate nutrient exchange and angiogenesis necessary to support bone formation and ongoing turnover (Li et al. 2019).

Current synthetic bone graft substitutes for clinical use are predominantly bioceramics, composed of calcium phosphate and bioactive glass. Among calcium phosphates, HA has excellent mechanical strength and osteoconductivity, but less osteoinductivity and biodegradability. In contrast, β-TCP lacks sufficient mechanical strength, with porosity in the range typically required for bone regeneration (50-90%) (Wagoner Johnson and Herschler 2011). For these reasons, the current clinical applications of synthetic bone graft substitutes are limited to filling small bone cavities, usually in non-load-bearing sites. Therefore, there is a great need to develop new synthetic bone graft materials that can improve the clinical treatment of difficult bone defects. A relatively low porosity of approximately 50% is usually required for bone graft substitutes to maintain sufficient strength for load-bearing purposes (Reitmaier et al. 2018).

A general issue is the limited bioactivity of synthetic bone graft substitutes. Most studies report the low bone formation of synthetic bone graft substitutes in defects of significant size, requiring the development of bone graft substitutes that combine strength and bioactivity to achieve adequate healing. As with other calcium phosphates, OCP and its composites with gelatin currently lack the mechanical strength to serve as a structural bone graft substitute, and further research should be conducted on composites with highly elastic scaffold materials that can improve this function (Grecula 2022). To investigate the defect size that can be treated with OCP, further animal studies are needed to understand the limits of the defect size that can be treated with OCP and how much mechanical stability is needed to maintain the regenerative ability of the OCP/Gel composite by varying the defect size and the strength of the structure. The mechanical properties of the regenerated bone should be tested and compared with those of the host bone. The field of alternative bone grafting is crowded; however, an optimal solution has not yet been found. Therefore, based on the promising results of the OCP/Gel composite (Hamada et al. 2022) and in consideration of its clinical application in dental reconstructive surgery (Kawai et al. 2020), although challenges to OCP, such as strength and maneuverability, a careful stepwise approach to clinical trials for orthopedic clinical response is warranted.

Clinical trials should start with a small case series using autologous bone grafting as a control. Another good starting point is a small randomized controlled trial applying the OCP/Gel composite to stable bone defects that occur after the removal of fracture treatment implants and curettage of benign bone lesions. The control group should include an autograft and/or another FDA-approved calcium phosphate substitute with established clinical and radiological results. If convincing results are observed in the first phase of the trial, subsequent clinical trials could be expanded to trauma or other more significant size defects encountered during reconstructive surgery.

Concluding Remarks

The authors discuss in detail the chemistry of OCP, the properties of OCP/Gel composites, and prospects for their clinical application in orthopedic surgery. The usefulness of OCP in drug delivery systems was also reviewed.

Focusing on OCP/Gel composites, which have been studied to optimize the properties of OCP as orthopedic biomaterials, the authors discussed the osteoconductive and osteoinductive performance, and their potential for treating refractory bone defects due to the superior osteogenic potential, while presenting results of preclinical studies. Current research requires the development of OCP composites with higher elasticity that can be used in load-bearing areas. Future research issues include the development of OCP that is strong enough to be used in load-bearing areas while maintaining the excellent osteogenic potential and biodegradability of OCP and its composites, and the development of a new OCP bone graft substitute carrying bioactive substances. The authors will continue their efforts to contribute to the development of orthopedic regenerative medicine by developing a bone graft substitute using OCP.

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Author Contributions

Mori Y., Hamai R., and Suzuki O. conceived the original idea. Mori Y., Hamai R., Aizawa T., and Suzuki O. performed the literature research and wrote the manuscript. All authors have read and approved the final submitted manuscript.

Conflict of Interest

The authors declare no conflict of interest.

References

Anada, T., Kumagai, T., Honda, Y., Masuda, T., Kamijo, R., Kamakura, S., Yoshihara, N., Kuriyagawa, T., Shimauchi, H. & Suzuki, O. (2008) Dose-dependent osteogenic effect of octacalcium phosphate on mouse bone marrow stromal cells. *Tissue Eng. Part A*, **14**, 965-978.

Arrington, E.D., Smith, W.J., Chambers, H.G., Bucknell, A.L. & Davino, N.A. (1996) Complications of iliac crest bone graft harvesting. *Clin. Orthop. Relat. Res.*, **329**, 300-309.

Baba, K., Shiwaku, Y., Hamai, R., Mori, Y., Anada, T., Tsuchiya, K., Oizumi, I., Miyatake, N., Itoi, E. & Suzuki, O. (2020) Chemical stability-sensitive osteoconductive performance of octacalcium phosphate bone substitute in an ovariectomized rat tibia defect. *ACS Appl. Bio Mater.*, **3**, 1444-1458.

Boanini, E., Torricelli, P., Fini, M., Sima, F., Serban, N., Mihailescu, I.N. & Bigi, A. (2012a) Magnesium and strontium doped octacalcium phosphate thin films by matrix assisted pulsed laser evaporation. *J. Inorg. Biochem.*, **107**, 65-72.

Boanini, E., Torricelli, P., Forte, L., Pagani, S., Mihailescu, N., Ristoscu, C., Mihailescu, I.N. & Bigi, A. (2015) Antiresorption implant coatings based on calcium alendronate and octacalcium phosphate deposited by matrix assisted pulsed laser evaporation. *Colloids Surface B Biointerfaces*, **136**, 449-456.

Boanini, E., Torricelli, P., Gazzano, M., Fini, M. & Bigi, A. (2012b) The effect of alendronate doped calcium phosphates on bone cells activity. *Bone*, **51**, 944-952.

Bohner, M., Santoni, B.L.G. & Dobelin, N. (2020) beta-tricalcium phosphate for bone substitution: synthesis and properties. *Acta Biomater.*, **113**, 23-41.

Chiba, S., Anada, T., Suzuki, K., Saito, K., Shiwaku, Y., Miyatake, N., Baba, K., Imaizumi, H., Hosaka, M., Itoi, E. & Suzuki, O. (2016) Effect of resorption rate and osteoconductivity of biodegradable calcium phosphate materials on the acquisition of natural bone strength in the repaired bone. *J. Biomed. Mater. Res. A*, **104**, 2833-2842.

Chickerur, N.S., Tung, M.S. & Brown, W.E. (1980) A mechanism for incorporation of carbonate into apatite. *Calcif. Tissue Int.*, **32**, 55-62.

Dawson, J.I. & Oreffo, R.O. (2008) Bridging the regeneration gap: stem cells, biomaterials and clinical translation in bone tissue engineering. *Arch. Biochem. Biophys.*, **473**, 124-131.

Fan, L.L., Zhang, Y.M., Hu, R., Lin, C.J., Shi, W. & Tian, Z.Q. (2020) Strontium substituted octacalcium phosphate coatings by electrochemical deposition and their dose-dependent bioactivities. *Mater. Lett.*, **272**, 127844.

Forte, L., Torricelli, P., Boanini, E., Gazzano, M., Fini, M. & Bigi, A. (2017) Antiresorptive and anti-angiogenetic octacalcium phosphate functionalized with bisphosphonates: an in vitro triculture study. *Acta Biomater.*, **54**, 419-428.

Forte, L., Torricelli, P., Bonvicini, F., Boanini, E., Gentilomi, G.A., Lusvardi, G., Della Bella, E., Fini, M., Nepita, E.V. & Bigi, A. (2018) Biomimetic fabrication of antibacterial calcium phosphates mediated by polydopamine. *J. Inorg. Biochem.*, **178**, 43-53.

Garrido, C.A., Lobo, S.E., Turibio, F.M. & Legeros, R.Z. (2011) Biphasic calcium phosphate bioceramics for orthopaedic reconstructions: clinical outcomes. *Int. J. Biomater.*, **2011**, 129727.

Giannoudis, P.V., Dinopoulos, H. & Tsiridis, E. (2005) Bone substitutes: an update. *Injury*, **36** Suppl 3, S20-27.

Grecula, M.J. (2022) CORR insights(R): octacalcium phosphate/gelatin composite (OCP/Gel) enhances bone repair in a critical-sized transcortical femoral defect rat model. *Clin. Orthop. Relat. Res.*, **480**, 2056-2058.

Habraken, W., Habibovic, P., Epple, M. & Bohner, M. (2016) Calcium phosphates in biomedical applications: materials for the future? *Materials Today*, **19**, 69-87.

Hamada, S., Mori, Y., Shiwaku, Y., Hamai, R., Tsuchiya, K., Baba, K., Oizumi, I., Kanabuchi, R., Miyatake, N., Aizawa, T. & Suzuki, O. (2022) Octacalcium phosphate/gelatin composite (OCP/Gel) enhances bone repair in a critical-sized transcortical femoral defect rat model. *Clin. Orthop. Relat. Res.*, **480**, 2043-2055.

Hamai, R., Tsuchiya, K. & Suzuki, O. (2019) Adsorption of serum albumin onto octacalcium phosphate in supersaturated solutions regarding calcium phosphate phases. *Materials* (*Basel*), **12**, 2333.

Handa, T., Anada, T., Honda, Y., Yamazaki, H., Kobayashi, K., Kanda, N., Kamakura, S., Echigo, S. & Suzuki, O. (2012) The effect of an octacalcium phosphate co-precipitated gelatin composite on the repair of critical-sized rat calvarial defects. *Acta Biomater.*, **8**, 1190-1200.

Henkel, J., Woodruff, M.A., Epari, D.R., Steck, R., Glatt, V., Dickinson, I.C., Choong, P.F., Schuetz, M.A. & Hutmacher, D.W. (2013) Bone regeneration based on tissue engineering conceptions - a 21st century perspective. *Bone Res.*, **1**, 216-248.

Hirayama, B., Anada, T., Shiwaku, Y., Miyatake, N., Tsuchiya, K., Nakamura, M., Takahashi, T. & Suzuki, O. (2016) Immune cell response and subsequent bone formation induced by implantation of octacalcium phosphate in a rat tibia defect. *RSC Adv.*, **6**, 57475-57484.

Honda, Y., Anada, T., Kamakura, S., Morimoto, S., Kuriyagawa, T. & Suzuki, O. (2009) The effect of microstructure of octacalcium phosphate on the bone regenerative property. *Tissue Eng. Part A*, **15**, 1965-1973.

Iijima, M., Nelson, D.G.A., Pan, Y., Kreinbrink, A.T., Adachi, M., Goto, T. & Moriwaki, Y. (1996) Fluoride analysis of apatite crystals with a central planar OCP inclusion: concerning the

role of F- ions on apatite/OCP/apatite structure formation. *Calcif. Tissue Int.*, **59**, 377-384.

Imaizumi, H., Sakurai, M., Kashimoto, O., Kikawa, T. & Suzuki, O. (2006) Comparative study on osteoconductivity by synthetic octacalcium phosphate and sintered hydroxyapatite in rabbit bone marrow. *Calcif. Tissue Int.*, **78**, 45-54.

Ishiko-Uzuka, R., Anada, T., Kobayashi, K., Kawai, T., Tanuma, Y., Sasaki, K. & Suzuki, O. (2017) Oriented bone regenerative capacity of octacalcium phosphate/gelatin composites obtained through two-step crystal preparation method. *J. Biomed. Mater. Res. B Appl. Biomater.*, **105**, 1029-1039.

Kamakura, S., Sasano, Y., Shimizu, T., Hatori, K., Suzuki, O., Kagayama, M. & Motegi, K. (2002) Implanted octacalcium phosphate is more resorbable than beta-tricalcium phosphate and hydroxyapatite. *J. Biomed. Mater. Res.*, **59**, 29-34.

Kamitakahara, M., Ishii, A., Matsubara, H., Kawashita, M., Furuya, M. & Kanetaka, H. (2021) Fabrication and evaluation of ascorbic acid phosphate-loaded spherical porous hydroxyapatite/octacalcium phosphate granules. *J. Ceram. Soc. Jpn.*, **129**, 60-65.

Kaneko, H., Kamiie, J., Kawakami, H., Anada, T., Honda, Y., Shiraishi, N., Kamakura, S., Terasaki, T., Shimauchi, H. & Suzuki, O. (2011) Proteome analysis of rat serum proteins adsorbed onto synthetic octacalcium phosphate crystals. *Anal. Biochem.*, **418**, 276-285.

Kawai, T., Kamakura, S., Matsui, K., Fukuda, M., Takano, H., Iino, M., Ishikawa, S., Kawana, H., Soma, T., Imamura, E., Kizu, H., Michibata, A., Asahina, I., Miura, K., Nakamura, N., et al. (2020) Clinical study of octacalcium phosphate and collagen composite in oral and maxillofacial surgery. *J. Tissue Eng.*, **11**, 2041731419896449.

Kinaci, A., Neuhaus, V. & Ring, D.C. (2014) Trends in bone graft use in the United States. *Orthopedics*, **37**, e783-788.

Kobayashi, K., Anada, T., Handa, T., Kanda, N., Yoshinari, M., Takahashi, T. & Suzuki, O. (2014) Osteoconductive property of a mechanical mixture of octacalcium phosphate and amorphous calcium phosphate. *ACS Appl. Mater. Interfaces*, **6**, 22602-22611.

Kovrlija, I., Locs, J. & Loca, D. (2021) Octacalcium phosphate: innovative vehicle for the local biologically active substance delivery in bone regeneration. *Acta Biomater.*, **135**, 27-47.

Koyama, S., Hamai, R., Shiwaku, Y., Kurobane, T., Tsuchiya, K., Takahashi, T. & Suzuki, O. (2022) Angio-osteogenic capacity of octacalcium phosphate co-precipitated with copper gluconate in rat calvaria critical-sized defect. *Sci. Technol. Adv. Mater.*, **23**, 120-139.

Li, C.F., Ge, X.L., Li, G.C., Gao, Q.C. & Ding, R. (2014) A facile hydrothermal method for synthesis of submillimeter-long octacalcium phosphate and hydroxyapatite as drug carriers with sustained release behaviors. *Adv. Powder Technol.*, **25**, 1661-1666.

Li, J.J., Dunstan, C.R., Entezari, A., Li, Q., Steck, R., Saifzadeh, S., Sadeghpour, A., Field, J.R., Akey, A., Vielreicher, M., Friedrich, O., Roohani-Esfahani, S.I. & Zreiqat, H. (2019) A novel bone substitute with high bioactivity, strength, and porosity for repairing large and load-bearing bone defects. *Adv. Healthc. Mater.*, **8**, e1801298.

Lodoso-Torrecilla, I., van den Beucken, J. & Jansen, J.A. (2021) Calcium phosphate cements: optimization toward biodegradability. *Acta Biomater.*, **119**, 1-12.

Ma, Z., Gao, C., Gong, Y., Ji, J. & Shen, J. (2002) Immobilization of natural macromolecules on poly-L-lactic acid membrane surface in order to improve its cytocompatibility. *J. Biomed. Mater. Res.*, **63**, 838-847.

Matsunaga, K. (2008) First-principles study of substitutional magnesium and zinc in hydroxyapatite and octacalcium phosphate. *J. Chem. Phys.*, **128**, 245101.

Matsunaga, K. & Murata, H. (2009) Strontium substitution in bioactive calcium phosphates: a first-principles study. *J. Phys. Chem. B*, **113**, 3584-3589.

Miyatake, N., Kishimoto, K.N., Anada, T., Imaizumi, H., Itoi, E. & Suzuki, O. (2009) Effect of partial hydrolysis of octacalcium phosphate on its osteoconductive characteristics. *Biomaterials*, **30**, 1005-1014.

Murakami, Y., Honda, Y., Anada, T., Shimauchi, H. & Suzuki, O. (2010) Comparative study on bone regeneration by synthetic octacalcium phosphate with various granule sizes. *Acta Biomater.*, **6**, 1542-1548.

Oizumi, I., Hamai, R., Shiwaku, Y., Mori, Y., Anada, T., Baba, K., Miyatake, N., Hamada, S., Tsuchiya, K., Nishimura, S.N., Itoi, E. & Suzuki, O. (2021) Impact of simultaneous hydrolysis of OCP and PLGA on bone induction of a PLGA-OCP composite scaffold in a rat femoral defect. *Acta Biomater.*, **124**, 358-373.

Reitmaier, S., Kovtun, A., Schuelke, J., Kanter, B., Lemm, M., Hoess, A., Heinemann, S., Nies, B. & Ignatius, A. (2018) Strontium(II) and mechanical loading additively augment bone formation in calcium phosphate scaffolds. *J. Orthop. Res.*, **36**, 106-117.

Saito, S., Hamai, R., Shiwaku, Y., Hasegawa, T., Sakai, S., Tsuchiya, K., Sai, Y., Iwama, R., Amizuka, N., Takahashi, T. & Suzuki, O. (2021) Involvement of distant octacalcium phosphate scaffolds in enhancing early differentiation of osteocytes during bone regeneration. *Acta Biomater.*, **129**, 309-322.

Sato, T., Anada, T., Hamai, R., Shiwaku, Y., Tsuchiya, K., Sakai, S., Baba, K., Sasaki, K. & Suzuki, O. (2019) 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 Biomater.*, **88**, 477-490.

Schroter, L., Kaiser, F., Stein, S., Gbureck, U. & Ignatius, A. (2020) Biological and mechanical performance and degradation characteristics of calcium phosphate cements in large animals and humans. *Acta Biomater.*, **117**, 1-20.

Shi, H., He, F. & Ye, J. (2016) Synthesis and structure of iron- and strontium-substituted octacalcium phosphate: effects of ionic charge and radius. *J. Mater. Chem. B*, **4**, 1712-1719.

Shi, H., Ye, X., Zhang, J., Wu, T., Yu, T., Zhou, C. & Ye, J. (2021) A thermostability perspective on enhancing physicochemical and cytological characteristics of octacalcium phosphate by doping iron and strontium. *Bioact. Mater.*, **6**, 1267-1282.

Shiwaku, Y., Anada, T., Yamazaki, H., Honda, Y., Morimoto, S., Sasaki, K. & Suzuki, O. (2012) Structural, morphological and surface characteristics of two types of octacalcium phosphate-derived fluoride-containing apatitic calcium phosphates. *Acta Biomater.*, **8**, 4417-4425.

Shiwaku, Y., Tsuchiya, K., Xiao, L. & Suzuki, O. (2019) Effect of calcium phosphate phases affecting the crosstalk between osteoblasts and osteoclasts in vitro. *J. Biomed. Mater. Res. A*, **107**, 1001-1013.

Singh, R., Singh, D. & Singh, A. (2016) Radiation sterilization of tissue allografts: a review. *World J. Radiol.*, **8**, 355-369.

Sugiura, Y., Munar, M.L. & Ishikawa, K. (2018) Fabrication of octacalcium phosphate block through a dissolution-precipitation reaction using a calcium sulphate hemihydrate block as a precursor. *J. Mater. Sci. Mater. Med.*, **29**, 151.

Suzuki, K., Honda, Y., Anada, T., Handa, T., Miyatake, N., Takahashi, A., Hosaka, M., Imaizumi, H., Itoi, E. & Suzuki, O. (2012) Stimulatory capacity of an octacalcium phosphate/gelatin composite on bone regeneration in a rabbit tibia defect model. *Phosphorus Research Bulletin*, **26**, 53-58.

Suzuki, O. (2010) Octacalcium phosphate: osteoconductivity and crystal chemistry. *Acta Biomater.*, **6**, 3379-3387.

Suzuki, O., Kamakura, S. & Katagiri, T. (2006a) Surface chemistry and biological responses to synthetic octacalcium phosphate. *J. Biomed. Mater. Res. B Appl. Biomater.*, **77**, 201-212.

Suzuki, O., Kamakura, S., Katagiri, T., Nakamura, M., Zhao, B., Honda, Y. & Kamijo, R. (2006b) Bone formation enhanced by implanted octacalcium phosphate involving conversion into

Ca-deficient hydroxyapatite. *Biomaterials*, **27**, 2671-2681.
Suzuki, O., Nakamura, M., Miyasaka, Y., Kagayama, M. & Sakurai, M. (1991) Bone formation on synthetic precursors of hydroxyapatite. *Tohoku J. Exp. Med.*, **164**, 37-50.
Suzuki, O., Nakamura, M., Miyasaka, Y., Kagayama, M. & Sakurai, M. (1993) Maclura pomifera agglutinin-binding glycoconjugates on converted apatite from synthetic octacalcium phosphate implanted into subperiosteal region of mouse calvaria. *Bone Miner.*, **20**, 151-166.
Suzuki, O., Yagishita, H., Yamazaki, M. & Aoba, T. (1995) Adsorption of bovine serum albumin onto octacalcium phosphate and its hydrolyzates. *Cells and Materials*, **5**, 45-54.
Tabata, Y. (2003) Tissue regeneration based on growth factor release. *Tissue Eng.*, **9** Suppl 1, S5-15.
Takami, M., Mochizuki, A., Yamada, A., Tachi, K., Zhao, B., Miyamoto, Y., Anada, T., Honda, Y., Inoue, T., Nakamura, M., Suzuki, O. & Kamijo, R. (2009) Osteoclast differentiation induced by synthetic octacalcium phosphate through receptor activator of NF-κB ligand expression in osteoblasts. *Tissue Eng. Part A*, **15**, 3991-4000.
Tomazic, B.B., Mayer, I. & Brown, W.E. (1991) Ion incorporation into octacalcium phosphate hydrolyzates. *J. Cryst. Growth*, **108**, 670-682.
Tung, M.S., Eidelman, N., Sieck, B. & Brown, W.E. (1988) Octacalcium phosphate solubility product from 4 to 37 ℃. *J. Res. Nat. Bur. Stand.* (*1977*), **93**, 613-624.
Veis, A. & Cohen, J. (1960) Reversible transformation of gelatin to the collagen structure. *Nature*, **186**, 720-721.
Wagoner Johnson, A.J. & Herschler, B.A. (2011) A review of the mechanical behavior of CaP and CaP/polymer composites for applications in bone replacement and repair. *Acta Biomater.*, **7**, 16-30.
Yamada, S., Heymann, D., Bouler, J.M. & Daculsi, G. (1997) Osteoclastic resorption of calcium phosphate ceramics with different hydroxyapatit/β-tricalcium phosphate ratios. *Biomaterials*, **18**, 1037-1041.