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Review Article

Octacalcium phosphate (OCP)-based bone substitute materials

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Summary The present article summarizes the characteristics of a synthetic octacalcium phosphate (OCP) and OCP-based materials. We previously established a method for a relatively large scale synthesis of OCP and showed that OCP enhances bone regeneration more than hydroxyapatite (HA) materials, including HA obtained through hydrolysis of OCP, coupled with material biodegradation if implanted in various bone defects. One of the OCP-based materials consisting of OCP and natural polymers, such as gelatin, induced a bone regeneration rate over 70% in critical sized rat calvaria defects, which approached the rate seen with autologous bone implantation. The bone regenerative properties observed for OCP-based materials could be due to the biological activity of OCP crystals that enhance *in vitro* osteoblast differentiation and osteoclast formation from precursor cells. OCP controls the environment around its own crystals, where osteoblastic cells encounter OCP during the progressive conversion to HA under physiological conditions. This process contributes to an increase in the biological activity of OCP, resulting in enhancing bone regeneration. Although the positive effect of OCP depends on the crystal stoichiometry and morphology, determined by the conditions used preparing OCP, it is probable that OCP-based materials could be good candidates for an advanced material compatible to autologous bone.

Contents

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1. Introduction

Autologous bone is recognized as the most osteoconductive and osteoinductive bone substitute material available for implantation in bone defects [1]. These observed properties of autologous bone are due to the presence of osteoblastic cells and growth factors, such as bone morphogenetic proteins (BMP), and matrix materials, such as collagen and hydroxyapatite (HA) crystals. However, in order to overcome the limitations of the availability of autologous bone and prevent the unnecessary pain of a second surgery in patients, synthetic biocompatible materials have widely been developed and used as alternatives for autologous bone to repair bone defects [2—9]. Calcium phosphate ceramic materials, such as sintered HA and β-tricalcium phosphate (β-TCP), have been extensively studied and clinically applied [3,7,10]. Sintered HA is classified as a relatively stable material chemically and has been shown to remain undissolved in bone defects over a long period of time, but provides better biocompatibility with the regenerative tissue [2,5,11]. In contrast, β-TCP is a resorbable material if implanted in bone defects [12,13], due to the intrinsic solubility at physiological pH [14], although the dissolution of this material is followed by an osteoclastic cellular phagocytotic response [13].

Recently, the property of resorbable materials *in vivo* has attracted the interest of material scientists and researchers in the field of tissue engineering. Such materials biodegrade and can be substituted by new bone over time through the process of bone remodeling [15]. The materials are chemically resorbable under physiological conditions, and the increased space made by the material dissolution is replaced with new bone formation [7,13,16]. This is advantageous for repairing bone defects; however, the biological response of cells to this type of material can be regarded as passive, although some materials, such as β-TCP, are not only dissolved through simple chemical dissolution, but also resorbed by osteoclastic cells [13,14]. The purpose of this review article is to describe the characteristics of octacalcium phosphate (OCP) and OCP-based composite materials, which were experimentally characterized in the laboratory. OCP materials are of biological interest because the materials themselves have a positive effect on bone forming cells similar to autologous bone. OCP has been postulated as a precursor of biological apatite crystals in bone as well as tooth dentin and enamel [17,18]. The osteoconductivity of synthetic OCP was first described through implantation onto mouse calvaria [19]. Recently, studies using synthetic OCP have intensified in order to elucidate the bone regenerative properties and establish an approach for using it in various bone defects [20—31].

2. Biodegradable calcium phosphate and OCP

2.1. Biodegradable calcium phosphates

Calcium phosphate ceramics that have been reported to biodegrade *in vivo* are summarized in Table 1. Acidic calcium phosphates, such as dicalcium phosphate anhydrous (DCPA) and OCP, are classified as soluble ceramics at neutral pH [4,14]. α-TCP [4,32] and amorphous calcium phosphate (ACP) [33—35] are recognized as highly soluble materials at neutral pH and have also been shown to biodegrade [32,36]. The biodegradability *in vivo* is in general considered to be associated with the solubility of calcium phosphate at physiological pH [4,14]. In addition, β-TCP is widely recognized as a biodegradable ceramic *in vivo* [7,13] although this material has been shown to start to dissolve in an experimental solution with a pH less than 6.0 [37]. Histological findings have revealed that some calcium phosphate ceramics can be resorbed by osteoclastic cells [8,13,23,25,38—41], including biphasic calcium phosphate (BCP) [40,41], which consists of two phases of HA and β-TCP, as well as carbonate-containing HA (carbonate HA) [8,42,43] and nano-HA [39]. HA is most stable chemically at physiological pH. However, the stability decreases as the non-stoichiometry increases [7], displaying Ca-deficiency and the presence of impurities, such as carbonate [44], in the structure. A decrease in the size of the crystals to a nanoscale level usually increases its dissolution and induces changes in the physicochemical properties, such as changes in the crystallinity [45]. The structure of OCP is stacked alternatively with hydrated layers [18]. Based on this structure, OCP has been proposed to be a precursor of biological apatite crystals in bone and tooth [17,18]. As shown in Table 1, the chemical formula of OCP is

Table 1 Calcium phosphate ceramics reported to show biodegradation *in vivo* after a single use.

Calcium phosphates	Abbreviation	Chemical formula	Ca/P molar ratio (theoretical)	*In vivo* condition reported	
				Implant form	Osteoclastic resorption
Dicalcium phosphate anhydrous	DCPA	$CaHPO_4$	1.0	Powdery [19]	Not examined
Octacalcium phosphate	OCP	$Ca_8H_2(PO_4)_6{\cdot}5H_2O$	1.33	Granules [19,24,27,29]	Resorbed
α-Tricalcium phosphate	α-TCP	$Ca_3(PO_4)_2$	1.5	Block [32]	Not reported
β-Tricalcium phosphate	β-TCP	$Ca_3(PO_4)_2$	1.5	Granules [7,13]/ block [12]	Resorbed
Amorphous calcium phosphate	ACP	$Ca_3(PO_4)_2{\cdot}nH_2O$	1.5	Coating [36]/ granules [19]	Not reported
Biphasic calcium phosphate	BCP	HA and β-TCP (in phase)	Dependent on ratio of HA and β-TCP	Granules [40,41]	Resorbed
Carbonate-containing hydroxyapatite	Carbonate HA	$Ca_{10}(PO_4, CO_3)_6(OH)_2$	Non-stoichiometric	Block [6]/deposit with polymers [8]	Resorbed
Nano-hydroxyapatite	Nano-HA	$Ca_{10}(PO_4)_6(OH)_2$	If stoichiometric: 1.67	Deposit with polymers [39]	Resorbed

$Ca_8H_2(PO_4)_6{\cdot}5H_2O$, which has a theoretical Ca/P molar ratio of 1.33. Interestingly, OCP exhibits variation in stoichiometry, and consequently, the Ca/P molar ratios vary from 1.23 to 1.37 [30,46–48]. These OCPs exhibit remarkable differences in osteoconductivity [46], which may be due to the physicochemical changes that occur in the synthesis conditions [49]. It has been proposed that the non-stoichiometric OCP structure has excess hydrogen, resulting in a non-stoichiometric chemical formula, $Ca_{16}H_{4+x}(PO_4)_{12}(OH)_x{\cdot}(10-x)H_2O$, which resembles the structure of HA even more closely than previously anticipated [50]. The preparation conditions may be critical for producing diversity in the chemical and physical properties of OCP, because OCP crystals exhibit plate-like morphologies with wide variation in their dimension [48,51–54]. Together, these findings demonstrate the remarkable differences in crystal size and direction of growth toward a particular axis, depending on the preparation conditions.

2.2. Chemical properties of OCP in medium

The solubility of calcium phosphates can be estimated by measuring the degree of supersaturation (DS) with respect to particular calcium phosphate phases [55–57]. Table 2 shows the DS values in the supernatant after soaking dicalcium phosphate dihydrate (DCPD), OCP, β-TCP, and HA in alpha essential minimal medium (α-MEM) for 72 h at 37 °C, as previously reported [58]. The DS can be expressed by dividing the ionic product by the solubility product from the objective calcium phosphate [55–57]. The DS is usually calculated using the analytical results, including the concentration of calcium (Ca^{2+}) and inorganic phosphate (Pi) ions as well as the pH of the solution [55–57,59]. The results showed that α-MEM was supersaturated with HA and OCP before the introduction of calcium phosphate materials, but undersaturated with DCPD, suggesting that α-MEM has the potential to form HA and OCP if seeded with crystals. The composition of α-MEM after the introduction of DCPD became saturated with respect to DCPD, and the introduction of OCP induced a slight supersaturated condition. In addition, the introduction of β-TCP and HA induced a relatively higher supersaturated condition with respect to OCP and HA. In particular, β-TCP induced a supersaturated condition that was higher than that of the α-MEM alone. Thus, calcium phosphates seeded in α-MEM may be deposited with newly formed HA and possibly OCP [58], although the crystal growth may also be affected by the kinetics of the specific calcium phosphate phase associated with the inhibitory effect of some of the ionic regulators, such as small amounts of magnesium and other factors [55,60–63].

Table 2 Degree of supersaturation of alpha essential minimal medium (α-MEM) after soaking calcium phosphate ceramics for 72 h at 37 °C.

Calcium phosphates	Degree of supersaturation		
	HA	OCP	DCPD
DCPD	4.9×10^{8}	1.8×10^{3}	6.7
OCP	3.2×10^{10}	7.5×10^{1}	2.1×10^{-1}
β-TCP	1.4×10^{12}	8.6×10^{2}	3.0×10^{-1}
HA	5.6×10^{11}	5.5×10^{2}	3.0×10^{-1}
α-MEM	8.8×10^{11}	2.7×10^{3}	7.5×10^{-1}

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3. Bone substitute materials showing possibly biologically active properties

It is of great interest to determine whether calcium phosphate ceramics positively promote osteogenesis, because recent studies have shown that some calcium phosphate ceramics have osteoinductive properties [64—66], including the capability to induce ectopic bone formation. It seems likely that the osteoinductive properties are due to the geometry of the materials and the surface nanostructure, which provides a site for osteoblastic cell attachment, migration, grow, and differentiation to form bone matrix [64—69], resulting in the onset of bone mineralization. In contrast, although such structural effects may be essential for the regeneration of new bone, the mechanism by which calcium phosphate materials themselves affect a biological response after implantation into bone defects has not been elucidated. Some studies have explored the effect of calcium phosphate materials on osteoblastic cells *in vitro*. HA ceramic particles ranging from submicron size to approximately 800 μm in diameter can influence the biological response of fibroblasts and myoblasts [70]. Calcium phosphate particles with various Ca/P molar ratios and in the nano- and micrometer ranges in size also have an influence on osteoblastic differentiation [71]. The β-TCP granules provide a scaffold for osteoblast colony formation over time on their surfaces [72]. This material also controls signaling of human osteoblasts, as increased α2 integrin subunit gene expression and activation of the mitogen-activated protein kinase (MAPK)/extracellular related kinase (ERK) signaling pathway has been observed [73]. These findings suggest that some calcium phosphates positively affect cellular function with regard to tissue generation; however, these studies have not shown the direct effects of calcium phosphate, and therefore we cannot exclude the possibility that the geometry affects cellular function. Therefore, further studies are needed to compare morphology, chemical composition, dose, and other parameters of the materials in order to fully elucidate these mechanisms.

4. Bone regenerative properties of OCP

4.1. Characteristics of bone formation induced by OCP implantation

Several lines of evidence have confirmed that OCP is an osteoconductive material that enhances bone regeneration in regions adjacent to the implanted OCP if used as a filling material in bone defects of various animal models [23—25,29,30,74]. One of the remarkable characteristics of OCP in bone regeneration is that osteoblasts aligned on an OCP implant initiate new bone deposition from a structure consisting of OCP particles and non-collagenous proteins, the latter of which originates from surrounding circulating serum proteins [19,75]. Interestingly, the initial bone matrix formed around OCP was shown to consist of fine filaments and small granular materials within the non-collagenous matrix at the ultrastructural level [19]. The structure was almost identical to the components of bone nodules previously described by Bernard and Pease [76], and considered to be a site that initiates intramembranous bone development [76—79]. Therefore, it is probable that OCP implantation into bone tissue may emulate the onset of bone formation, at least regarding the morphological features of the initial bone deposition [19]. This initial step is followed by additional bone formation, which is characterized by collagen formation and accompanied by apatite crystal deposition [79,80], namely, the progress of bone mineralization.

4.2. Osteoblastic and osteoclastic cells observed around OCP

Fig. 1 shows a typical *in vivo* cellular response induced by OCP implantation into a bone defect [23], showing new bone formation by osteoblasts and OCP biodegradation through the direct resorption of osteoclast-like cells. This figure is a histological section of an OCP granule implanted for 4 weeks intramedullary in a 3 mm diameter defect created in the cortex of the femoral metaphysic of a rabbit femur using undecalcified specimen stained with hematoxylin and eosin (H&E). The OCP granules used were 500—1000 μm in diameter. Osteoblasts were directly aligned on the OCP granule surface as well as on the newly formed osteoid bone matrix. Osteoblasts were cuboidal in shape, indicating that active synthesis of bone matrix collagen was occurring. In addition, osteoclast-like multinuclear giant cells were present between cuboidal osteoblasts, which were on the bone matrix deposited onto the OCP surface. An osteoclast-like cell was in contact with the two osteoblasts and attached directly to the OCP surface for resorption (Fig. 1, upper section, arrow). Osteoclast-like cells also resorbed the

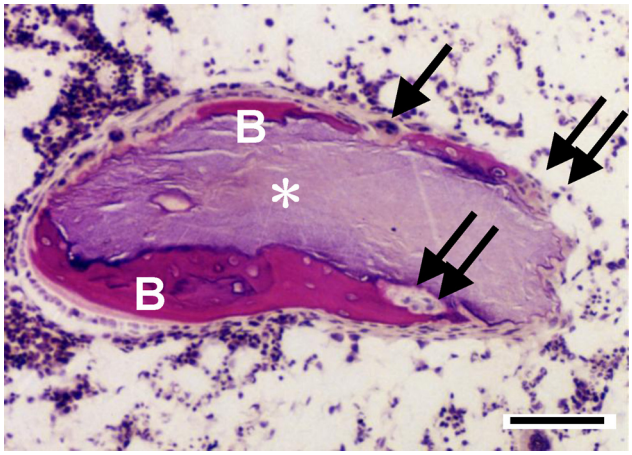

Figure 1 Undecalcified, hematoxylin and eosin (H&E) stained histological section of an OCP granule having a 500—1000 μm diameter, implanted in the intramedullary canal of a rabbit femur at 4 weeks. Aligned cuboidal osteoblasts formed bone matrix (non-calcified osteoid bone matrix) around the OCP granule. Calcified bone matrix underneath the osteoid tissue can be observed because of the use of the undecalcified tissue. Osteoclasts (arrow and double arrows) were resorbing directly on the surface of the OCP granule. An osteoclast (arrow) was in direct contact with two osteoblasts, indicating a close association and coupling between osteoblasts and osteoclasts. The details of the experiments have been reported in Imaizumi et al. [23]. Asterisk: OCP; B: newly formed bone. Bar = 100 μm. All procedures in the experiment were approved by the Animal Research Committee of Tohoku University.

OCP surface (Fig. 1; upper section, double arrow) and interfaced between the OCP and new bone (Fig. 1, lower section, double arrow). These multinuclear osteoclast-like cells were confirmed to be TRAP-positive cells [23]. Moreover, the bone formation induced by OCP implantation not only occurs at the margins of the bone defect, but also frequently initiates directly from the OCP surface [19,24,30,75]. Biodegradation by direct resorption of osteoclast-like cells has been mostly observed in OCP implantations examined through various animal bone defect models, such as mouse and rat calvarias as well as rat and rabbit femurs [23,25,38,46,81,82]. Thus, OCP is recognized as a biodegradable material that promotes simultaneous bone formation by osteoblasts.

4.3. Distinctive features of bone formation by OCP implantation

Fig. 2 shows an undecalcified histological section of OCP granules surrounded by cancellous bone that were formed in the bone marrow space of a rabbit femur at 2 weeks post-implantation. The experimental model was the same as that shown in Fig. 1. Some of the OCP granules were encapsulated with new bone that had grown from cortical bone or cancellous bone in the bone marrow space. As described above, although osteoclast-like cells were able to biodegrade OCP, most of the OCP granules were still present at this stage. The rate of degradation of the granules is a function of the granule size [82,83]. The number of TRAP-positive osteoclast-like cells increased as the OCP granule size increased from 53—300 and 300—500 to 500—1000 μm in diameter if implanted in mouse critical-sized calvaria defects [82]. This tendency was associated with the amount of bone regeneration that occurred around OCP granules [82]. However, the bone regeneration occurring on a material OCP/collagen composite was enhanced as the granule size decreased in the presence of TRAP-positive cells [83]. The TRAP activity around OCP granules was much higher than that of HA granules when implanted onto mouse calvaria, while the amount of bone formation around the granules was much

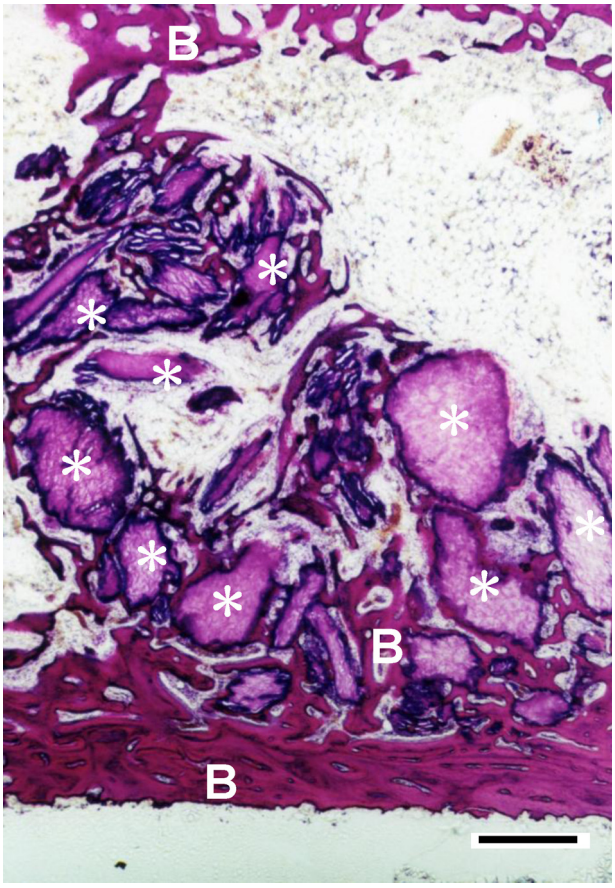

Figure 2 Undecalcified, H&E stained histological section of OCP granules, having a 500—1000 μm diameter and implanted in the intramedullary canal of rabbit femur at 2 weeks. The OCP granules were surrounded by cancellous bone near repaired cortical bone. The details of the experiments have been reported in Imaizumi et al. [23]. Asterisk: OCP; B: newly formed bone. Bar = 500 μm. All procedures in the experiment were approved by the Animal Research Committee of Tohoku University.

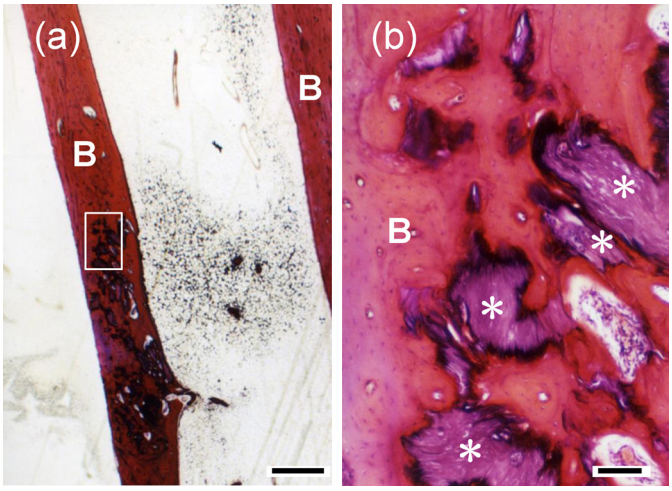

Figure 3 Undecalcified, H&E stained histological section of OCP granules having a 500—1000 μm diameter, implanted in the intramedullary canal of a rabbit femur at 12 weeks (a and b). The OCP granules were almost resorbed in the bone marrow space (a). The debris from OCP granules were embedded in the repaired cortical bone (b). The details of the experiments have been reported in Imaizumi et al. [23]. Asterisk: OCP; B: newly formed bone. Bars = 2 mm (a) and 200 μm (b). Figure (b) is a magnified view of (a) marked by the rectangle. All procedures in the experiment were approved by the Animal Research Committee of Tohoku University.

higher for OCP than HA [25]. These results suggest that the degree of osteoclastic resorption of OCP may be associated with the amount of new bone stimulated by OCP [82]. The tissue formation shown in Fig. 2 included reactive bone formation through the creation of the defect, which is usually observed in the medullary site [23,46]. The reactive bone formation resulted in enhanced remodeling of bone marrow tissue accompanied by the complete resorption of OCP granules from the medullary site. However, in general, the OCP granules remained mostly within the repaired cortical bone encapsulated as debris (Fig. 3a and b). This may be one of the characteristics of OCP biodegradation if used in long bone, although the biodegradable properties of OCP through osteoclast-like cellular resorption appear to be the same when implanted into intramembranous bone, such as calvaria bone [25,81,82]. Therefore, OCP is a material that can be remodeled together with bone.

5. Phase conversion of OCP *in vivo* and *in vitro* and the relation to tissue fluid interaction

It has been reported that body fluids are almost saturated with respect to the OCP phase from studies of calcium phosphate solubilities [84] and the equilibrium of human serum [85]. X-ray diffraction analysis confirmed that implanted OCP tends to gradually convert to HA over time in various bone sites or subcutaneous sites [19,30,75,86]. Furthermore, Fourier transform infrared spectroscopy (FTIR) verified that the incubation of OCP in medium also facilitates conversion to the HA phase [30]. OCP conversion into the HA phase was accompanied by calcium ion consumption into the crystals and inorganic phosphate (Pi) ion release from the crystals [59]. Although the mechanism to promote OCP hydrolysis into HA has not been fully characterized, it is conceivable that physiological fluids include very small amount of fluoride ions [55], which is a strong ionic promoter of OCP hydrolysis and works at very low concentrations [87], in these physiological conditions. Circulating serum proteins, such as α2HS-glycoproteins, can be adsorbed by OCP *in vivo* [75]. The advancement of OCP hydrolysis, which has been studied using OCP and its OCP hydrolyzates as adsorbents, modulates the adsorption affinity of bovine serum albumin [48]. Recent proteomic analyses confirmed that OCP can adsorb over one hundred proteins from rat serum [88]. In addition, proteins involved in bone metabolism, such as apoliporoteins, were identified [88], suggesting the possibility that the proteins adsorbed onto OCP influence bone regeneration by OCP *in vivo* [88—91].

6. *In vitro* cellular response to OCP

When mouse bone marrow stromal ST-2 cells were cultured on culture plates coated with OCP, the OCP significantly stimulated the differentiation of ST-2 cells into osteoblastic cells to a greater extent than HA [30]. The OCP-mediated differentiation was enhanced in a dose-dependent manner, while HA did not exhibit such an effect [20]. The mRNA expression of alkaline phosphatase (ALP), osterix, and type I collagen was significantly up-regulated by OCP in a dose-dependent manner [20]. Another distinguishing characteristic regarding the cellular response to OCP was the OCP-mediated enhancement of osteoclast formation [31]. Osteoclast formation was examined by co-culturing bone marrow cells (osteoclast precursor cells) and osteoblastic cells in the absence of $1,25(OH)_2D_3$ in the culture media, which is an essential factor needed to up-regulate the receptor activator of NF-κB ligand (RANKL) expression in osteoblasts [31]. Fig. 4 shows the precise appearance of TRAP-positive osteoclast-like cells on OCP coated plates (Fig. 4a). When OCP was absent in the culture, TRAP-positive cells did not form in the absence of $1,25(OH)_2D_3$ (Fig. 4b). Moreover, the osteoblasts expressed RANKL, an osteoclast differentiation factor, when incubated with OCP [31]. These results demonstrated that OCP is capable of inducing osteoclast formation by activating osteoblasts *in vitro* [31]. In order to examine osteoclast attachment onto the OCP surface, mature osteoclasts were formed from co-cultures in the presence of $1,25(OH)_2D_3$ and then placed onto OCP-coated plates (Fig. 4c). Actin filament formation was observed in

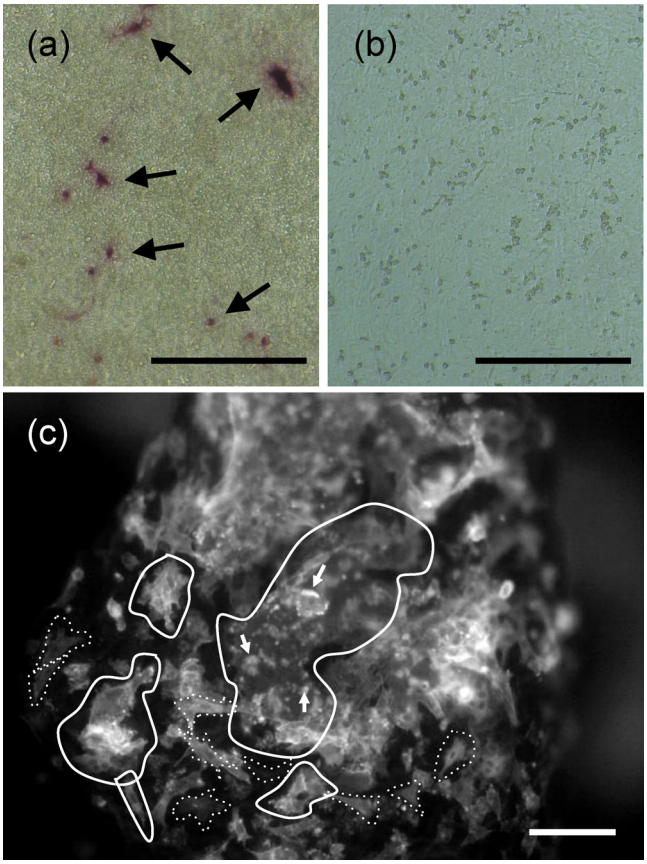

Figure 4 Observation of osteoclastic cells on OCP-coated plates. Co-cultures of mouse bone marrow cells and osteoblastic cells with OCP (a) and without OCP (control experiment) (b) for 6 days, showing formation of TRAP-positive osteoclastic cells (arrows) only if incubated with OCP (a). These co-cultures were grown in the absence of $1,25(OH)_2D_3$, which is an essential factor for up-regulating RANKL. Mature osteoclasts were placed on OCP-coated plates and further incubated for 24 h (c). The circled solid lines indicate osteoclastic cells developing actin filaments (arrows) on OCP. The circled dotted lines indicate osteoblastic cells that are most likely present underneath other cells. The actin filaments were labeled with rhodamine-conjugated phalloidin. The details of the experiments have been reported in Takami et al. [31]. Bars = 100 μm (a and b) and 50 μm (c).

osteoclasts grown on OCP, indicating that osteoclasts are relatively firmly attached onto the OCP surface. Together, these results suggest that OCP is a material that stimulates cells, and in particular osteoblastic cells, to enhance new bone formation by osteoblasts and its own biodegradation by osteoclasts, which is advantageous in the physiological bone remodeling process [15].

7. Possible mechanism of OCP-stimulated bone regeneration

One possible mechanism of OCP-stimulated bone regeneration is summarized in Fig. 5, which is hypothesized based on experimental evidence. The biological responses of OCP both *in vitro* and *in vivo* were compared with the OCP hydrolyzate prepared by the hydrolysis of the original OCP in hot water [30]. OCP hydolyzate had a Ca/P molar ratio 1.46 compared to the stoichiometric 1.67 of HA but showed single HA phase in its structure. OCP hydrolyzate, namely Ca-deficient HA, maintained the original plate-like OCP morphology even after the hydrolysis. From these material characteristics, Ca-deficient HA obtained *via* OCP, would be a veritable control material to investigate as to how OCP responds to osteoblastic cells or bone tissues [30]. OCP implanted in rat calvaria defect was progressively converted to apatitic phase as observed previously in the implantation onto mouse calvaria [19]. OCP enhanced the

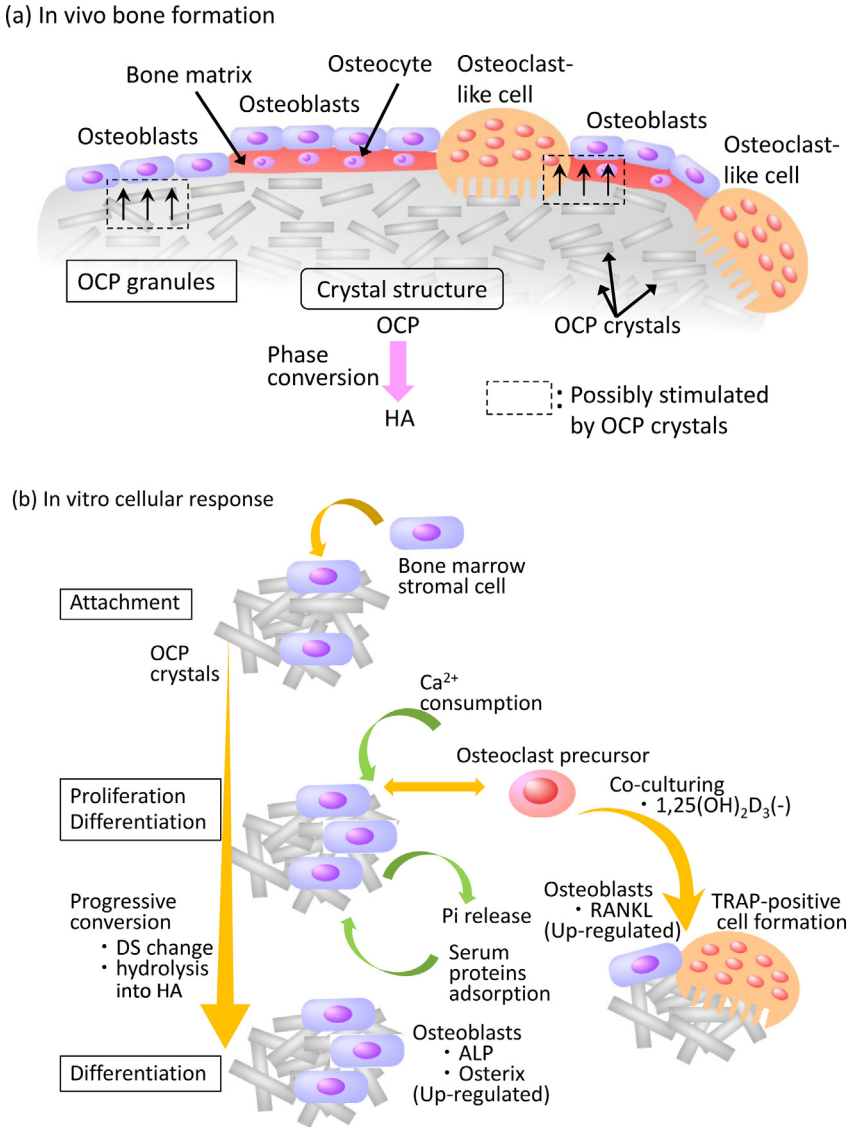

Figure 5 Schematic view of bone formation by OCP-based *in vivo* observations (a) and the possible mechanism for induction of osteoblastic differentiation and osteoclast formation by OCP crystals based on *in vitro* experimental findings (b). Osteoblastic differentiation is enhanced during a process of OCP—HA conversion. The up-regulation of osteoblast differentiation markers, such as alkaline phosphate (ALP) and osterix, is induced by OCP crystals. Osteoclast formation is induced by co-culturing the cells with osteoblastic cells and bone marrow cells even in the absence of $1,25(OH)_2D_3$. The up-regulation of RANKL in osteoblasts is induced by the OCP crystals. The process of OCP to HA conversion occurs progressively but very slowly, and induces physicochemical changes, including Ca^{2+} consumption and inorganic phosphate (Pi) ions release as well as elevated adsorption affinity of serum proteins, such as α2HS-glycoproteins.

bone regeneration in rat calvaria defect significantly more than OCP hydrolyzate did. OCP tended to enhance osteoblastic cell differentiation more than OCP hydrolyzate *in vitro* [30]. The effect of which was confirmed later in quantitative analysis of the expression of osteoblast differentiation markers [20]. As explained in Fig. 1, bone formation by OCP granule implantation is usually accompanied with bone matrix synthesis by osteoblasts and biodegradation of OCP by osteoclast-like cells [23,25,30,38,46]. It is highly probable that the osteoblasts that are attached onto the OCP granule surface may be stimulated by each OCP crystal, which forms an aggregate of the granules (Fig. 5a). Fig. 5b summarizes the mechanism of OCP-stimulated bone formation. Bone marrow stromal cells attach onto OCP crystals and proliferate [30,59], and the OCP crystals enhance osteoblastic differentiation [20,30]. Osteoclast formation from adjacent bone marrow osteoclast precursor cells is also induced by osteoblasts due to the OCP-induced up-regulation of RANKL [31]. These cellular responses advance during the OCP conversion into HA [20,30]. The conversion process induces physicochemical alterations around the OCP crystals, including ionic exchanges of Ca^{2+} and Pi ions [20,59], with the change of DS value, as well as serum protein adsorption [48,92]. However, the osteoconductivity of OCP is remarkably controlled by the stoichiometry (a variety of chemical composition) of OCP [46] and the crystal microstructure [81]. Non-stoichiometric OCP, having a Ca/P molar ratio of 1.37, which is a slightly higher Ca/P molar ratio compared to the stoichiometric 1.33 and a product generated from the early stage of the OCP hydrolysis in an experimental hot water incubation, significantly increases the osteoconductivity of the original OCP [30,46]. In contrast, the large OCP crystals, which grow toward the long axis of the crystals, markedly suppress the osteoconductivity of OCP [81].

8. OCP-based materials

There are two aspects to consider when preparing composite materials with OCP: the moldability and increasing the osteoconductivity of OCP [51,93,94]. OCP-based materials developed to date have considered these aspects [51,93,94]. Due to the inclusion of a large number of water molecules in the structure [18,95,96], the phase of OCP cannot be maintained during sintering, unlike HA or β-TCP ceramics. Therefore, combining OCP with other materials, such as polymers, are required to form larger three-dimensional implant bodies. The second aspect to be considered is how well the OCP crystals are dispersed within the matrix materials, which may increase the number of sites available for bone development initiation, resulting in enhancement of bone regeneration [51,97]. Although several studies have shown that OCP coating on titanium or titanium alloy raises the osteoconductivity of the original metal surfaces [21,74,98–102], the use of OCP-based composite materials is favorable from the view point of the biodegradation coupled with new bone formation, even in implants with a large volume used for larger bone defects. In regard to the stability of OCP-based materials, including the stability of the OCP crystal itself, the characteristics of the materials can be maintained as long as stored under drying condition and even after the various sterilizations, such as electron beam irradiation [20,47,75,103].

8.1. OCP—gelatin composite

Composite materials composed of OCP and gelatin (Gel) molecules have been recently developed through the co-precipitation of OCP together with various concentrations of Gel molecules [51]. Gel is a random coiled molecule that is derived from denatured collagen [104]. Gel preserves a cellular attachment motif [105], and reconstituted materials are extensively used in biomedical applications [106], including scaffolds [107,108]. Gel materials are known to be highly biodegradable compared to collagen [104]. This is because collagen biodegrades into telopeptides through decomposition to Gel molecules [104]. OCP/Gel composites containing OCP up to 40 wt% were obtained [51]. After cross-linking of the Gel matrix in the composites through dehydrothermal treatment, the resulting composite materials were highly porous and contained homogenously dispersed OCP [51]. The OCP crystals elongated toward the long axis were found to be closely associated with the Gel matrix [51]. Fig. 6a shows an example of an OCP/Gel composite (40 wt% of OCP) molded as rod-like implants. Scanning electron microscopy (SEM) showed that the OCP/Gel composite had pores that were approximately 500 μm in diameter (Fig. 6b); however, mercury intrusion porosimetry determined the pore size to be in the range of 10—500 μm in diameter [51]. Importantly, a rat calvaria critical-sized defect that was experimentally created with a 9 mm diameter and not repaired spontaneously was sufficiently repaired by the implantation of the OCP/Gel composite (40 wt% of OCP; 9 mm in diameter and 1 mm thick) after 16 weeks [51]. Fig. 6c shows the soft x-ray photograph with a highly radiopacity within the defect corresponding to new bone formation. Histomorphometric analysis revealed that the newly formed bone area was estimated to be 71% of the defect area [51], which is close to the value attained by autograft (85% of the defect area) [109] or implantation of a chitosan gel composite seeded with mesenchymal stem cells (MSCs) and bone morphogenetic proteins (BMP-2) (80% of the defect area) in similar critical-sized calvaria defects [110]. Thus, the OCP/Gel composite is a material that efficiently repairs intramembranous bone defects [51]. The efficiency of the OCP/Gel composite for repairing a long bone defect (4 mm diameter in rabbit tibia) [97], which is frequently used as an orthopedic bone defect model, was also assessed. Although the control group (defect only) was not sufficiently bridged by the repaired bone (Fig. 6d), the implantation of OCP/Gel (40 wt% of OCP; 4 mm in diameter and 5 mm thick) induced new bone formation that was qualitatively better than the control group 2 weeks after the implantation into the defect (Fig. 6d and e). In addition, the OCP/Gel composite appeared to almost completely biodegrade (Fig. 6e). Based on these findings, it seems likely that the OCP/Gel composite is a material that potentially has regenerative capabilities that approach those of autologous bone.

8.2. OCP—collagen composite

Re-constituted collagen (Col) is also a cell-attaching matrix protein, and therefore its spongy nature has been widely used as scaffolding material, often in combination with calcium phosphates [8,39,94,111—115]. Nano-HA crystals deposited on self-assembled Col fibril-based HA/Col composites have been developed as a bone tissue-mimicking material [39] that displays excellent bone regenerative and biodegradable

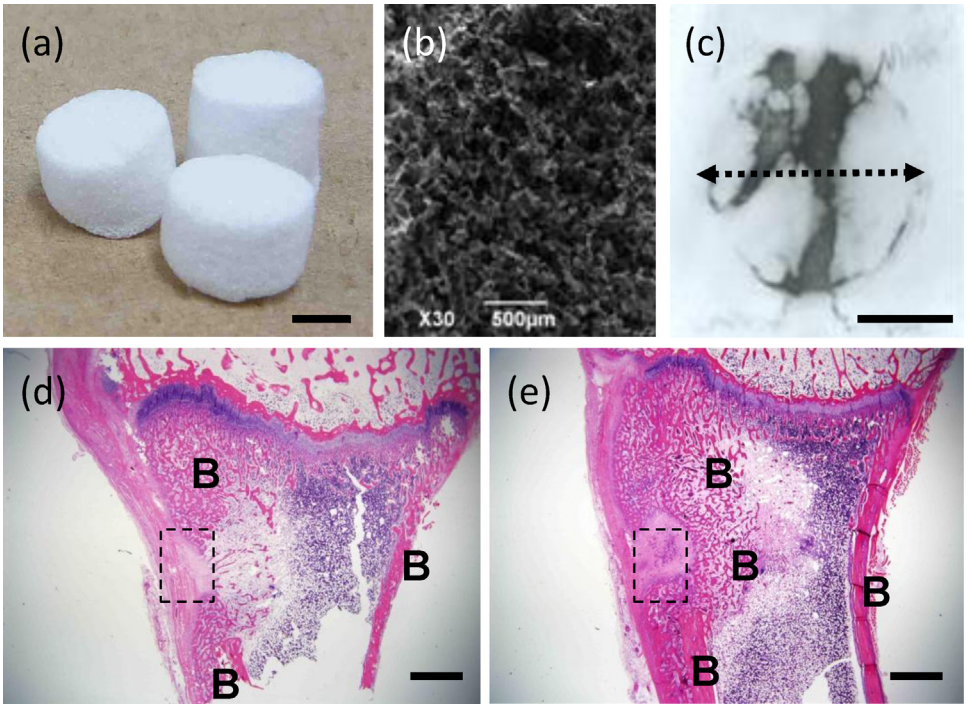

Figure 6 Examples of bone regenerative properties of an OCP-based material consisting of OCP-precipitated gelatin (Gel) molecules (OCP/Gel composite) in bone defects. (a) Rod-like OCP/Gel composites; (b) SEM observation of an OCP/Gel composite showing porous characteristics; (c) soft X-ray photograph of rat critical-sized calvaria defects at 16 weeks after the implantation of an OCP/Gel disk having a 9 mm diameter and 1 mm thickness, showing high radiopacity, indicating active bone regeneration; (d) control group (defect only) and (e) experimental group (OCP/Gel implantation) of decalcified, H&E stained histological sections of an OCP/Gel composite having a 4 mm diameter and 5 mm thickness, implanted in the intramedullary canal of a rabbit tibia at 2 weeks. The section shows remarkable bone repair in the OCP/Gel implantation group (e) and markedly less bone formation in the control group. The details of the experiments have been reported in Handa et al. [51] and Suzuki et al. [97]. The arrow (shown by a dotted line) in (c) indicates the defect created before repair. Bars = 4 mm (a), 500 μm (b), 4 mm (c), and 3 mm (d and e). All procedures in the experiment were approved by the Animal Research Committee of Tohoku University.

properties [8,39]. The effect of the OCP and Col composite on repairing bone tissue has been previously reported for rabbit long bone defects [113]. Recently, the effect of an OCP/Col composite consisting of OCP granules, which have been shown to have osteoconductive properties as described above, and porcine dermis-derived atelo-Col, has been assessed in intramembranous bone defects in the field of oral surgery using critical-sized defects in different animal models, such as rat and dog [94,103,116]. The disk-shaped sponge of the OCP/Col (77 wt% of OCP) material enhanced bone regeneration more than the OCP granules alone [94], indicating the synergistic effect of the Col matrix, although the disk of Col alone did not enhance bone formation [94]. The OCP/Col composite increased the bone regenerative properties in an OCP dose-dependent manner from 23 wt% to 83 wt% [117] and OCP granule size-dependent manner from 53—300 μm to 500—1000 μm [83] in rat calvaria defects. Moreover, the OCP/Col (77 wt% of OCP) composite seeded next to rat bone marrow-derived MSCs further enhanced bone regeneration in a rat calvaria defect [118]. In contrast, mechanical stress loaded onto the OCP/Col material induced excessive osteoclastic resorption of its own materials, resulting in a complete resorption of the materials without bone regeneration [119]. However, alleviation of the mechanical stress using a stress-shielding support material restored the bone regenerative property of OCP/Col [120]. Therefore, OCP/Col is a material that has greater bone regenerative properties than OCP alone due to the synergistic effect of the Col matrix.

8.3. OCP—alginate composite

Alginate (Alg) is well-recognized as a useful hydrogel material for various cellular encapsulations because Alg molecules do not provide cellular-attaching sites, unlike Gel and Col molecules, and therefore the cells are not induced to differentiate [121,122]. OCP/Alg composites were developed using OCP-co-precipitated Alg molecules [93]. The pore size of the composites ranging from 6 to 52 μm markedly affected *in vivo* bone regeneration of mouse critical-sized calvaria defects and *in vitro* osteoblastic cell attachment [93]. Although the effect of seeding stem cells with the OCP/Alg composite has not been examined as yet, the effect of OCP crystals in an Alg matrix, if combined with stem cells such as MSCs, is of great interest in the tissue engineering field.

8.4. Characteristics of OCP-based materials compared to calcium phosphate cement materials

The crystals of HA converted from the implanted OCP onto rat calvaria in the form of OCP/Col composite have been

attributed to be a biomimetic carbonate-containing apatite crystals [119]. Although the capability of OCP to form osteoclasts from the precursor cells on the surface [31] could be the primary cause to enhance the biodegradation of OCP [23], the formation of carbonated-HA from OCP *in vivo* may strengthen the material replacement with newly formed bone [8,42,43]. Various injectable and osteoconductive calcium phosphate cements have been developed and reported to be effective in enhancing bone formation if putting them into bone defects [4,123,124]. A cement mixing tetracalcium phosphate (TTCP) with DCPA can be hardened *in vivo* as carbonated-HA, resulting in becoming to show the biodegradable property [125]. Brushite-forming cements, originated from β-TCP and monocalcium phosphate monohydrate (MCPM) or phosphoric acid, are also recognized as osteoconductive and biodegradable materials which are slowly converted to carbonated-HA [126,127]. The calcium phosphate cement materials that contain OCP phase in its composition have also been developed from the mixture of α-TCP and DCPA [128,129] or α-TCP, MCPM, calcium carbonate and phosphate solution [130,131] as filling agents for the defects in bone and tooth. Our previous studies suggest that the biological activity of OCP is induced during a physicochemical OCP—HA conversion process [19,30] in particular at the early stage of the conversion in OCP [46]. However, further study is required to elucidate the precise mechanism concerning the osteoconductivity induced in the present OCP-based materials in relation to the osteoconductivity observed in other calcium phosphate cement materials.

9. Conclusion

It is clear that OCP exhibits a stimulatory effect on the activity of osteoblasts during the conversion into HA in physiological environments [20,30,31]. However, the osteoconductive properties of OCP crystals vary greatly depending on the OCP preparations [49]. This may be due to variation in the stoichiometry [46] and the crystal morphological features [81] of OCPs that are obtained in the preparations, which most likely occurs through controlled crystal growth and the tendency for slight hydrolysis during the preparation based on the condition used [19,26,132—135]. Although the osteoconductive property of OCP is highly dependent on such physicochemical properties of the crystals that are obtained, OCP-based materials, if prepared in well-controlled conditions, could be good candidates for an advanced material that approaches to autologous bone.

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