35th Annual International Conference of the IEEE EMBS
Osaka, Japan, 3 - 7 July, 2013

Synthetic octacalcium phosphate: a possible carrier for mesenchymal stem cells in bone regeneration

Osamu Suzuki and Takahisa Anada

Abstract— **The present paper reviews biomaterial studies of synthetic octacalcium phosphate (OCP) as a scaffold of osteoblastic cells. OCP crystals have been suggested to be one of precursor phases in hydroxyapatite (HA) crystal formation in bone and tooth. The recent intensive biomaterials and tissue engineering studies using synthetic OCP disclosed the potential function of OCP as a bioactive material as well as synthetic HA materials due to its highly osteoconductive and biodegradable properties. In vitro studies showed that OCP crystals exhibit a positive effect on osteoblastic cell differentiation. In vivo studies confirmed that the materials of OCP in a granule forms and OCP-based composite materials with natural polymers, such as gelatin and collagen, enhance bone regeneration if implanted in various model bone defects with critical-sized diameters, defined as a defect which does not heal spontaneously throughout the lifetime of the animals. One of particular characteristics of OCP, found as a mechanism to enhance bone regeneration in vivo, is a process of progressive conversion from OCP to HA at physiological conditions. The OCP-HA conversion is accompanied by progressive physicochemical changes of the material properties, which affects the tissue reaction around the crystals where osteoblastic cells are encountered. Mesenchymal stem cells (MSCs) seeded in an OCP-based material enhanced bone regeneration in the rat critical-sized calvaria defect more than that by the material alone. The overall results reveal that OCP crystals have an effect on osteoblastic cell differentiation including the differentiation of MSCs in vivo. The evidence collected experimentally in the laboratory was presented.**

I. Introduction

Octacalcium phosphate (OCP; $Ca_8H_2(PO_4)_6 \cdot 5H_2O$) is one of precursor phases in hydroxyapatite (HA; $Ca_{10}(PO_4)_6(OH)_2$) formation from supersaturated calcium and phosphate solutions in vitro [1]. From this, OCP has been advocated as a precursor of apatite crystals in bone mineralization in vivo [2]. Recently, this calcium phosphate, if synthesized in a specified condition [3-5], has become recognized as one of osteoconductive and biodegradable material as a synthetic bone substitute if implanted in experimentally created various animal bone defects [6-8]. The rationale for the use of OCP crystals in the bone substitute materials have been well presented by in vitro studies that OCP crystals enhance osteoblast differentiation with the increase of expression of the marker genes, such as alkaline phosphatase (ALP) and osterix [9], and induce osteoclast formation from co-culturing of bone marrow cells (osteoclast precursor cells) and osteoblasts with the increase of expression of RANKL in osteoblasts [10] which is a osteoclast inducing factor. OCP tends to convert to HA in vivo [3,6] as well as in vitro. The process of OCP-HA conversion induces the physicochemical properties of the crystals, such as the solubility and protein adsorption on them [11-13]. The OCP-HA conversion is closely associated with the stimulatory capacity of OCP since the HA obtained from OCP hydrolysis in vitro experimental solution does not show any stimulatory response and therefore does not enhance bone formation [6]. These evidence obtained encouraged us to use OCP crystals as a scaffold for exogenous osteoblastic cells in bone regeneration. The osteocondutivity of OCP has been reported in a study by its implantation onto mouse calvaria for the first time [3]. OCP is classified as a bioactive and osteoconductive ceramic material [14] as well as bone bonding materials, such as bioglass [15], glass-ceramic A-W [16], HA [17,18] and β-tricalcium phosphate (β-TCP) [19].The present paper summarizes and reviews the property of OCP and OCP-based materials as bone substitute materials and also as scaffold materials for introducing osteoblastic cells, such as mesenchymal stem cells (MSCs) in bone tissue engineering.

*Resrach supported by part by grants-in-aid (17076001, 19390490, 23390450, 23659909, and 23106010) from the Ministry of Education, Science, Sports, and Culture of Japan.

Osamu Suzuki is with the Division of Craniofacial Function Engineering, Tohoku University Graduate School of Dentistry, Sendai 980-8575, JAPAN (phone: +81-22-717-7635; fax: suzuki-o@m.tohoku.ac.jp; e-mail: suzuki-o@m.tohoku.ac.jp).

Takahisa Anada is with the Division of Craniofacial Function Engineering, Tohoku University Graduate School of Dentistry, Sendai 980-8575, JAPAN (e-mail: anada@m.tohoku.ac.jp).

II. OCP and OCP based Materials

A. OCP

OCP is a calcium phosphate which has a triclinic crystal system and a theoretical Ca/P molar ratio 1.33 [1]. We have synthesized more than one kind of OCP with distinct non-stoichiometric compositions, ranging from Ca/P molar ratio 1.23 to 1.37, and distinct crystallinity [4]. These OCP crystals had different osteconductivity: an OCP with Ca/P molar ratio 1.37 showed highest bone formation rate in a comparative study in which the granules of OCP and its hydrolyzed apatitic product with Ca/P molar ratio 1.48 were implanted in rat tibia defect [4]. The microstructure of OCP crystals is another factor to control the osteoconductivity of OCP [5]. OCP exhibits a plate-like morphology in general and tends to grow toward the long axis of the crystals depending on the preparation conditions in the syntheses [7]. The hydrolysis of OCP produces HA without any phases other than OCP but tends to form non-stoichiometric Ca-deficient HA with lower Ca/P molar ratios than the stoichiometric ratio [6,12]. Table 1 summarizes the structure estimated by X-ray diffraction (XRD) and Ca/P molar ratios of OCP and its hydrolyzates prepared.

TABLE I. SYNTHETIC OCTACALCIUM PHOSPHATE AND HYDROXYAPATITE CRYSTLAS

Crystals	Abbreviation	Phase by XRD	Ca/P molar ratio
Octacalcium phosphate	OCP, stoichiometric	OCP	1.33[1)]
Octacalcium phosphate	OCP, non-stoichiometric	OCP	1.23-1.37[3-7)]
Octacalcium phosphate, hydrolyzate	OCP hydrolyzate	HA	1.48 [6,12)]

B. OCP Based Materials

OCP composites with natural polymers, such as gelatin [20], collagen [21] and alginate [22], were prepared for the use of bone substitute materials and scaffolds for seeding the osteoblastic cells. OCP has a large amount of water molecules in the structure [1] therefore cannot be sintered without decomposing its original crystal structure with increasing the temperature, unlike sintered ceramics, such as HA materials. OCP becomes moldable by combining with the polymer materials into the desired shapes. Table 2 summarizes the OCP composites prepared in the laboratory and the osteoblastic cells investigated with the composites. Porcine dermis derived acid extracted gelatin and collagen were selected as cell attaching matrices to form the OCP composites. Although alginate is not cell attaching matrix, it was used as a matrix for the OCP composite, because alginate in hydrogel form has been extensively used as a cell containing material that the cells included is expected to deliver to the objective site in the tissue repair [23,24]. OCP/gelatin (OCP/Gel) and OCP/Alginate (OCP/Alg) composites were prepared through co-precipitation [20,22]. Briefly, OCP was precipitated trough a wet method previously reported [3] but in the presence of various concentrations of gelatin or alginate molecules.

TABLE II. OCP COMPOSITES PREPARED AND OSTEOBLASTIC CELLS

Composites	Preparation	Cells examined in vitro
OCP/Gelatin [20)] (OCP/Gel)	Co-precipitation with OCP crystals	Mouse bone marrow stromal ST-2 cells
OCP/Alginate [22)] (OCP/Alg)	Co-precipitation with OCP crystals	Mouse bone marrow stromal ST-2 cells
OCP/Collagen [28)] (OCP/Col)	Mixing with OCP granules	Rat bone marrow cells

OCP/Collagen (OCP/Col) was made by mixing the OCP, synthesized solely and then prepared into a granule form, having 300 to 500 μm in diameter, with atelo-collagen [21]. These composites were molded, lyophilized and then cross-linked dehydrothermally (in gelatin and collagen composites). The disks of the composites were made respectively and further used for in vitro osteoblastic cell seeding or in vivo implantation experiments of bone regeneration. These composites were spongy forms and showed porous structure, the characteristics of which were brought about by the polymer matrices although OCP granules were also porous in nature due to the lose aggregation of the plate-like OCP crystals.

III. BONE TISSUE AND CELLULAR RESPONSES TO OCP COMPOSITES

A. OCP/Gel Composite

The osteoconductive property of OCP/Gel composites were examined in rabbit tibia bone defect [25] and in critical-sized defect of rat calvaria [20]. Both defects were well repaired by the implantation of the OCP/Gel composites. In the rabbit tibia defect, while the control defect was not repaired sufficiently, the implanted group enhanced cortical bone regeneration over the defect even at 2 weeks after the implantation [25]. OCP/Gel composite almost biodegraded therefore the composite was not observed anymore histologically. The osteconductive and the biodegradable properties of OCP/Gel composite were reproduced in the rat critical-sized calvaria defect that the regeneration rate over 70% and the remnant rate approximately only 3% were recorded histomorphometrically at 16 weeks after the implantation [20]. Mouse bone marrow derived ST-2 cells were well invaded in OCP/Gel disks in vitro study even after one day of the seeding. 60 to 70% of the total cells incubated were attached onto the composite [20]. The results suggest that OCP/Gel composite may be a bone substitute material that the host osteoblastic cells can easily be invaded resulting in the enhancement of bone regeneration stimulated by OCP crystals within the gelatin matrix [20, 25].

B. OCP/Alg Composite

The osteoconductive property of OCP/Alg composite was examined in critical sized defect of mouse calvaria [22]. In spite of the cell non-attaching matrix, the implantation of OCP/Alg composite regenerated new bone throughout the calvaria defect in accordance with the composite biodegradation until 3 weeks. The control alginate alone implantation did not induce any bone formation within the same periods. ST-2 cells were also well attached in OCP/Alg disks in vitro study even after 3 days of the seeding. However, the attachment rate increased markedly depending on the pore size of the composites that was made in the material preparation through distinct centrifugation from 3,000 to 15,000 rpm of the composites at the time of the cross-linking in the presence of calcium ions [22]. The results suggest that OCP/Alg composite may be a bone substitute material that the host osteoblastic cells can easily be invaded resulting in the enhancement of bone regeneration stimulated by OCP crystals within the alginate matrix [22].

C. OCP/Col Composite

The osteoconductive property of OCP/Col composite was examined in the calvaria critical size defects in rat and dog [21,26,27]. The composite consisted from OCP granules and collagen matrix enhanced bone regeneration in the rat calvaria defect more than OCP granules alone [21]. The bone regeneration rate increased with increasing the content of OCP in collagen matrix [26]. The dose-dependent stimulatory capacity of OCP in OCP/Col was identical to the previous

results obtained in vitro that OCP crystals enhance osteoblastic differentiation in a dose-dependent manner of OCP [9]. The osteoconductivity of this materials was also reproduced in the dog calvaria defect [27], showing the bone bridge across the defect with 20mm in diameter.

IV. OCP/Col Composite with Mesenchymal Stem Cells

Since OCP crystals reveal the stimulatory effect on osteoblastic cell differentiation, we examined the effect on the differentiation of MSCs [28]. Bone marrow derived cells isolated from Wistar rat long bones were pre-incubated in osteogenic medium in the presence of basic fibroblast growth factor (bFGF). The cells, expressing high alkaline phosphatase activity and therefore assigned as MSCs, were seeded on OCP/Col disks for an additional day. The disks of OCP/Col/MSCs were implanted in the critical-sized calvaria defects of 12-weekold male Wistar rats. The calvaria retrieved at 4 and 8 weeks were analyzed radiographically and histomorphometrically for the effect of MSCs within OCP/Col on bone regeneration. The radiographic analysis showed that while the control untreated group did not induce any radiopacities, the OCP/Col/MSCs implantation group increased them significantly (Fig.1). Because the OCP/Col/MSCs enhance significantly bone regeneration more than OCP/Col alone in the defects, it was apparent that the differentiation of MSCs and subsequent bone regeneration by the MSC are enhanced by the presence of OCP crystals.

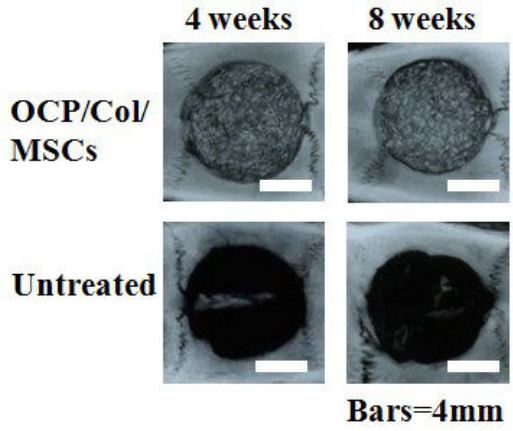

Figure 1. Radiographs of rat calvaria defect implanted by OCP/Col/MSCs and untretaed (cntrol experiment) at 4 and 8 weeks. Reproduced with kind permission from eCM journal, Figure 3 (modified) in Kawai T et al., "The effect of synthetic octacalcium phosphate in a collagen scaffold on the osteogenicity of mesenchymal stem cells, " Eur. Cell. Mater., vol.22, pp.124-136, 2011 [28].

V. Conclusion

The present review paper summarizes the characteristics of OCP based materials as scaffolds to seed the osteoblastic cells for bone tissue engineering. Recent our studies further disclosed that OCP crystals promote odontoblast differentiation [29] and have an influence on chondrogenic differentiation [30]. Based on our experimental evidence, the use of OCP materials could be recommended as the scaffolds for the cell seeding especially for the hard tissue forming cells, such as osteoblastic cells, including MSCs [28]. However, further study regarding the crystal chemistry of OCP is required for suitable use in the cell scaffolding since the physicochemical properties of OCP crystals regulate in essence the osteoconductive properties in vivo [4,5,7].

Acknowledgment

This study was supported in part by grants-in-aid (17076001, 19390490, 23390450, 23659909, and 23106010) from the Ministry of Education, Science, Sports, and Culture of Japan, the Uehara Memorial Foundation, the Suzuken Memorial Foundation, and Iketani Science and Technology Foundation.

References

[1] W.E. Brown, M. Mathew, M.S. Tung, "Crystal chemistry of octacalcium phosphate," Prog. Cryst. Growth Charact. vol.4, pp.59-87, 1981.

[2] W.E. Brown, "Crystal growth of bone mineral," Clin. Orthop. Relat. Res. vol.44, pp.205-220, 1966.

[3] O. Suzuki, M. Nakamura, Y. Miyasaka, M. Kagayama, and M. Sakurai, "Bone formation on synthetic precursors of hydroxyapatite," Tohoku J. Exp. Med., vol.164, pp.37-50, 1991.

[4] N. Miyatake, K.N. Kishimoto, T. Anada, H. Imaizumi, E. Itoi, and O. Suzuki, " Effect of partial hydrolysis of octacalcium phosphate on its osteoconductive characteristics," Biomaterials vol.30, pp.1005-1014, 2009.

[5] Y. Honda, T. Anada, S. Kamakura, S. Morimoto, T. Kuriyagawa, and O. Suzuki, "The effect of microstructure of octacalcium phosphate on the bone regenerative property," Tissue Eng. Part A, vol.8, pp.1965-1973, 2009.

[6] O. Suzuki, S. Kamakura, T. Katagiri, M. Nakamura, B. Zhao, Y. Honda, and R. Kamijo, "Bone formation enhanced by implanted octacalcium phosphate involving conversion into Ca-deficient hydroxyapatite," Biomaterials, vol.27, pp.2671-2681, 2006.

[7] O. Suzuki, "Octacalcium phosphate: osteoconductivity and crystal chemistry," Acta Biomater., vol.6, pp.3379-3387, 2010.

[8] O. Suzuki, "Octacalcium phosphate (OCP)-based bone substitute materials," Jpn. Dent. Sci. Rev., 2013 in press (10.1016/j.jdsr.2013.01.001).

[9] T. Anada, T. Kumagai, Y. Honda, T. Masuda, R. Kamijo, S. Kamakura, N. Yoshihara, T. Kuriyagawa, H. Shimauchi, and O. Suzuki, "Dose-dependent osteogenic effect of octacalcium phosphate on mouse bone marrow stromal cells," Tissue Eng. Part A, vol.14, pp. 965-978, 2008.

[10] M. Takami, A. Mochizuki, A. Yamada, K. Tachi, B. Zhao, Y. Miyamoto, T. Anada, Y. Honda, T. Inoue, M. Nakamura, and O. Suzuki, and R. Kamijo, "Osteoclast differentiation induced by synthetic octacalcium phosphate through RANKL expression in osteoblasts," Tissue Eng. Part A, vol.15, pp. 3991-4000, 2009.

[11] O. Suzuki, S. Kamakura and T. Katagiri, "Surface chemistry and biological responses to synthetic octacalcium phosphate," J. Biomed. Mater. Res. Appl. Biomater., vol.77B, pp.201-212, 2006.

[12] O. Suzuki, H. Yagishita, M. Yamazaki, and T. Aoba, "Adsorption of bovine serum albumin onto octacalcium phosphate and its hydrolyzates," Cells Mater., vol. 5:45-54, 1995.

[13] Y. Shiwaku, T. Anada, H. Yamazaki, Y. Honda, S. Morimoto, K. Sasaki, and O. Suzuki, "Structural, morphological and surface characteristics of two types of octacalcium phosphate-derived fluoride-containing apatitic calcium phosphates," Acta Biomater., vol. 8, pp.4417-4425, 2012.

[14] A. Oyane, X. Wang, Y. Sogo, A. Ito, and H. Tsurushima, “Calcium phosphate composite layers for surface-mediated gene transfer,” Acta Biomater., vol.8, pp.2034-2046, 2012.
[15] L.L. Hench, R.J. Splinter, W.C. Allen, and T.K. Greenlee, “Bonding mechanisms at the interface of ceramic prosthetic materials,” J. Biomed. Mater. Res., vol.2, pp.117–141, 1971.
[16] T. Kokubo, M. Shigematsu, Y. Nagashima, T. Tashiro, T. Nakamura, T. Yamamuro, et al., “Apatite and wollastonite containing glass-ceramics for prosthetic application,” Bull. Inst. Chem. Res. Kyoto Univ., vol. 60, pp.260–268, 1981.
[17] H. Aoki, “Science and medical applications of hydroxyapatite,” Tokyo: Takayama Press System Center Co. Inc., 1991.
[18] M. Jarcho, J.F. Kay, K.I. Gumaer, R.H. Doremus, and H.P. Drobeck, “Tissue, cellular and subcellular events at a bone-ceramic hydroxylapatite interface,” J. Bioeng . vol.1, pp.79–92, 1977.
[19] S. Kotani, Y. Fujita, T. Kitsugi, T. Nakamura, T. Yamamuro, C. Ohtsuki, and T. Kokubo, “Bone bonding mechanism of b-tricalcium phosphate,” J. Biomed. Mater. Res. vol.25, pp.1303–1315, 1991.
[20] T. Handa, T. Anada, Y. Honda, H. Yamazaki, K. Kobayashi, N. Kanda, S. Kamakura, S. Echigo, and O. Suzuki, “The effect of an octacalcium phosphate co-precipitated gelatin composite on the repair of critical-sized rat calvarial defects,” Acta Biomater., vol.8, pp.1190-1200, 2012.
[21] S. Kamakura, K. Sasaki, Y. Honda, T. Anada, and O. Suzuki, “Octacalcium phosphate combined with collagen orthotopically enhances bone regeneration,” J. Biomed. Mater. Res. B Appl. Biomater, vol.79, pp.210-217, 2006.
[22] T. Fuji, T. Anada, Y. Honda, Y. Shiwaku, H. Koike, S. Kamakura, K .Sasaki, and O. Suzuki, “Octacalcium phosphate-precipitated alginate scaffold for bone regeneration,” Tissue Eng. Part A, vol.15, pp. 3525-3535, 2009.
[23] C.C. Barrias, M. Lamghari, P.L. Granja, M.C. Sa Miranda, and M.A. Barbosa, “Biological evaluation of calcium alginate microspheres as a vehicle for the localized delivery of a therapeutic enzyme,” J. Biomed. Mater. Res. A, vol.74, pp.545-552, 2005.
[24] H.J. Kong, M.K. Smith, and D.J. Mooney, “Designing alginate hydrogels to maintain viability of immobilized cells,” Biomaterials, vol. 24: pp.4023-4029, 2003.
[25] K. Suzuki, Y. Honda, T. Anada, T. Handa, N. Miyatake, A. Takahashi, M. Hosaka, H. Imaizumi, E. Itoi, and O. Suzuki, “Stimulatory capacity of an octacalcium phosphate/gelatin composite on bone regeneration in rabbit tibia defect model,” Phosphorus Res. Bull. vol.26 (Special Issue), pp. 53-58, 2012.
[26] T. Kawai, T. Anada, Y. Honda, S. Kamakura, K. Matsui, A. Matsui, K. Sasaki, S. Morimoto, S. Echigo, and O. Suzuki, “Synthetic octacalcium phosphate augments bone regeneration correlated with its content in collagen scaffold,” Tissue Eng. Part A, vol.15, pp.23-32, 2009.
[27] T. Kawai, K. Matsui, S. Iibuchi, T. Anada, Y. Honda, K. Sasaki, S. Kamakura, O. Suzuki, and S. Echigo, “Reconstruction of critical-sized bone defect in dog skull by octacalcium phosphate combined with collagen,” Clin. Implant Dent. Relat. Res., vol.13, pp.112-123, 2011.
[28] T. Kawai, T. Anada, T. Masuda, Y. Honda, Y. Sakai, Y. Kato, S. Kamakura, S. Echigo, and O. Suzuki, “The effect of synthetic octacalcium phosphate in a collagen scaffold on the osteogenicity of mesenchymal stem cells,” Eur. Cell. Mater., vol.22, pp.124-136, 2011.
[29] X. Wang, T. Suzawa, T. Miyauchi, B. Zhao, R. Yasuhara, T. Anada, N. Nakamura, O. Suzuki, and R. Kamijo. “Synthetic Octacalcium phosphate enhanced reparative dentin formation via induction of odontoblast differentiation,” J. Tissue Eng. Regen. Med., 2013 in press (doi.10.1002/term.1669).
[30] I. Shibuya, K. Yoshimura, Y. Miyamoto, A. Yamada, M. Takami, T. Suzawa, D. Suzuki, N. Ikumi, F. Hiura, T. Anada, O. Suzuki, and R. Kamijo, “Octacalcium phosphate suppresses chondrogenic differentiation of ATDC5 cells,” Cell. Tissue Res., 2013 in press (doi.10.1007/s00441-012-1548-8).