Chapter 10
Efficacy of Calcium Phosphate-Based Scaffold Materials on Mineralized and Non-mineralized Tissue Regeneration

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Abstract Calcium phosphate materials have been advocated as useful implantable scaffolds for bone tissue engineering. We have reported that synthetic octacalcium phosphate (OCP) is capable of enhancing differentiation of hard tissue-forming cells including osteoblastic cells, osteoclast precursor cells, and odontoblastic cells. The differentiation of mesenchymal stem cells is promoted to form new bone in the presence of OCP with atelo-collagen in vivo condition. The stimulatory capacity of OCP to conduct new bone increases with the copresence of amorphous calcium phosphate (ACP). Physical and chemical analyses of the materials suggested that the bioactivity of such hydroxyapatite (HA) precursor phases is induced as a result of the progressive change of chemical property of these materials during the hydrolysis into HA under physiological environment. The composite materials composed of OCP and natural polymers are capable of repairing not only mineralized tissues but also non-mineralized tissue. The form of OCP-based materials and the tissue responses to the materials will be summarized.

Keywords Calcium phosphate • Octacalcium phosphate • Scaffold • Tissue regeneration

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10.1 Introduction

Calcium phosphate ceramics, such as sintered hydroxyapatite (HA, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) and $\beta$-tricalcium phosphate ($\beta$-TCP, $\beta$-$\text{Ca}_3(\text{PO}_4)_2$), have widely been used as filling materials for various bone defects due to the superior osteoconductivity which is capable of bonding to bone tissue without intervention of connective tissues [1]. HA and $\beta$-TCP have been used as implantable scaffold materials with mesenchymal stem cells (MSCs) for bone regeneration [2]. These calcium phosphate materials provide suitable site where osteoblastic cells can attach, proliferate, and differentiate [2]. The physicochemical property of calcium phosphate materials affects the cellular responses to some extent [3, 4]. Octacalcium phosphate (OCP, $\text{Ca}_8\text{H}_2(\text{PO}_4)_6\cdot5\text{H}_2\text{O}$) is a non-sintered calcium phosphate material which was first proven to directly bond to bone tissue by onlaying it on mouse calvaria [5]. OCP has been regarded as a precursor phase in HA formation from supersaturated calcium and phosphate solutions with respect to HA and is therefore suggested to be a precursor to bone apatite crystals [6, 7] as well as amorphous calcium phosphate (ACP, $\text{Ca}_3(\text{PO}_4)_2\cdot\text{nH}_2\text{O}$) [8]. OCP shows diversity regarding the stoichiometry [9] and the microstructure [10] depending on the preparation condition [11], which controls its bioactivity in vitro and in vivo [11]. This article summarizes the bioactivity of OCP and the composite materials with natural polymers as scaffold materials we have reported previously.

10.2 Mineralized and Non-mineralized Tissue Responses

OCP displays a unique osteoconductive property which tends to biodegrade and be followed by new bone formation if implanted in various bone defects, including the defects in intramembranous bone and long bones in various animal models [11, 12]. The efficacy of OCP has recently been reported by implanting its composite with collagen in human maxilla after cystectomy [13]. The stimulatory capacity of OCP could be induced by the environmental changes around the crystals, including calcium ion concentration change, due to the progressive hydrolysis from OCP to HA under physiological conditions [5, 14, 15]. The hydrolysis of OCP to HA is enhanced if ACP coexists with OCP, and the bone regenerative capacity of OCP is augmented by mechanically mixing OCP with ACP in rat critical-sized calvaria defect [16]. A composite of OCP with gelatin matrix, prepared through a wet synthesis, showed a greater biodegradable property coupled with a greater bone regenerative property if the material was implanted in rat critical-sized calvaria defect [17, 18]. The in vivo studies indicated that OCP/gelatin composite repairs not only rabbit tibia defect model [19] but also reforms infraspinatus tendon insertion using rabbit rotator cuff tear model [20].
10.3 Matrix Materials for Calcium Phosphate

Various calcium phosphate composites with natural polymers have been developed for bone and other tissue engineering (Table 10.1). Collagen (Col) is the major component of extracellular matrix proteins in bone, and its reconstituted Col has been utilized as matrix materials for calcium phosphates. HA/Col composites have been prepared by different processes, such as direct physical mixing [21, 22], chemical deposition of HA [23], or biomimetic mineralization [24]. Biodegradable calcium

| Natural polymers | Calcium phosphates | Composites | Notes (references) |
|------------------|--------------------|------------|--------------------|
| Collagen         | HA                 | Sponge     | Chemical deposition [23] |
|                  | Nano HA            | Sponge     | Physical mixture [22] |
|                  |                    | Nanofiber  | Physical mixture [21] |
|                  |                    | Fiber      | Biomimetic mineralization [24] |
|                  | OCP                | Sponge     | Chemical deposition [26] |
|                  | β-TCP              | Sponge     | Physical mixture [25] |
| Gelatin          | HA                 | Particles  | Chemical deposition [29] |
|                  |                    | Sponge     | Chemical deposition [30] |
|                  |                    |            | Porogen leaching [31] |
|                  |                    | Film       | Physical mixture [28] |
|                  | OCP                | Sponge     | Chemical deposition [17] |
|                  |                    |            | Chemical deposition and physical mixture [18] |
|                  | β-TCP              | Sponge     | Physical mixture [49] |
| Chitosan         | HA                 | Sponge     | Biomimetic mineralization [36] |
|                  | Nano HA            | Sponge     | Physical mixture [35] |
|                  |                    |            | Chemical deposition [33] |
|                  | β-TCP              | Sponge     | Physical mixture [37] |
| Silk fibroin     | HA                 | Sponge     | Physical mixture [34] |
|                  |                    | Film       | Biomimetic mineralization [50] |
|                  | Ca-deficient HA    | Particles  | Chemical deposition [32] |
| Alginate         | HA                 | Sponge     | Physical mixture [40] |
|                  |                    |            | Biomimetic mineralization [39] |
|                  |                    | Beads      | Chemical deposition [41] |
|                  | OCP                | Sponge     | Chemical deposition [42] |
|                  |                    |            | Physical mixture [43] |
|                  |                    | Beads      | Physical mixture [45] |
|                  | β-TCP              | Beads      | Physical mixture [38] |
| Hyaluronic acid  | HA                 | Hydrogel   | Physical mixture [46] |
|                  | OCP                | Hydrogel   | Physical mixture [48] |
|                  | β-TCP              | Hydrogel   | Physical mixture [47] |
phosphates/Col composites, such as OCP/Col and β-TCP/Col, have also been obtained using coprecipitation methods [25, 26] and by physical mixing [27]. Gelatin (Gel) is a denatured form of collagen and has the advantage of higher biodegradability. HA/Gel composites have been obtained in various forms, not only in porous form but also in film and particle forms [28–31]. OCP/Gel composites have been prepared by physical mixing and coprecipitation [17, 18]. Other natural polymers, such as chitosan and silk fibroin, have also been combined with calcium phosphates [32–37].

Alginate (Alg) is a natural polysaccharide obtained from brown algae and applied for wound healing and drug delivery due to its biocompatibility. HA/Alg and β-TCP/Alg have been prepared as porous scaffolds or beads [38–41]. Our group has developed OCP/Alg porous scaffolds [42, 43]. Although it is known that Alg is not able to interact with mammalian cells [44], OCP/Alg was capable of enhancing osteoblastic cell attachment and bone formation depending on the pore size and the porosity of the composites [42]. OCP/Alg beads including osteoblastic cells have a potential to activate and deliver osteoblastic cells from the beads to the local sites of bone defects [45]. Hyaluronic acid (HyA) is a major component of the extracellular matrix in the connective tissue. HyA hydrogel can be used as injectable materials filling bone defects directly and has been reported to promote bone regeneration [46, 47]. OCP/HyA is a bone substitute material which was proven to show the injectable and bioactive properties [48].

10.4 Cell Responses to Calcium Phosphate Materials

We have reported that OCP induces osteoblastic differentiation of mouse stromal cells [15, 51]. When mouse ST2 cells were cultured on the OCP or HA coatings, expression of osteogenic markers, such as type I collagen, alkaline phosphatase, and osterix, was enhanced on the OCP coating plates in an OCP dose-dependent manner [51]. We also reported that OCP enhanced alkaline phosphatase (ALP) activity of mouse mesenchymal stem cell line D1 cells in a three-dimensional cell culture system compared to HA and β-TCP [52]. When rat dental pulp cells were cultured on the OCP or HA coatings, OCP promoted their odontoblastic differentiation more than HA, as confirmed by ALP activity, mineralization, and enhancement of dentine sialophosphoprotein expression [53]. Furthermore, OCP is capable of inducing osteoclast formation from bone marrow cells in vitro in the presence of osteoblasts without vitamin D3 [54].

OCP can gradually convert into the crystal structure of apatite in the physiological conditions [11]. The conversion induces the release of phosphate ions to the periphery of the crystals and the uptake of calcium ion into the crystals [14]. We demonstrated that these changes in ion concentration have effects on osteoblastic differentiation through the phosphorylation of p38 MAP kinase [55], migration of macrophage-like cells [56], and osteoclastic differentiation by the increase of the expression of RANKL in osteoblasts [54].
10.5 Conclusion

We have designed OCP-based materials and reported their tissue regenerative properties not only in the calcified tissues but also in the noncalcified tissue. The physicochemical properties of OCP induced in the conversion process from OCP to HA could be involved in promoting osteoblastic and osteoclastic differentiation and bone formation and reformation of infraspinatus tendon insertion. The composite materials of OCP with natural polymers, described in this article, could be used as scaffold materials for mineralized tissue regeneration and potentially for non-mineralized tissue regeneration.

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