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Application of Octacalcium Phosphate Collagen Composite to Bone Defects in Humans: A Long-Term Observational Study

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Abstract

Clinical research on octacalcium phosphate collagen (OCP/Col) application in bone defect has demonstrated its high bone regeneration ability. Herein, we conducted a long-term study involving the clinical evaluation of OCP/Col-applied cases. A successful outcome was confirmed in two patients followed up for 7 years and 6.5 years. Both cases showed stable radiographic findings of the bone tissue without any abnormal findings of the oral cavity. To our knowledge, this is the first report to confirm the long-term stability and safety of newly formed bone after OCP/Colimplantation.

Keywords: Bone regeneration; Bone substitute material; Long-term observation

Introduction

Certain calcium phosphate cement materials have been used as bone substitute materials for bone augmentation [1-3]. However, the use of these materials was found to lead to conditions, such as infection [4,5] or lower osteo conductivity, compared with the use of the autologous bone [6]. Therefore, a study of the long-term stability of substitute materials is imperative for reconstructive surgery. Octacalcium Phosphate (OCP) has been recognized as a bone substitute material with high osteo conductivity both *in vitro and in vivo* [7,8] and has been recommended as a precursor of biological apatite in bones and teeth [9]. In addition, direct evidence of the presence of OCP in the central part of human dentine crystals has been demonstrated, and apatite has been detected in the outermost layers of the same crystals [10] as well as in the porcine enamel [11] and sutures of the mouse calvaria during intramembranous osteogenesis [12]. The osteogenic potential of OCP was confirmed for the first time in 1991 by implantation under the periosteum of the mouse calvarium [7]. Subsequently, several studies were conducted on the bone regeneration ability of OCP, and repair of bone defect using OCP was confirmed in rats [13] and rabbits [14]. Moreover, the bone regeneration ability of OCP has been confirmed to be superior to that of hydroxyapatite and beta-tricalcium phosphate both in *vitro and in vivo* [15-17]. Since OCP is a granular material of inferior operability, a combination of OCP and atelocollagen (OCP/Col) was used for improving its operability [18]. Compared with OCP alone, OCP/Col yielded improved operability as well as bone regeneration ability [18]. On the basis of translational research for clinical application, some experiments have been performed on the bone regeneration ability of OCP/Col in various dog models of bone defects, such as tooth extraction hole [19], critical-sized calvarial bone defect [20], artificial alveolar cleft [21], and mandibular bone defect [22]. Successful bone repair was confirmed using OCP/Col in each experiment.

OCP/Col was first clinically applied in 10 cases between April 2011 and September 2013 as part of clinical research approved by the Research Ethics Committee of the Graduate School of Dentistry, Tohoku University. OCP/Col was used in five cases of tooth extraction socket and five cases of cystectomy cavity, and the effective bone healing of these bone defects was observed without infection or allergic reaction. The results of the first application of OCP/Col and the efficacy of OCP/Col have been previously reported [23-25]. Another group has also reported the clinical use of OCP granules for the defect after resection of fibrous dysplasia at the mandible and generation of space by

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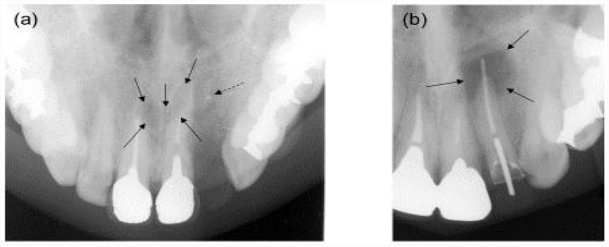

Figure 1: X-ray images of two cases before OCP/Col implantation. (a) Case 1. Arrows indicate a heart-shaped transmission region as a nasopalatine duct cyst in this X-ray image. Dotted arrow indicates punctiform foreign matter. (b) Case 2. Arrows indicate a radicular cyst.

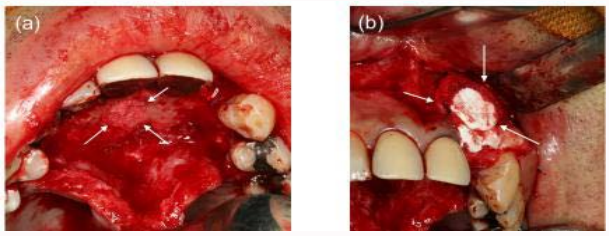

Figure 2: Images of OCP/Col discs implantation in case 1. (a) After the extraction of the nasopalatine duct cyst, OCP/Col discs were implanted. (b) After curettage of the tooth extraction cavity at the left upper lateral incisor region, the OCP/Col discs were implanted. Arrows indicate OCP/Col discs implanted in a bone defect.

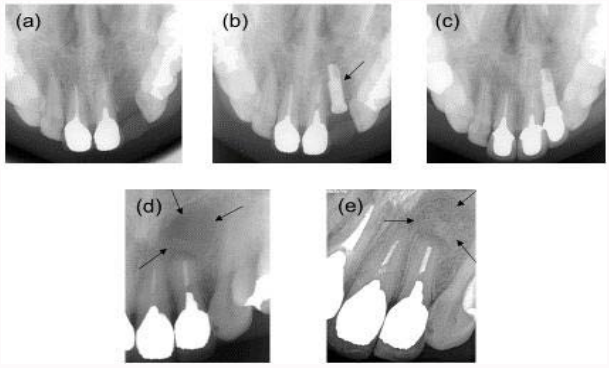

Figure 3: X-ray images of two cases after OCP/Col implantation. (a) Case 1. Heart-shaped transmission region disappeared at 6 months. (b) Case 1 at 1 year and 3 months. Arrow indicates the dental implant. (c) Case 1 at 7 years. Showing no significant change. (d) Case 2 at 1 year. Arrows indicates the cystectomy region. (e) Case 2 at 6 years and 6 months. Bone-like tissue was confirmed at the cystectomy region (arrows).

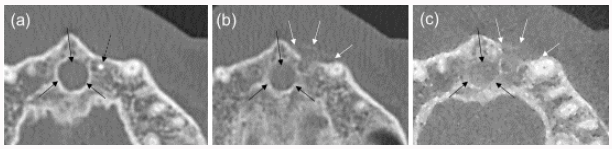

Figure 4: Horizontal CT scans of case 1. (a) Before OCP/Col implantation. (b) At 3 months after OCP/Col implantation. (c) At 6 months after OCP/Col implantation. Radiopacity increased at the OCP/Col implantation region. Black arrows indicate the nasopalatine duct cyst region. Dotted black arrow indicates foreign body. White arrows indicate the newly formed bone using OCP/Col at the curettage region.

sinus floor elevation [26]. However, these reports have demonstrated the short-term progress after treatments using OCP, and no long-term progress has been reported thus far. In the present study, we investigated the long-term progress of two cases treated using OCP/Col for bone defects.

Materials and Methods

Preparation of OCP/Col

OCP was prepared by mixing calcium and phosphate solutions as previously described [7]. Sieved OCP granules (particle size range: 300-500 μm) obtained from dried OCP were sterilized by heating at 120°C for 2 hr. Our previous study showed that such heating does not affect the physical properties of OCP granules, such as crystalline structure or specific surface area [27] although it has been reported that increasing the temperature to >100°C can induce the collapse of OCP structure because of dehydration [28,29]. Collagen was prepared from NMP collagen PS (Nippon Meat Packers, Tsukuba, Ibaraki, Japan), a lyophilized powder of pepsin-digested atelocollagen isolated from porcine dermis. NMP collagen PS was dissolved in distilled water and adjusted to a final concentration of 3% with a pH of 7.4. OCP/Col was prepared from NMP collagen PS and OCP granules. OCP was added to concentrated collagen and mixed well. The weight percentage of OCP in OCP/Col was 77%. This OCP/Col mixture was then lyophilized and the discs were moulded (9-mm diameter, 1-mm thickness). The moulded OCP/Col was subjected to de hydro thermal treatment (150°C, 24 hr) in the Vacuum Drying Oven DP32 (Yamato Scientific, Tokyo, Japan) and then sterilized using 5-kGy electron beam irradiation.

Cases

The study trial protocol was approved by the Ethics Committee of Tohoku University Graduate School of Dentistry (reference numbers 20-27 and 24-31). In this study, case1 was a 45-year-old man who was tentatively diagnosed with a nasopalatine duct cyst of approximately 10-mm diameter using X-ray examination (Figure 1a). Additionally, the failure of tooth extraction cavity healing because of the presence of a punctiform foreign matter in the upper left lateral incisor region was confirmed (Figure 3a, 4a). OCP/Col was implanted into each defect after cystectomy at the nasopalatine duct region and curettage of tooth extraction cavity at the left upper lateral incisor region under general anaesthesia (Figure 2a, 2b). Histological examination revealed a nasopalatine duct cyst. At 1 year of OCP/Col implantation, a titanium dental implant (Brånemark System® Mk III Groovy; Nobel Biocare Japan K.K., Tokyo, Japan) was inserted into the new bone region at the left upper lateral incisor region. The final prosthesis was set and the occlusion was reconstructed at 2 years after OCP/Col implantation. The patient is presently under observational follow-up at ongoing visit to our hospital for his dental implant. Case 2 was a 37-year-old man diagnosed with a radicular cyst of approximately 8-mm diameter at the left upper lateral incisor region based on X-ray examination (Figure 1b). OCP/Col was implanted into the defect after cystectomy and apicoectomy under local anaesthesia. Histological examination revealed a radicular cyst. After the study, this patient visited the hospital for dental check-up because of injury to the maxillary region, where OCP/Col was implanted.

Radiographic and clinical examination

Laboratory and radiographic examinations were performed before cystectomy and at 1 and 7 days and 1, 3, 6 and 12 months after OCP/Col implantation. Next, radiographic examination was performed whenever deemed necessary. Computed Tomography (CT) was performed before cystectomy and at 3 or 6 months after OCP/Col implantation. The Regions of Interest (ROIs) were defined

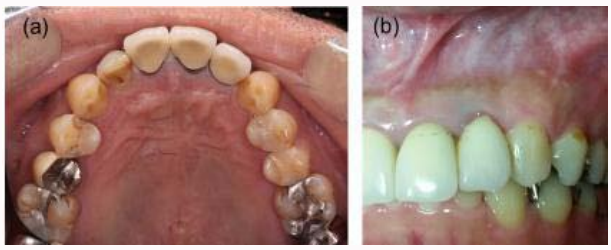

Figure 5: Images of the treated region in each case. (a) Case 1 at 7 years. (b) Case 2 at 6 years and 6 months. No abnormal findings observed.

at the centre of the augmented region. ROI was circular, with 5-mm diameter. CT values of ROIs were measured using software (We View Open-Pacs series, Hitachi Medical Corp., Tokyo, Japan); all values were reported as mean ± standard deviation. In intraoral findings, the presence or absence of abnormalities, such as inflammation and infection was confirmed during each visit.

Result

Radiographic examination

Although OCP/Col has low radiopacity under normal x-ray conditions, radiopacity at the OCP/Col implantation region was noted to increase with time for up to 6 months after cystectomy in each case. In case 1, a heart-shaped transmission region was observed before OCP/Col implantation at the nasopalatine duct region and alveolar bone absorption was detected at the left upper lateral incisor region on X-ray (Figure 1a). However, these findings disappeared at 6 months after OCP/Col implantation (Figure 3a). At 1 year and 3 months, a dental implant body was confirmed at the site repaired with newly formed bone using OCP/Col (Figure 3b). CT images indicated hard tissue formation with radiopacity at 3 and 6 months after OCP/Col implantation; CT findings also confirmed that the foreign matter was removed and that the tooth extraction cavity was healed with the bone-like tissue (Figure 4b, 4c). CT values of the cystectomy and curettage regions were respectively 300.12 ± 52.05 and 248.63 ± 89.99 at 3 months after OCP/Col implantation (preoperative values were respectively 121.37 ± 74.03 and 157.27 ± 90.23). The corresponding values of OCP or OCP/Col were 130-140 HU. At 6 months, these values increased to 300.12 ± 52.05 and 378.31 ± 56.35, respectively. Even though the surgical sites were confirmed by X-ray examination at every 6 months, no abnormal findings were noted, and the dental implant was stable until 7 years after OCP/Col implantation (Figure 3c). Moreover, in case 2, the X-ray transmission image became unclear at 1year after implantation (Figure 3d), which is in concordance with the results, obtained in a previous study [23]. Additionally, the radiopacity further increased, and the boundary with the surrounding bone became unclear at 6 years and 6 months after OCP/Col implantation (Figure 3e).

Clinical examination

No abnormal healing, infection and allergic reaction were observed in any of the treated regions until the last clinical examination (Figure 5a, 5b). Laboratory examination revealed no abnormal findings, and no infection occurred in any case. A slight increase in C-reactive protein level was observed only after cystectomy. No swelling or pain was recorded in the treated regions until the last clinical examination. Moreover, no abnormal findings, such as scar tissues or loss of surrounding teeth, were observed in any case.

Discussion

We have previously applied OCP/Col in humans for the first time [23]. Subsequently, another study group has reported the clinical application of OCP granules [26]. Both these studies have confirmed bone regeneration using OCP materials. In previous *in vivo* studies, the use of OCP resulted in improved bone regeneration through complex formation with collagen [18]; therefore, OCP/Col was selected as a bone substitute material in this study. OCP/Col was implanted in the bone defects of 10 patients and no abnormal findings were observed until 1 year after implantation [25]. According to the protocols followed in this clinical research, the follow-up was performed only up to 1 year, and observation after this period was performed in only one case of dental implantation. Owing to the regular follow-up of the dental implant status, continuous confirmation of the OCP/Col implantation region was possible for case 1. During the 7-year follow-up period, no infection at the treated region or formation of neoplastic lesions was observed and no adverse effects on the surrounding teeth, such as mobility or loss, were confirmed; thus, it can be considered that OCP/Col was safely absorbed and replaced in the body. Furthermore, the dental implant inserted into the OCP/Col implantation region were stable, and the newly formed bone using OCP/Col demonstrated an affinity to the dental implant. A recent study has demonstrated similar stability with the simultaneous implantation of OCP/Col and dental implant body *in vivo* [30]. In case 2, long-term progress could be confirmed by examination because the patient visited the hospital due to an injury at the site treated in this clinical research. This patient was not continuously followed up thereafter. However, no abnormal findings were observed in X-ray and intraoral examination at 6 years and 6 months after OCP/Col implantation, and stable results were confirmed in the region treated using OCP/Col. Moreover, the newly formed bone using OCP/Col did not mutate and remained stable for a long time in this patient.

Reportedly, other materials, such as hydroxyapatite, are not completely absorbed in the body and cause infection [4,5]. Several studies have been performed or improving absorption and osteo conductivity by combining with other materials [31,32], incorporating other factors [33,34] or using mesenchymal stem cells [35,36]. However, OCP can irreversibly convert to bone-like apatite *in vitro* [8], and OCP/Col can form a new bone without remaining the phase of OCP because complete conversion from OCP to bone-like apatite has been confirmed directly using a micro-beam X-ray diffraction analysis in situ for the corresponding area of the implanted OCP in both bone and subcutaneous tissues [7,27]. In this study, the boundary between newly formed bone and the surrounding bone remained unclear until the last X-ray examination. Because OCP/Col does not remain as a foreign matter, the newly formed bone using OCP/Col may be stable.

Bone regeneration using OCP/Col or OCP granules has been reported in the clinical setting, but the long-term progress remains unknown. Even though we analyzed only two cases in this study, we could confirm, for the first time, the long-term treatment course using OCP/Col and indicate its safety and stability. Recently, a clinical trial of OCP/Col has been performed as a prospective, multi-centre, single-arm study. In this clinical trial, OCP/Col has been used in patients undergoing dental implantation and in those with cleft palate. In this study, however, the data have been collected over a short period until the confirmation of bone regeneration although these cases generally require long-term management. Therefore, in

the future, long-term courses of several cases need to be observed for validating of the stability of OCP/Col.

Conclusion

This present study confirmed for the first time the long-term stability and safety of newly formed bone using octacalcium phosphate collagen composite in two cases.

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