Clinical study of octacalcium phosphate and collagen composite in oral and maxillofacial surgery

Tadashi Kawai[1] iD **, Shinji Kamakura[3], Keiko Matsui[1],**
Masayuki Fukuda[4], Hiroshi Takano[4], Mitsuyoshi Iino[5],
Shigeo Ishikawa[5], Hiromasa Kawana[6,7], Tomoya Soma[6],
Eisaku Imamura[8], Hideki Kizu[9], Aya Michibata[10], Izumi Asahina[11],
Keiichiro Miura[11], Norifumi Nakamura[12], Toshiro Kibe[12],
Osamu Suzuki[13] and Tetsu Takahashi[1]

Abstract

Octacalcium phosphate and its collagen composite have been recognized as bone substitute materials possessing osteoconductivity and biodegradation properties. We evaluated the effectiveness of octacalcium phosphate and its collagen composite used for bone augmentation in major oral and maxillofacial surgeries in a clinical trial. Octacalcium phosphate and its collagen composite were used in cases of sinus floor elevation in 1- and 2-stage, socket preservation, cyst, and alveolar cleft procedures. A total of 60 patients were evaluated for effectiveness after the implantation of octacalcium phosphate and its collagen composite. Although sinus floor elevation in 1-stage, cyst, and alveolar cleft cases met the criteria for the judgment of success, sinus floor elevation in 2-stage and socket preservation groups did not meet the criteria in the initial evaluation. However, an additional evaluation for reconfirmation revealed the effectiveness of octacalcium phosphate and its collagen composite in those groups, and all evaluation results ultimately indicated the success of this clinical trial. Therefore, this clinical trial suggested that application of octacalcium phosphate and its collagen composite for oral and maxillofacial surgery was safe and effective and that octacalcium phosphate and its collagen composite could be a bone substitute candidate instead of autologous bone.

[1]Department of Oral Medicine and Surgery, Division of Oral and Maxillofacial Surgery, Tohoku University Graduate School of Dentistry, Sendai, Japan

[2]Department of Reconstructive Oral and Maxillofacial Surgery, Division of Oral and Maxillofacial Surgery, School of Dentistry, Iwate Medical University, Morioka, Japan

[3]Department of Bone Regenerative Engineering, Division of Regenerative and Biomedical Engineering, Tohoku University Graduate School of Biomedical Engineering, Sendai, Japan

[4]Department of Dentistry and Oral Surgery, Akita University Graduate School of Medicine, Akita, Japan

[5]Department of Dentistry, Oral and Maxillofacial-Plastic and Reconstructive Surgery, School of Medicine, Yamagata University, Yamagata, Japan

[6]Department of Dentistry and Oral Surgery, Division of Oral and Maxillofacial Surgery, School of Medicine, Keio University, Tokyo, Japan

[7]Department of Oral and Maxillofacial Implantology, Kanagawa Dental University, Kanagawa, Japan

[8]Department of Oral and Maxillofacial Surgery, Yokohama General Hospital, Kanagawa, Japan

[9]Department of Dentistry and Oral Surgery, Tachikawa Hospital, Tachikawa, Japan

[10]Department of Oral Surgery, Shizuoka City Shimizu Hospital, Shizuoka, Japan

[11]Department of Regenerative Oral Surgery, Medical and Dental Sciences, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan

[12]Department of Oral and Maxillofacial Surgery, Field of Oral and Maxillofacial Rehabilitation, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan

[13]Department of Craniofacial Engineering and Regeneration, Division of Craniofacial Function Engineering, Tohoku University Graduate School of Dentistry, Sendai, Japan

Corresponding author:
Tetsu Takahashi, Department of Oral Medicine and Surgery, Division of Oral and Maxillofacial Surgery, Tohoku University Graduate School of Dentistry, 4-1, Seiryo-machi, Aoba-ku, Sendai 980-8575, Miyagi, Japan.
Email: tetsu@dent.tohoku.ac.jp

Introduction

In oral and maxillofacial, orthopedic, and plastic surgeries, it is important to restore shape and function by augmenting bone for congenital bone defects or those acquired by injury. Hydroxyapatite (HA),[1] β-tricalcium phosphate (β-TCP),[2,3] and xenogeneic grafts, such as bovine bone,[4] have been clinically used as bone substitutes. Although these materials have osteoconductive properties and are effective for filling bone defects, their ability of bone formation may be insufficient in some cases.[5–9] In addition, they remained as remnants without being resorbed or took long time to resorb in the body. Autologous bone grafting has been performed as the first choice in cases where it is necessary to compensate for large bone defects and acquire normal physiological functions as bones,[10,11] whereas it has disadvantages such as surgical invasion requiring bones, namely, the iliac bone, which are collected from other parts of the body, and the amount that can be harvested is insufficient.[12–14]

In 1962, octacalcium phosphate (OCP) was suggested as a precursor of biological apatite crystal in bones and teeth.[15] OCP was detected in porcine enamel,[16] human dentin,[17] and mouse calvaria[18] as an intermediate to those apatite matrices. The higher osteoconductive property of synthetic OCP, accompanying the structural change to apatite phase, was confirmed first in 1991 in comparison with other calcium phosphate materials, including non-sintered HA materials.[19] Furthermore, OCP has been demonstrated to promote osteoblastic cell differentiation in vitro[19,20] and facilitate bone regeneration in vivo.[21] OCP exerts osteoconductivity with progressive and irreversible conversion to bone-like apatite crystals in vivo.[22] Furthermore, OCP enhances bone regeneration and is resorbed in vivo more than HA or β-TCP.[23] However, OCP has limited usability because it is produced in granular form owing to its chemical structure. To solve this problem, OCP was combined with atelocollagen in a previous study.[24] The combination of OCP granules and atelocollagen (OCP/Col) improved not only usability but also bone regeneration more than OCP granules alone.[24] Furthermore, several canine bone defect models were used to investigate the bone regeneration by OCP/Col as translational research. The results in these studies indicated the following: OCP/Col can be converted to normal bone tissue in critical-sized bone defects in the calvarium without remaining as a foreign body,[25,26] OCP/Col can retain the shape of the alveolar bone after tooth extraction without bone resorption,[27] the gap in the alveolar bone cleft can be filled with newly formed bone after OCP/Col implantation,[28,29] a permanent tooth can normally erupt in the newly formed bone after OCP/Col implantation in the extraction socket of deciduous teeth,[30] OCP/Col can facilitate bone augmentation in the mandibular alveolar bone defect with titanium mesh,[31] and newly formed bone after OCP/Col implantation can have the same osseointegration for titanium dental implant surfaces as autologous bone.[32]

The first clinical application of OCP/Col has been conducted in the bone defect caused by tooth extraction or cystectomy in 10 patients in a clinical study of bone regenerative therapy by OCP/Col composites (registered with JPRN-UMIN000004655 in the University Hospital Medical Information Network in Japan (UMIN) and International Clinical Trials Registry Platform Search Portal of the World Health Organization, from 2011 to 2013 at Tohoku University Hospital),[33,34] as a preclinical trial. The first objective of this study was to investigate the safety of OCP/Col in clinical use. The second objective was to investigate the efficacy of this material when implanted into a bone defect. This preclinical trial suggested that OCP/Col can be safely used and enhances bone regeneration in human bone defects and can be a good bone substitute material.[35] Based on these results, this study was designed as a clinical trial of phase 3 for many patients at multiple institutions to demonstrate the effectiveness of OCP/Col for cases requiring bone regeneration (Figure 1).

Materials and methods

Preparation of OCP/Col

OCP and OCP/Col were prepared as previously described,[19,36] and OCP/Col was molded into 9-mm-diameter and 1.5-mm-thick disks or 9-mm-diameter and 10-mm-thick cylinders, and then subjected to dehydrothermal treatment (Figure 2(a) and (b)). Before implantation, OCP/Col was scanned with a Microfocus X-ray computed tomography (CT) system (Scan Xmate-E090; Comscantecno Co., Ltd., Kanagawa, Japan), with settings of 90 kV and 0.1 mA, and the image data were calculated using a three-dimensional image analysis system (TRI/3D-BON; Ratoc System Engineering, Tokyo, Japan) as described previously.[36]

Material characteristics

Figure 2(c) shows that OCP/Col is a porous material as revealed by scanning electron microscopy images. OCP/Col has approximately 92% porosity and bimodal peaks of 48 μm (main pores) and 0.3 μm (minor pores), pore volume of 6.3 cm^{-3}/g, and specific surface area of 17.8 m^2/g, as reported previously.[37] The minor peak assigned to 0.3 μm
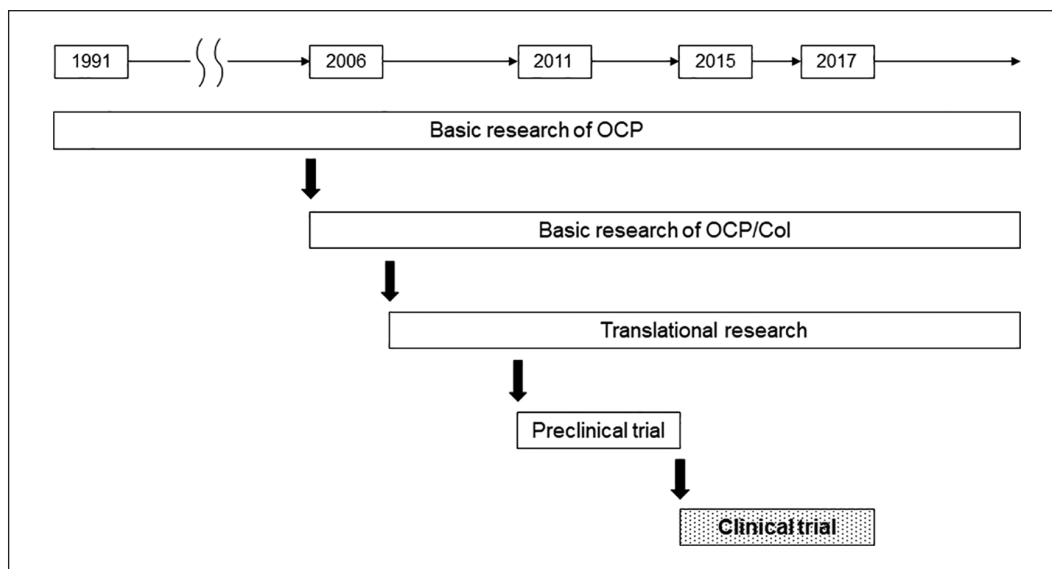
Figure 1. Roadmap chart for OCP/Col research. Research on OCP began in 1991, and research on OCP/Col began in 2006. Following preclinical research, this trial was conducted from 2015 to 2017.
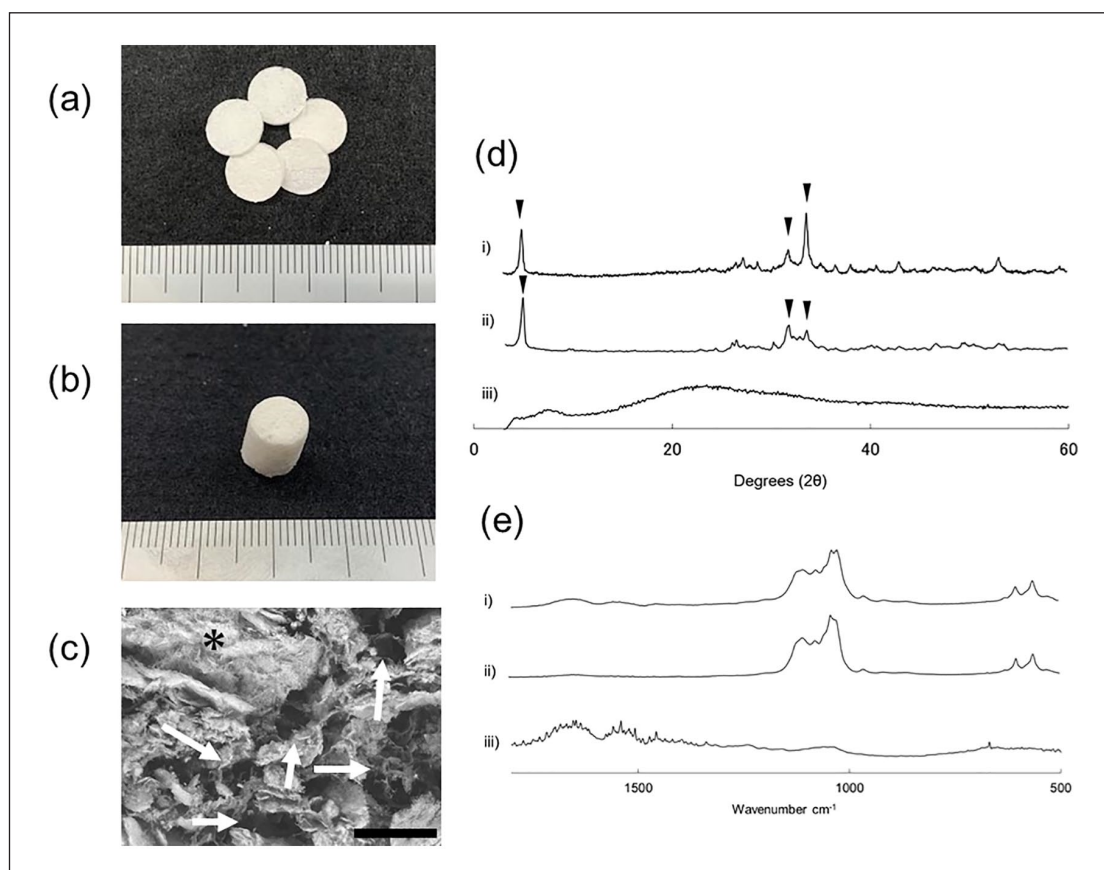

Figure 2. Characteristics of OCP/Col. (a) Picture of disk- type OCP/Col, 9 mm in diameter and 1.5 mm thick. (b) Cylinder type, 9 mm in diameter and 10 mm thick. (c) Scanning electron microscopy images of OCP/Col. Original magnification = 1000×, bars = 50 μm. White arrows indicate pores, and an asterisk indicates OCP granule of OCP/Col. OCP/Col has approximately 92% porosity and bimodal peaks of 48 μm (main pores) and 0.3 μm (minor pores), pore volume of 6.3 cm^{-3}/g, and specific surface area of 17.8 m^2/g. (d) X-ray diffraction pattern of (i) OCP/Col, (ii) OCP, and (iii) collagen. The primary (100) peak at $2\theta = 4.7$ is identified in OCP/Col and OCP. Other reflections of OCP are also confirmed in OCP/Col. Black triangles indicate OCP reflection. (e) Fourier transform infrared spectroscopy of (i) OCP/Col, (ii) OCP, and (iii) collagen.

diameter pores in OCP/Col has been recognized to be the pores that originated from OCP granule itself within Col matrix of OCP/Col in our subsequent study of OCP materials.[38] OCP/Col has little radiopacity, and the CT values of OCP/Col before implantation ranged from 130 to 140 HU.[33] The primary (100) peak at $2\theta = 4.7$ is identified in OCP/Col by examining the X-ray diffraction pattern, which is the same as OCP (Figure 2(d)). The features of Fourier transform infrared spectroscopy of OCP/Col is indicated in Figure 2(e) with those of OCP and collagen.

Design of the clinical trial and participants

Our study was a phase 3, prospective, multicenter, single-arm study of OCP/Col for a guided bone regeneration clinical trial, which was registered with the medical information network in Japan (UMIN; registration numbers JPRN-UMIN000018192). The protocol of this clinical trial was approved by the Institutional Review Board of the Pharmaceuticals and Medical Devices Agency in Japan (reference number OCTC-14001). Toyobo Co., Ltd. sponsored this clinical, single-arm, non-randomized intervention study, which was performed at nine hospitals in Japan. In the first clinical evaluation in this clinical trial, we assessed the safety of OCP/Col used to fill bone defects after OCP/Col implantation by clinical examinations and analysis of adverse events. The second clinical evaluation focused on the radiographical, histological, and clinical effectiveness of OCP/Col as a bone substitute material. The subjects were patients undergoing sinus floor elevation for dental implant treatment, socket preservation for dental implant treatment, cystectomy of the jaw, and bone grafting at alveolar cleft. Male and female patients aged 20–70 years were included in sinus floor elevation, socket preservation, and cyst cases. Moreover, male and female alveolar cleft patients aged 7 years or more were included. Exclusion criteria included severe hepatic or renal diseases, heart disease, drug allergy, bone metabolism diseases such as osteoporosis, history of bisphosphonate administration, systemic administration of steroids or immunosuppressants, anticoagulant therapy, suffering from malignant tumor within 5 years, pregnant or lactating woman, uncontrolled diabetes, or smokers.

The target number of patients was set to 60, of whom 40 underwent sinus floor elevation (2-stage method set to ⩾30 people); 10, socket preservation; 5, a jaw cyst procedure; and 5, alveolar cleft surgery. These numbers were set in the anticipation of fluctuations and omissions in the clinical trial period. All patients included in our study provided written informed consent. After confirming the presence or absence of abnormality on screening, OCP/Col was used in each case and clinical evaluation started.

Procedures

Sinus floor elevation procedure was divided into 1-stage (length between alveolar crest and sinus floor ⩾5 mm) and 2-stage (length <5 mm) methods, because if the length would be <5 mm, the initial fixation of the implant body might have been insufficient. According to standard methods, the gingiva and periosteum were ablated and a window was formed in the lateral wall of the maxillary sinus. The Schneiderian membrane was ablated from the sinus floor and lifted upward to make space for OCP/Col implantation. In cases of Schneiderian membrane perforation, if the perforation was <5 mm, the operation was continued as it was, whereas if the perforation was ⩾5 mm or if the surgeon judged that the operation could not be continued, it was canceled.

In the 1-stage method, OCP/Col was implanted into the space by sinus membrane elevation, and dental implants were spontaneously placed at the missing tooth region (Figure 3(a)). Approximately 6 months postoperatively, the abutments were exchanged, and prosthetic treatment was started. In the 2-stage method, only sinus floor elevation was performed using OCP/Col during the first operation. Approximately 6 months after OCP/Col implantation, the dental implants were placed at the missing tooth region as a second operation. After 6 months, the abutments were exchanged and prosthetic treatment was started. The dental implants to be used were made of titanium, and no implant with bioactive coating was used. The implants were placed in accordance with the method instructed by each implant manufacturer.

In socket preservation cases, after tooth extraction and curettage of the socket, OCP/Col was spontaneously implanted into the tooth extraction hole, and the socket was sutured closely. Approximately 6 months after OCP/Col implantation, the dental implants were placed at the missing tooth region. After 6 months, the abutments were exchanged, and then prosthetic treatment was started for installation of the superstructure.

In cystectomy cases, after the gingiva and periosteum were ablated and the surrounding bone was removed, the jaw cysts were extirpated. The defect created by cystectomy was filled with OCP/Col spontaneously. Then, the gingiva and periosteum were repositioned and sutured closely.

In alveolar cleft cases, a recipient space was formed in accordance with standard bone grafting procedures. OCP/Col was implanted into the alveolar bone defect. Then, the defect was covered with gingiva and periosteum and sutured closely.

Clinical laboratory examination

Subjective symptoms and objective findings were investigated from OCP/Col implantation to the end of the observation period or until discontinuation in each case. When OCP/Col implantation was discontinued, as much as possible, the investigation was continued up to 6 months after implantation. Laboratory tests were conducted at screening, within 1 week and at 3 and 6 months after OCP/Col implantation, and at the time of discontinuation. The tests
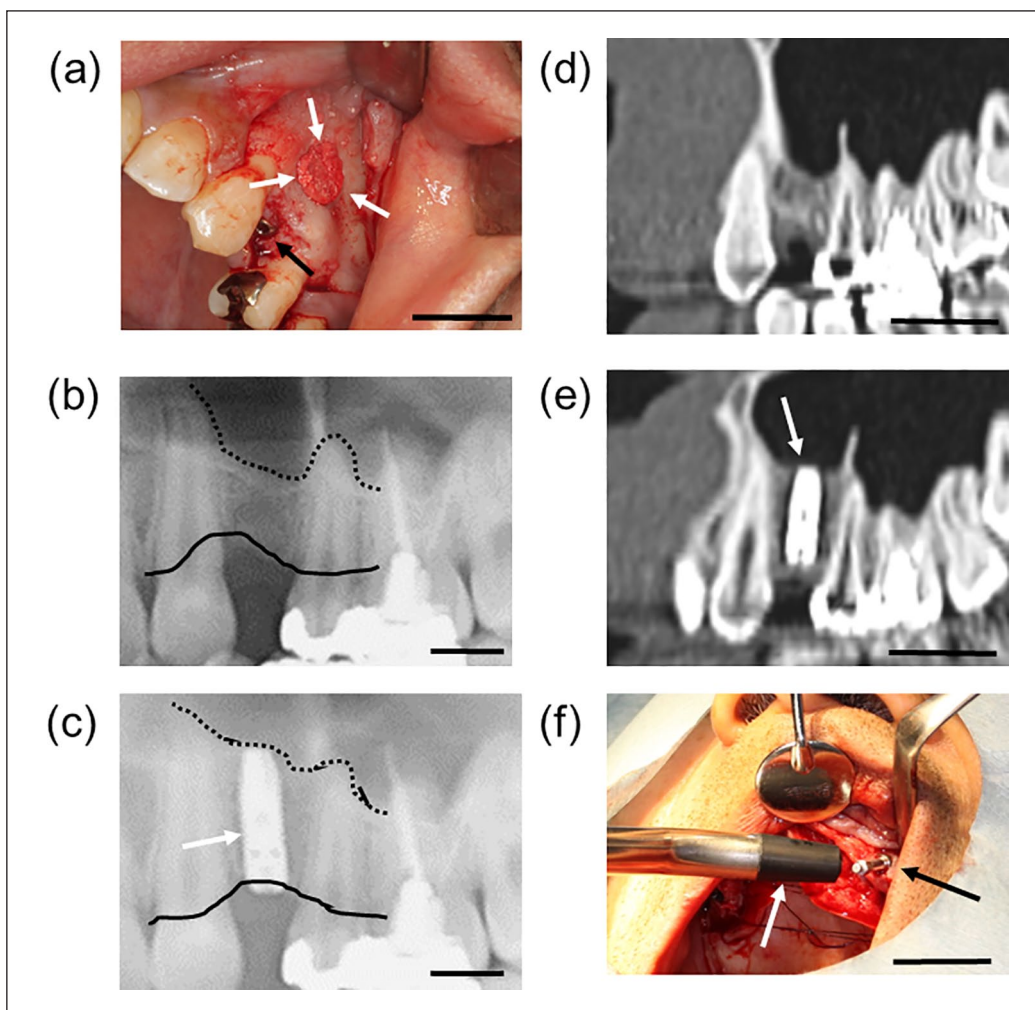
Figure 3. Pictures of this clinical trial. (a) During surgery of a sinus floor elevation in the 1-stage case, gingiva and periosteum were ablated, and a window was prepared by scraping bone. After that, sinus membrane was ablated from sinus floor and elevated to make space for filling OCP/Col. White arrows indicate OCP/Col implanted in the space by sinus floor elevation. Black arrow indicates dental implant head placed from alveolar crest. Bar = 10 mm. (b) X-ray of this patient before OCP/Col implantation and (c) at 6 months after dental implant and OCP/Col implantation. Black lines indicate alveolar crest and dot black lines indicate sinus floor (sinus membrane exists along the sinus floor). The distance from alveolar crest to sinus floor before operation was 6 mm. However, the distance increased to 10 mm at 6 months after operation. A white arrow indicates a dental implant. Bars = 5 mm. (d) CT picture before OCP/Col implantation and (e) at 6 months after dental implant and OCP/Col implantation. The wide black part above is the space of the maxillary sinus. A white arrow indicates a dental implant. Hard tissue converted from OCP/Col was observed around the root of the dental implant. There was no abnormal finding such as thickening of the sinus mucosa or absorption of surrounding bone. Bars = 10 mm. (f) During measurement of ISQ value, a black arrow indicates a SmartPeg connected dental implant and a white arrow indicates the analyzer probe placed close to the SmartPeg. The resonance frequency measured by the Osstell system is expressed as ISQ value ranging from 0 to 100. It is considered that the stability of the dental implant is obtained with an ISQ value of 60 or more. Bar = 20 mm.

were performed for blood cells, inflammatory markers, liver function, kidney function, electrolytes, and urine. In addition, human chorionic gonadotropin was examined in females who could be pregnant.

Radiographical examination

Intraoral or panoramic radiographs were taken before OCP/Col implantation, within 1 week and at 3 and 6 months after OCP/Col implantation, and at the time of discontinuation. In the sinus floor elevation in the 1-stage group, the radiographical examination was added at 4 weeks after installation of the superstructure. In the sinus floor elevation in 2-stage and socket preservation groups, the radiographical examination was added at ≥4 weeks after installation of the superstructure as an additional examination.

CT scans were performed before OCP/Col implantation and at 3 and 6 months after OCP/Col implantation

(Figure 3(b)). CT value, vertical bone width, and bone regeneration rate were evaluated by CT. CT values were evaluated in each case. The region of interest (ROI) was circular and 5, 3, or 1 mm in diameter, including no foreign body and normal bone. The ROIs were defined at the center of the augmented region. In the sinus floor elevation in the 1-stage group, the ROIs were defined around the dental implants. Vertical bone width was evaluated in sinus floor elevation in 1- and 2-stage, and socket preservation groups. For sinus floor elevation, it was measured from the alveolar crest to the sinus floor. For socket preservation, it was measured from the alveolar crest to tooth apex (bottom of tooth extraction socket). Bone regeneration rate was calculated in cystectomy and alveolar cleft groups. In the horizontal section, the ratio of newly formed bone in the original bone defect area was calculated at the cross section of the center of the bone defect.

Histological examination

Bone biopsy was performed in the sinus floor elevation in 2-stage and socket preservation groups. Before dental implant placement, the newly formed bones were collected using a trephine bur with 2.4 mm diameter from the alveolar crest of the site where the dental implants are to be placed in each case. The samples were fixed with 4% para-formaldehyde in 0.1 M phosphate-buffered saline, pH 7.4, for a few days and decalcified in 10% ethylenediaminetetraacetic acid in 0.01 M phosphate buffer, pH 7.4, for 2–4 weeks at 4°C. The samples were dehydrated in a graded series of ethanol concentrations and embedded in paraffin. Each sample was then sectioned to 5 μm thickness, stained with hematoxylin and eosin, and photographs were taken with a photomicroscope. The presence or absence of newly formed bone and abnormal findings was evaluated.

Initial fixation and micromobility of dental implant examination

Initial fixation of dental implants was evaluated as the torque value by Newtron-1 (Kyoto Tool Co., Ltd., Kyoto, Japan). The torque values were measured at the end of the dental implant placement using the instrument in the sinus floor elevation in 2-stage and socket preservation groups. A wrench was attached to the dental implant, which was manually rotated to evaluate the maximum torque value at that time.

The micromobility of dental implants was evaluated by the Osstell system (Osstell™; Integration Diagnostics AB, Göteborg, Sweden) immediately after placement of the dental implants and when the abutments were exchanged. A "SmartPeg" (Integration Diagnostics AB), which is an aluminum metal rod with a magnet attached to its top, was screwed into each dental implant. The SmartPeg was tightened manually to approximately 5 N cm in accordance with the manufacturer's guidelines. The analyzer probe was then placed close to the SmartPeg in the same direction, perpendicular to the long axis, to standardize the experimental procedure. The SmartPeg was excited by a magnetic pulse generated by the measurement probe, which produced a vibrational signal that was detected by the handheld instrument. The resonance frequency measured by the Osstell system was expressed as an implant stability quotient (ISQ) value ranging from 0 to 100. Three measurements were taken per implant, and the lowest value was recorded as the final ISQ. Figure 3(f) shows the image of measurement of ISQ value at dental implant placement.

Evaluation of dental implant treatment

Regarding the effect of dental implant treatment accompanied by bone augmentation with OCP/Col, the occlusion relationship at the installation of the superstructure and after 4 weeks was evaluated in the sinus floor elevation in the 1-stage group. The same examination was performed in the sinus floor elevation in 2-stage and socket preservation groups as an additional examination at ⩾4 weeks.

Major and secondary evaluation items and judgment

Major and secondary evaluation items are shown in Table 1. The major evaluation item of the whole clinical trial was "the ratio of the major evaluation result 'good' of each subject patient is ⩾70 in total."[39]

The major evaluation item of the sinus floor elevation in the 1-stage group was "success of dental implant treatment at 4 weeks after installation of the superstructure." The success of dental implant treatment was assessed by confirmation of infection, surrounding inflammation, mobility of the implant, pain, sensory disorder, and bone resorption around the dental implant body by X-ray (Table 2). As a judgment criterion, 6, 5, and ⩽4 points were "good," "slight poor," and "poor," respectively. The secondary evaluation items of 1-stage sinus floor elevation were (1) success of the dental implant treatment before installation of the superstructure (at 24 weeks after OCP/Col implantation), (2) evaluation of CT value at 24 weeks after implantation of OCP/Col, (3) evaluation of vertical bone width change before and at 24 weeks after OCP/Col implantation, and (4) evaluation of ISQ value immediately after dental implant placement and before installation of the superstructure. Secondary evaluation item (1) was judged in the same way as the major evaluation item. In secondary evaluation item (2), since the CT value of OCP/Col was 130–140 HU, ⩾150 HU was judged as "good" and <150 HU was judged as poor.

The major evaluation items of the sinus floor elevation in 2-stage and socket preservation groups were (1) osteogenesis effect by biopsy diagnosis at 24 weeks after implantation

Table 1. Evaluation items in this clinical trial.

	(a) Major evaluation items
Whole clinical trial	The ratio of the major evaluation result "good" of each subject patient is 70% or more in total
Sinus floor elevation in the 1-stage group	Success of dental implant treatment at 4 weeks after installation of superstructure
Sinus floor elevation in 2-stage and socket preservation groups	(1) Osteogenesis effect by biopsy diagnosis at 24 weeks after implantation of OCP/Col (dental implant placement) (2) Evaluation based on the maximum torque value at 24 weeks after implantation of OCP/Col (dental implant placement)
Cyst and alveolar cleft	Evaluation of CT value at 24 weeks after implantation of OCP/Col
	(b) Secondary evaluation items
Sinus floor elevation in the 1-stage group	(1) Success of dental implant treatment before installation of superstructure (at 24 weeks after OCP/Col implantation) (2) Evaluation of CT value at 24 weeks after implantation of OCP/Col (3) Evaluation of vertical bone width change before and at 24 weeks after OCP/Col implantation (4) Evaluation of ISQ value immediately after dental implant placement and before installation of superstructure
Sinus floor elevation in 2-stage and socket preservation groups	(1) Evaluation of CT value at 24 weeks after implantation of OCP/Col (2) Evaluation of vertical bone width change before and at 24 weeks after OCP/Col implantation (3) Evaluation of ISQ value at 24 weeks after OCP/Col implantation (dental implant placement)
Cyst and alveolar cleft	Evaluation of bone regeneration rate by CT image
	(c) Additional evaluation item
Sinus floor elevation in 2-stage and socket preservation groups	Success of dental implant treatment at 4 weeks or more after installation of superstructure

OCP/Col: octacalcium phosphate and its collagen composite; CT: computed tomography; ISQ: implant stability quotient.

of OCP/Col (at dental implant placement) and (2) evaluation based on the maximum torque value at 24 weeks after implantation of OCP/Col (at dental implant placement). In major evaluation item (1), results were regarded as "good" if newly formed bone was recognized and there was no histological abnormality, whereas if newly formed bone was not recognized or histologically abnormal, results were regarded as poor. In major evaluation item (2), results were judged "good" if the torque value was $\geq 20\,N\,cm$ and poor if it was $<20\,N\,cm$. In sinus floor elevation in 2-stage and socket preservation cases, both major items (1) and (2) were evaluated as "good," and major evaluation was judged as "good" overall. The secondary evaluation items of sinus floor elevation in 2-stage and socket preservation were (1) evaluation of CT value at 24 weeks after implantation of OCP/Col, (2) evaluation of vertical bone width change before and at 24 weeks after OCP/Col implantation, and (3) evaluation of ISQ value at 24 weeks after OCP/Col implantation (at the dental implant placement). The additional evaluation item of 2-stage sinus floor elevation and socket preservation was "success of implant treatment at ≥ 4 weeks after installation of the superstructure."

The major evaluation item of the cyst and alveolar cleft groups was "evaluation of CT value at 24 weeks after implantation of OCP/Col," and the secondary evaluation item was "evaluation of bone regeneration rate by CT image" (judged "good" and "poor" if the newly formed bone rate was more than half and was less than half, respectively).

Statistical analysis method

There was a plurality of sites to be treated by OCP/Col, and the site to be analyzed as the full analysis set (FAS) and the per protocol set (PPS) conforming to the trial protocol was set as a predetermined site by the investigator. In addition, analysis was performed for all teeth sites where OCP/Col was implanted (Parts). FAS was defined as all cases from which OCP/Col implantation was performed, except for those in which the efficacy was not evaluated at all. Non-compliant cases in which evaluation based on this clinical trial protocol could not be performed sufficiently were excluded from FAS and were defined as PPS. In each item judged "good" or "poor," the ratio determined to be "good,"

Table 2. Check items of success of dental implant treatment.

Check items		Criteria of judging points	
		1 point	0 point
a.	Infection	No noticeable infection	Remarkable infection (pus discharge)
b.	Surrounding inflammation	No noticeable inflammation	Remarkable inflammation (swelling and redness)
c.	Mobility of dental implant	No mobility	Confirmation of mobility
d.	Pain	No noticeable pain	Confirmation of remarkable pain (Take painkillers more than three times a day for more than 2 days) (Exclude cases where there are no associations such as pain in the masticatory muscles and temporomandibular joints)
e.	Sensory disorder	No sensory disorder	Confirmation of sensory disorder (Hypoesthesia, hyperesthesia, and paresthesia) (Exclude cases where there are no associations such as sensory disorder in the masticatory muscles and temporomandibular joints)
f.	Confirmation of bone absorption of dental implants with X-ray	No noticeable absorption	Confirmation of remarkable absorption (One-quarter or more bone absorption around the dental implant)

As a judgment criterion, 6 points were "good"; 5, "slight poor"; and ⩽4, "poor."

that is, the effective ratio, and the two-sided confidence intervals (CIs) were calculated. In the evaluation of vertical bone width change before and at 24 weeks after OCP/Col implantation, the average value of the vertical bone width before and at 6 months after OCP/Col implantation and its two-sided CIs were calculated. Furthermore, the difference between before and at 6 months after OCP/Col implantation was calculated, and the average value and its two-sided CIs were calculated. For evaluation of ISQ values, the average value of the ISQ values and the two-sided CIs were calculated.

Results

Whole clinical trial

In this clinical trial, consent was obtained from 75 patients, 9 were excluded on the basis of screening examination and 66 were registered. One of the 66 patients did not undergo OCP/Col implantation due to deviation from the eligibility criteria. Among the remaining 65 patients who underwent OCP/Col implantation surgery, 1 who underwent sinus floor elevation in 1-stage surgery stopped this clinical trial before OCP/Col implantation due to perforation of the Schneiderian membrane, 60 completed this clinical trial, and 2 who underwent socket preservation stopped due to the occurrence of adverse events, and the remaining 2 patients were treated as cases of serious non-compliance with implementation criteria and were excluded from all analyses because the superstructure was installed by dentists who were not responsible for this clinical trial and belonged to a hospital that was not this clinical trial site.

Adverse events appeared in all 60 patients who completed this clinical trial evaluation; most of the events were common, such as pain, swelling, increasing white blood cell count, and increasing C-reactive protein levels.

In the sinus floor elevation in the 2-stage group, two patients who underwent the socket lift procedure at dental implant placement and two others who underwent treatment prohibited for combination were excluded from PPS.

In the additional examination, after obtaining consent from 33 of the 40 patients who completed the sinus floor elevation in 2-stage and socket preservation procedures, all completed additional tests. Seven patients who did not provide consent included one who refused, one who could not visit the hospital, three who did not have the superstructure, one who lost the dental implant, and one who refused consent and lost the dental implant. In the sinus floor elevation in the 2-stage group, two patients who underwent the socket lift procedure at dental implant placement and one who underwent treatment prohibited for combination were excluded from PPS. Table 3 shows the breakdown of effectiveness analysis group. The average age of patients was 54.0 (range, 7–68), 59.0 (range, 20–67), 58.5 (range, 40–68), 51.5 (range, 42–61), 51.0 (range, 40–57), and 9.0 (range, 7–16) years for the whole clinical trial at FAS, sinus floor elevation in 1-stage, 2-stage, socket preservation, cyst, and alveolar cleft at FAS groups, respectively. There were 28 males and 32 females at FAS. Table 4 presents the summary of participating patients and institutions in this clinical trial.

Figure 4(a) and (b) show the percentage of "good" results overall for major evaluation items and at additional evaluation, respectively. The ratio of "good" for the whole clinical trial major evaluation item was 50.0% (95% CI,

Table 3. Breakdown of effectiveness analysis group.

Item	FAS	PPS	Parts
Major evaluation item of whole clinical trial	$N=60$	$N=56$	$N=107$
Sinus floor elevation in the 1-stage group			
Major evaluation items	$n=7$	$n=7$	$n=14$
Secondary evaluation item (1)	$n=7$	$n=7$	$n=14$
Secondary evaluation item (2)	$n=7$	$n=7$	$n=14$
Secondary evaluation item (3)	$n=7$	$n=7$	$n=14$
Secondary evaluation item (4)	$n=7$	$n=7$	$n=14$
Sinus floor elevation in the 2-stage group			
Major evaluation items	$n=32$	$n=28$	$n=66$
Secondary evaluation item (1)	$n=30$	$n=28$	$n=80$
Secondary evaluation item (2)	$n=32$	$n=28$	$n=85$
Secondary evaluation item (3)	$n=32$	$n=28$	$n=85$
Additional evaluation	$n=25$	$n=22$	$n=55$
Socket preservation			
Major evaluation items	$n=8$	$n=8$	$n=12$
Secondary evaluation item (1)	$n=7$	$n=7$	$n=12$
Secondary evaluation item (2)	$n=8$	$n=8$	$n=13$
Secondary evaluation item (3)	$n=7$	$n=7$	$n=13$
Additional evaluation	$n=6$	$n=6$	$n=10$
Cyst			
Major evaluation item	$n=5$	$n=5$	$n=6$
Secondary evaluation item	$n=5$	$n=5$	$n=6$
Alveolar cleft			
Major evaluation item	$n=8$	$n=8$	$n=9$
Secondary evaluation item	$n=8$	$n=8$	$n=9$

FAS: full analysis set; PPS: per protocol set.

Table 4. Summary of participating patients and institutions.

Institutions	Age	Patients	Male	Female	Oral and maxillofacial models (patients number)	Additional examination (patients number)
Tohoku University Hospital Akita University Hospital Yamagata University Hospital Keio University Hospital Yokohama General Hospital Tachikawa Hospital Shizuoka City Shimizu Hospital Nagasaki University Hospital Kagoshima University Hospital	54.0 (range, 7–68)	60	28	32	Sinus floor elevation in the 1-stage group (7) Sinus floor elevation in the 2-stage group (32) Socket preservation (8) Cyst (5) Alveolar cleft (8)	Sinus floor elevation in the 2-stage group (26) Socket preservation (7)

36.8–63.2) for FAS, 51.8% (95% CI, 38.0–65.3) for PPS, and 46.7% for Parts. Summary of this clinical trial is shown in Figure 4(c)–(f).

Sinus floor elevation in 1-stage group

In sinus floor elevation in the 1-stage group, as infection was confirmed in one case, the ratio of "good" with FAS or PPS in the major evaluation item was 85.7% (95% CI, 42.1–99.6) and that of Parts was 92.3%, while the "slight poor" ratios were 14.3% and 7.1%, respectively. The ratio of "good" with FAS or PPS in secondary item (1) was 100.0% (95% CI, 59.0–100.0) and that of Parts was 100.0%, whereas for secondary item (2), as the CT value of one case indicated approximately 70 HU, "good" ratio for FAS or PPS was 85.7% (95% CI, 42.1–99.6), and the average CT value was 266.9 ± 108.4 HU (standard deviation (SD)). For Parts, the "good" ratio was 92.9% (average CT value, 268.9 ± 81.9). In secondary item (3), average vertical bone widths before and at 24 weeks after OCP/Col implantation were 4.4 ± 1.3 mm (95% CI, 3.3–5.6) and 12.4 ± 2.1 mm (95% CI, 10.4–14.3), respectively, for FAS or PPS and 4.9 ± 1.5 mm and 13.0 ± 2.5 mm, respectively, for Parts. The average of the changes in vertical bone width was
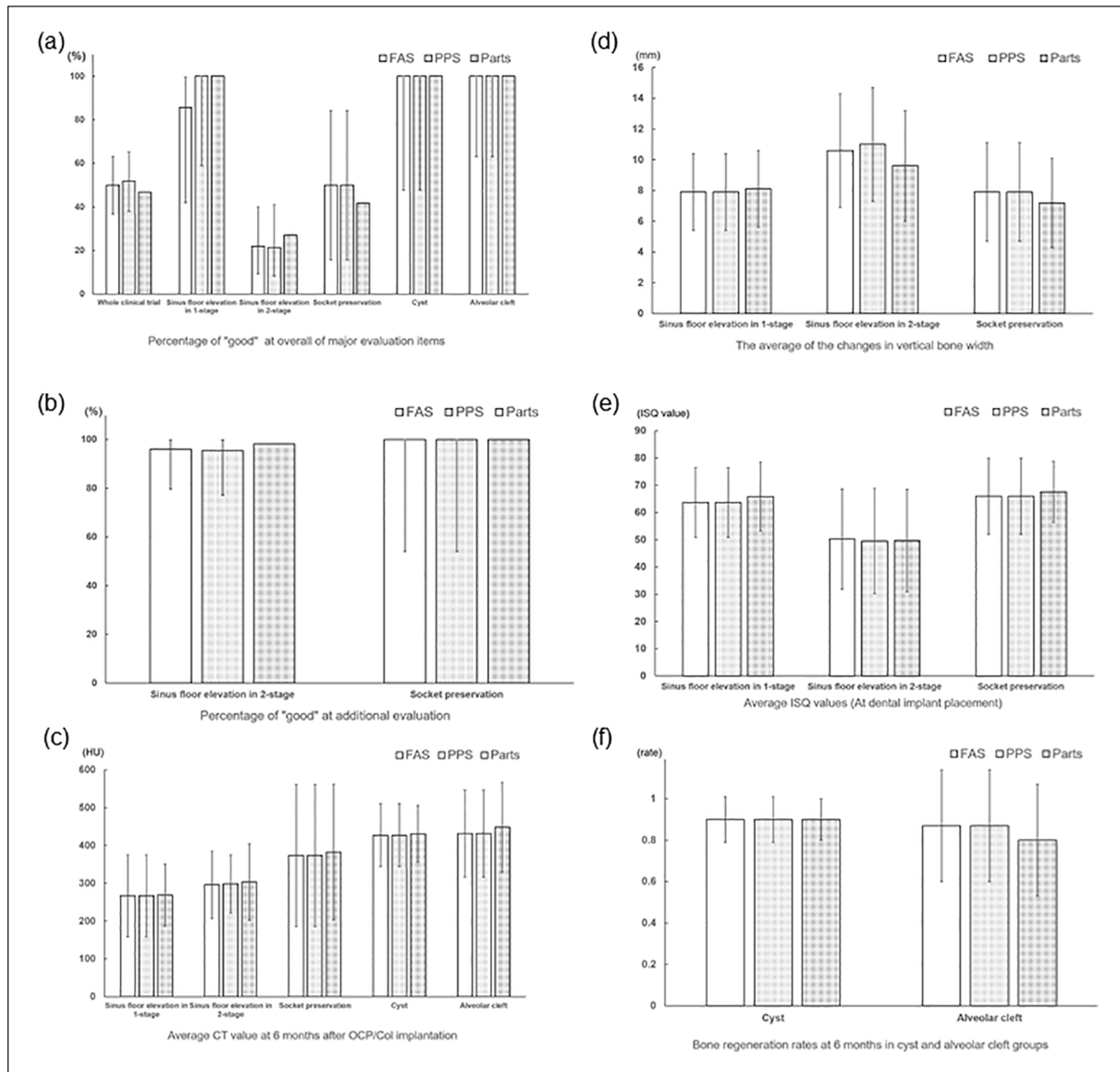
Figure 4. Results of clinical trials. (a) The percentage of "good" results overall for major evaluation items. For the judgment of "good" or "poor," evaluation items were set for each case. Cases of sinus floor elevation in the 1-stage group were evaluated based on the presence or absence of the findings in Table 2. Other groups were evaluated according to the measurement results such as CT values and torque value. Overall, the percentage of "good" of the whole clinical trial was less than 70% due to low results in the sinus floor elevation in the 1-stage and 2-stage groups. (b) The ratio of "good" in additional examination for the sinus floor elevation in 2-stage and socket preservation groups. Those results were more than 70% in both groups. Including these results of the additional examination, the percentage of "good" of the whole clinical trial was over 70%, and the criteria for judgment of success of this trial were met. (c) The results of average CT value 6 months after OCP/Col implantation in each group. All results were over 150 HU, which were considered normal cancellous bone. (d) The average of the changes in vertical bone width for dental implants. As the vertical bone width before OCP/Col implantation in sinus floor elevation in the 2-stage group was very thin, the amount of change increased compared with other groups. (e) The results of average ISQ values at dental implant placement. The value of sinus floor elevation in the 2-stage group was lower than in the other groups. This was probably because the majority was newly formed bone and less mature bone. (f) The results of bone regeneration rates after 6 months in cyst and alveolar cleft groups. The defects in both groups were nearly entirely replaced with newly formed bone. Error bars of (a) and (b) represent 95% CI of FAS and PPS, and error bars of (c)–(f) represent standard deviation. FAS, full analysis set; PPS, per protocol set; Parts, all teeth sites where OCP/Col was implanted.

7.9 ± 2.5 mm (95% CI, 5.6–10.3) and 8.1 ± 2.5 mm, respectively. For secondary item (4), average ISQ values immediately after dental implant placement and before installation

of the superstructure were 63.7 ± 12.8 (95% CI, 51.9–75.5) and 69.4 ± 9.6 (95% CI, 60.5–78.3), respectively, for FAS or PPS and 65.9 ± 12.6 and 70.3 ± 10.6, respectively, for
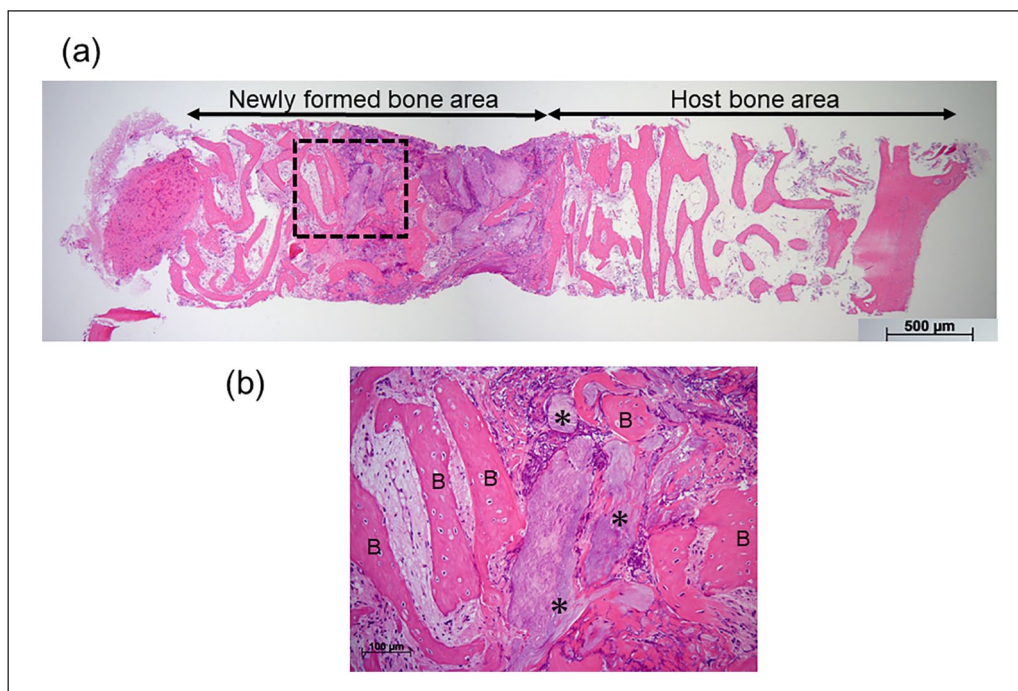
Figure 5. Histological images of sinus floor elevation in the 2-stage group. (a) Overview of a specimen stained with hematoxylin and eosin. Left bilateral arrow indicates the OCP/Col implantation area (newly formed bone area). Right side is host bone. Newly formed bone spread at OCP/Col implantation area. (b) Magnified view of a region marked by dotted square in (a). Newly formed bone was observed around remaining implants. There was no scar tissue or infiltration of inflammation cells.
*=remaining implant; B=newly formed bone.

Parts. There was a slightly poor judgment in one case, and the basis for the judgment was infection.

Figure 3(b)–(e) shows X-ray and CT pictures before and at 6 months after operation of a case. The distance from alveolar crest to sinus floor increased and there was no abnormal finding such as thickening of the sinus mucosa or absorption of surrounding bone.

Sinus floor elevation in 2-stage group

In the sinus floor elevation in the 2-stage group, six cases were indicated "poor" in histological analysis; however, these included four non-evaluated cases. Torque values of 25 cases were <20 N cm. The ratios of "good" for major evaluation items (1) and (2) and overall were 81.3% (95% CI, 63.6–92.8), 21.9% (95% CI, 9.3–40.0), and 21.9% (95% CI, 9.3–40.0), respectively, for FAS; 82.1% (95% CI, 63.1–93.9), 21.4% (95% CI, 8.3–41.0), and 21.4% (95% CI, 8.3–41.0), respectively, for PPS; and 81.8%, 27.1%, and 25.8%, respectively, for Parts. Figure 5 shows the specimen stained with hematoxylin and eosin. Newly formed bone was observed at OCP/Col implanted region without the infiltration of inflammation cells or scar. For secondary item (1), "good" ratio and average CT values were 96.7% (95% CI, 82.8–99.9) and 295.8 ± 89.1 HU, respectively, for FAS; 100.0% (95% CI, 87.7–100.0) and 298.3 ± 77.2 HU, respectively, for PPS; and 96.3% and 303.3 ± 101.5 HU,

respectively, for Parts. For secondary item (2), average vertical bone width before and at 24 weeks after OCP/Col implantation was 2.4 ± 1.3 (95% CI, 2.0–3.0) and 13.0 ± 3.8 (95% CI, 12.0–15.0) mm, respectively, for FAS; 2.6 ± 1.3 (95% CI, 2.0–3.0) and 13.5 ± 3.6 (95% CI, 12.0–16.0) mm, respectively, for PPS; and 3.5 ± 2.3 and 13.1 ± 3.5 mm, respectively, for Parts. Average changes in vertical bone width were 10.6 ± 3.7 (95% CI, 9.0–13.0), 11.0 ± 3.7 (95% CI, 9.0–14.0), and 9.6 ± 3.6 mm, for FAS, PPS, and Parts, respectively. For secondary item (3), average ISQ values immediately after dental implant placement were 50.2 ± 18.4 (95% CI, 42.9–57.5), 49.5 ± 19.3 (95% CI, 41.4–57.7), and 49.7 ± 18.8, respectively.

For additional item, one case was not evaluated, and this case was included in "poor." The ratios of "good" and "poor" were 96.0% (95% CI, 79.6–99.9) and 4.0%, respectively, for FAS; 95.5% (95% CI, 77.2–99.9) and 4.5%, respectively, for PPS; and 98.2% and 1.8%, respectively, for Parts.

Socket preservation

For socket preservation, one case was not evaluated histologically, and this case was included in "poor." Torque values of four cases were <20 N cm. The ratio of "good" for the major evaluation items (1) and (2) and overall was 87.5% (95% CI, 47.3–99.7), 50.0% (95% CI, 15.7–84.3),

and 50.0% (95% CI, 15.7–84.3), respectively, for FAS or PPS and 83.3%, 46.2%, and 41.7%, respectively, for Parts. For secondary item (1), the ratio of "good" and average CT values was 100.0% (95% CI, 59.0–100.0) and 373.4 ± 188.3 HU, respectively, for FAS or PPS and 100.0% and 382.8 ± 179.6 HU, respectively, for Parts. For secondary item (2), average vertical bone widths before and at 24 weeks after OCP/Col implantation were 11.0 ± 7.2 mm (95% CI, 5.0–17.0) and 18.9 ± 8.6 mm (95% CI, 11.7–26.1), respectively, for FAS or PPS and 12.6 ± 6.5 mm and 19.8 ± 7.8 mm, respectively, for Parts. Average change in vertical bone width was 7.9 ± 3.2 (95% CI, 5.2–10.5) for FAS or PPS and 7.2 ± 2.9 mm for Parts. For secondary item (3), average ISQ value immediately after dental implant placement at FAS or PPS was 66.0 ± 13.9 (95% CI, 53.2–78.8) or 67.6 ± 11.2, respectively.

For an additional item, the "good" ratio was 100.0% (95% CI, 54.1–100.0) for FAS or PPS and 100.0% for Parts.

Cyst

For cystectomy, the ratio of "good" with FAS or PPS in the major evaluation item was 100.0% (95% CI, 47.8–100.0), and the average CT value ± SD was 427.4 ± 83.3 HU, while those of Parts were 100.0% and 431.0 ± 75.0 HU. For secondary items, the "good" ratio and bone regeneration rates were 100.0% (95% CI, 47.8–100.0) and 0.90 ± 0.11 for FAS or PPS and 100.0% and 0.90 ± 0.10 for Parts.

Alveolar cleft

For alveolar cleft cases, the ratio of "good" and average CT values were 100.0% (95% CI, 63.1–100.0) and 431.8 ± 115.5 HU, respectively, for FAS or PPS in the major evaluation item and 100.0% and 448.2 ± 118.8 HU, respectively, for Parts. For secondary item, one case showed a bone regeneration rate of 0.32. The ratio of "good" and average rate was 87.5% (95% CI, 47.3–99.7) and 0.87 ± 0.27, respectively, for FAS or PPS and 88.9% and 0.80 ± 0.27, respectively, for Parts.

Discussion

Some bone substitute materials, such as HA, β-TCP, and xenogeneic grafts, are being currently used clinically.[1–4,40] However, they are not permitted for bone augmentation in dental implant treatment or alveolar defect according to Japan's Pharmaceutical Affairs Law. Moreover, the purpose of this study was to prove that the effectiveness was non-inferior in comparison with autologous bone or other materials for the approval of OCP/Col; this clinical trial was designed as a single-arm study because no approved

material for dental implant treatment was available during the trial in Japan and autologous bone was associated with secondary invasion and infection risk. Recently, Cytrans® (carbonate apatite; GC, Tokyo, Japan) has been approved for dental implant treatment in Japan.[41] However, bone substitute material has not yet been approved for alveolar defect. In this clinical trial, evaluations of the newly formed bone was performed approximately 6 months after OCP/Col implantation because previous studies have demonstrated that bone formation by OCP/Col was sufficiently confirmed at 6 months after implantation.[25] Because the evaluation items were different for each subject, the target number of subjects was set to 60 as the number that could be conducted when considering the fluctuation of the number of analysis groups and omission.

In this clinical trial, the sinus floor elevation groups contained many cases and large bone augmentation volumes. Because the survival rate of dental implants with sinus floor elevation procedures using autologous bone was reported to be 61.2%–100% (median, 80.6%),[39] the criteria for judgment of success of this clinical trial were set if the percentage of "good" was ≥70%.

The rate of "good" in major evaluation items of the whole clinical trial was <70% (50% for FAS, 51.8% for PPS, and 46.7% for Parts), and this result did not meet the criteria for judgment of success in this clinical trial. The subjects who did not meet such criteria underwent sinus floor elevation in 2-stage and socket preservation procedures. Major evaluation item (1) (biopsy diagnosis) met the criteria, but major evaluation item (2) (maximum torque value) did not. However, the additional items met the criteria for judgment of success in each subject. "Maximum torque value" sometimes has been used as an index indicating the degree of initial fixation of the dental implant. The timing of superstructure installation and loading must be decided according to cases, and the initial fixation has not been required depending on the timing of loading. Therefore, it was considered that the success or failure of the dental implant treatment was not judged by the maximum torque value. Regarding autogenous bone grafting as a conventional treatment method, it has also been reported that maximum torque values have been <20 N cm in the bone augmentation region. Ogawa et al.[42] have reported no failure at an average of 3.9 years of observation in seven cases after dental implant placement at the augmented region by sinus floor elevation using autologous bone and platelet-rich plasma if the maximum torque values were <20 N cm. Norton[43] has reported that the maximum torque values of 68 dental implants at placement without the necessity of bone augmentation were ≥25 N cm in 47 bodies, ≥20 N cm in 11, ≥15 N cm in 7, and ≥10 N cm in 3, and the failures of dental implant treatment included 1 body of ≥25 N cm and 1 of ≥15 N cm. Based on these findings, it was judged that the success of dental implant treatment was more appropriate than the

maximum torque value as a judgment criterion for the success of sinus floor elevation at 2-stage and socket preservation procedures. Therefore, if the additional evaluation item was included, the percentage judged to be "good" in the major evaluation items was 85.7% by sinus floor elevation in 1-stage, 96.0% by sinus floor elevation in 2-stage, 100.0% by socket preservation, 100.0% by cyst, and 100.0% by alveolar cleft, and these met the criteria for judgment of success of this clinical trial.

Since the maximum torque value at dental implant placement did not have sufficient strength required for the initial fixation, it was thought that the appropriate loading timing was selected after sufficient consideration of bone quality and bone volume as well as existing therapy. If the appropriate judgment could not be done, there was a possibility of dropping the dental implant and reoperation would be necessary. However, the effectiveness of OCP/Col was confirmed, and the use of OCP/Col could reduce the amount of autologous bone harvesting and minimize invasion at secondary operative sites. Since the therapeutic effect in cystectomy and alveolar cleft defect was also confirmed, the anticipated benefit could be thought to exceed the assumed risk.

Histological examination was performed in 40 cases of sinus floor elevation in 2-stage and socket preservation groups. Six cases of sinus floor elevation in the 2-stage group and one case of socket preservation group were indicated to be "poor" in evaluation. However, these seven cases included five cases that were not evaluated histologically, and two indicating abnormal findings were considered to be caused by inflammation or absorption of OCP/Col. Remaining implants were observed with newly formed bone. Previous studies have indicated that the remaining implants are converted to biological apatite, leaving no OCP feature.[25,26]

CT value was used for the evaluation of bone formation in this clinical trial. CT value of OCP or OCP/Col was approximately 130–140 HU.[33] It has been defined that the CT value of bone capable of dental implant placement is 150 HU or more.[44] Therefore, it was considered that conversion to bone-like tissue occurs if the CT value increases.

The patients undergoing dental implant treatment with sinus floor elevation or socket preservation were of older age and included female patients. Considering the effects of bone metabolism and menopause, these factors generally were considered to be disadvantageous for bone regeneration.[45,46] However, sufficient efficacy was confirmed in this clinical trial, and it was considered that the influence of age and sex was low. Also, as bone grafting to the alveolar cleft defect is mainly used in children, OCP/Col has been used for young patients and the effect was confirmed in this clinical trial. These results demonstrated that OCP/Col could be applicable to young and elderly patients. Miura et al.[47] have reported a summary of sinus floor elevation cases conducted in Nagasaki University in

this trial. Furthermore, this study also demonstrated the effectiveness in the field of oral and maxillofacial surgery including other cases.

Adverse events appeared in all 60 patients who underwent OCP/Col implantation. However, all the events were listed in this clinical trial, and most of them were pain, swelling, increasing white blood cell count, and increasing C-reactive protein levels; these also occur during normal treatment. We considered that there were no special adverse events due to OCP/Col because there were no lethal or serious adverse events. Two cases showed OCP/Col leakage that was considered to be caused by insufficient closure (suturing) of the wound site, and we judged that there was no causal relationship with OCP/Col. From these results, we suggest that the use of OCP/Col as a bone substitute material is safe for oral and maxillofacial surgery.

Conclusion

In this study, OCP/Col was used for bone augmentation as a clinical trial in sinus floor elevation in 1- and 2-stage, socket preservation, cyst, and alveolar cleft cases, and the effectiveness and safety of OCP/Col were evaluated. Although sinus floor elevation in 1-stage, cyst, and alveolar cleft cases met the criteria for the judgment of success, sinus floor elevation in 2-stage and socket preservation groups did not meet the criteria. However, additional evaluation revealed the effectiveness of OCP/Col, and these groups also met the criteria for judgment of success. As a result, it was suggested that bone augmentation by OCP/Col was effective for clinical use.

Author contributions

T.K. contributed to conception, design, surgery, data collection, analysis, interpretation, writing the original draft, and final approval of the version to be submitted. S.K. contributed to conception, design, analysis, interpretation, critically revised manuscript, and final approval of the version to be submitted. K.M. contributed to conception, design, analysis, critically revised manuscript, and final approval of the version to be submitted. M.F. contributed to conception, design, surgery, data collection, and final approval of the version to be submitted. H.T. contributed to surgery, data collection, and final approval of the version to be submitted. M.I. contributed to conception, design, surgery, data collection, and final approval of the version to be submitted. S.I. contributed to surgery, data collection, and final

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ORCID iD

Tadashi Kawai 🆔 https://orcid.org/0000-0002-2712-1895

Supplemental material

Supplemental material for this article is available online.

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