Mini Review

Tissue engineered bone; Application for implant surgery

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The concept of bone tissue engineering, which began in the early 1980s, has seen tremendous growth in the numbers of research studies. One of the key areas of research has been in the field of implant surgery, where the challenge is to produce the perfect tissue-engineered bone construct. This practical review summarizes applied state-of-the-art research in the important translational research that has already been initiated in Nagoya University Hospital. The topics that will be covered include, clinical applications in alveolar regeneration for dental implant, tumors surgery and distraction osteogenesis. Although significant challenges remain, there exists an exceptional opportunity to translate basic research in tissue engineering technologies into viable clinical treatments for bone regeneration in implant surgery.

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INTRODUCTION

Implant surgeons face oftenly the clinical situation in which bone regeneration is needed. In Japan alone, the number of bone grafting procedures is estimated to be 50000 per year. The tremendous need for bone tissue in numerous clinical situations and the limited availability of suitable bone grafts are driving the development of new approaches to bone repair. In the past the "gold standard" bone graft materials is autologous bone graft and this is limited in supply and its harvesting is associated with significant morbidity[1,2]. Approximately 8% of iliac grafts result in major complications such as infection, blood lose, nerve injury, short and long term-pain, and functional deficit.

The use of allo-grafts avoids donor site issues but these grafts are associated with risks of infection and possible immune response of the most tissue[3], which can lead to high rates of complications[4-6]. Thus, there is a trend toward tissue engineering as an alternative to the traditional techniques in bone repair. Langer and Vacanti defined tissue engineering as "an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ[7]".

Regeneration of the bone tissue is the most studied field in tissue engineering. According to the concept, equivalents of the bone tissue can be obtained by targeted osteo-genic differentiation of multiponent mesenchymal stem cells (MSC) of the bone marrow (BM). MSC predifferentiated towards osteogenic lineage are applied on biocompossible materials maintaining osteoinduction and possessing sufficient osteoconductive properties[8] transplanted into the bone defect area.

The first clinical attempt to use the osteogenic bone marrow Stem Cell (MSC) in human was in 1980 when Quatro et al. reported the successful use of expanded autologous MSC seeded on macroporous (HA) scaffolds, in combination with external fixation, in the treatment of three patients with large segmental bone defects (range, 4-7cm). In Japan, Ohgushi et al. proposed the concept of hard tissue repair consisting of culture expansion of marrow-derived MSC, differentiation of these MSC into osteoblasts, and then fabrication of bone matrix on various substrata. They have shown that the cultured bone demonstrated immediate bone-forming capability after in vivo implantation; these researchers have used this concept in clinical applications since 2002. An example of this is the implantation of cell-seeded alumina ceramic total ankle prostheses in patients to facilitate osseointegration.

In the field of oral region, creation of bone equivalents have been aggressively beyond the scope of experimental numerous experimental study the possibility of effective reconstructed the bone tissue using various biodegradable material and MSC[9-11]. Because the required bone tissue in this field is relatively small compared with that in other fields.

The tissue engineered bone carrying MSC from the BM tissue was performed for implant surgery at the department of Oral Surgery in Nagoya University Hospital from 2002 in accordance with the research protocol approved by the Nagoya University Ethics committee (Permission No.172) and in compliance with Helsinki Declaration (2000).

In this review article we present the human application of tissue engineered bone in oral maxillofacial region and its clinical usability will be discussed.

TISSUE ENGINEERED BONE

Cell Preparation

Mesenchymal stem cells (MSC) were isolated from the patient's iliac crest marrow aspirates (10 mL) according to the reported method[12]. Briefly, the basal medium, low-glucose Dulbeccdo's Modified Eagle's Medium, and growth supplements (50 mL of serum, 10 mL of 200 mML-glutamine, and 0.5 mL of penicillin-streptomycin mixture containing 25 units of penicillin and 25 μg of streptomycin) were purchased from Cambrex Inc. (Walkersville, MD). Three supplements, dexamethasone, sodium β-glycerophosphate, and L-ascorbic acid 2-phosphate, for inducing osteogenesis were purchased from Sigma Chemical Co. (St. Louis, MO). The cells were incubated at 37°C in a humidified atmosphere containing 95% air and 5% CO^2. The MSCs were replated at densities of 3.1×10^3 cells/cm^2 in 0.2 mL/cm^2 of control medium. The differentiated MSCs were confirmed by detecting alkaline phosphatase activity using *p*nitrophenylphosphatase as a substrate.

In culture, MSCs were trypsinized and used for implanting. For the safety of cultured cell, the culture media were examined for contaminations of bacterium, fungus, and mycoplasma before transplantation.

Platelet-Rich Plasma Preparation

Preoperative hematological assessments included a complete blood count with platelet levels. The result-

ing pellet of platelets (PRP) was extracted 1 day before surgery. The PRP was isolated in a 200-mL collection bag containing the anticoagulant citrate under a sterilized condition at the blood transfusion service department of Nagoya University Hospital, Japan. Briefly, the blood was first centrifuged for 10 minutes at 350*g*. Subsequently, the yellow plasma containing the buffy coat, which contained the platelets and leukocytes, was removed. A second centrifugation at 3500*g* for 10 minutes was performed to combine the platelets into a single pellet and the plasma supernatant, which was plateletpoor plasma and contained relatively few cells, was removed. The buffy coat/ plasma fraction (PRP) was resuspended in 20 mL of residual plasma and used in the platelet gel.

Tissue Engineered Bone Preparation

The PRP was stored at 22°C in a conventional shaker until used. Human thrombin in a powder form (5000 units) was dissolved in 5 mL of 10% calcium chloride in a separate sterile cup. Next, 3.5 mL of PRP, MSCs (1.0 ×107 cell/mL), and air were aspirated into a 5-mL sterile syringe. In a second 2.5 mL syringe, 500 µL of the thrombin/calcium chloride mixture was aspirated. The cells were resuspended directly into the PRP. The 2 syringes were connected with a T connector and the plungers of the syringes were alternatively pushed and pulled allowing the air bubble to transverse the 2 syringes. Within 5 to 30 seconds, the contents assumed a gel-like consistency as the thrombin affected the polymerization of the fibrin to produce an insoluble gel. (Fig. 1)

TISSUE ENGINEERED BONE AND IMPLANT

In the field of implant surgery, bone availability is the key to successful placement of endosseous implants in the posterior maxilla and mandible. When the thickness of the bone between the sinus and alveolar crest is less than 5 mm, increasing the thickness of the alveolar sinus floor through grafting is necessary to support the required length of implants. On the other hand, the distance from the mandibular canal is a critical condition to avoid serious nerve injury during implant installation. In a case with insufficient alveolar bone, vertical ridge augmentation through onlay grafting is needed to increase the alveolar bone height.

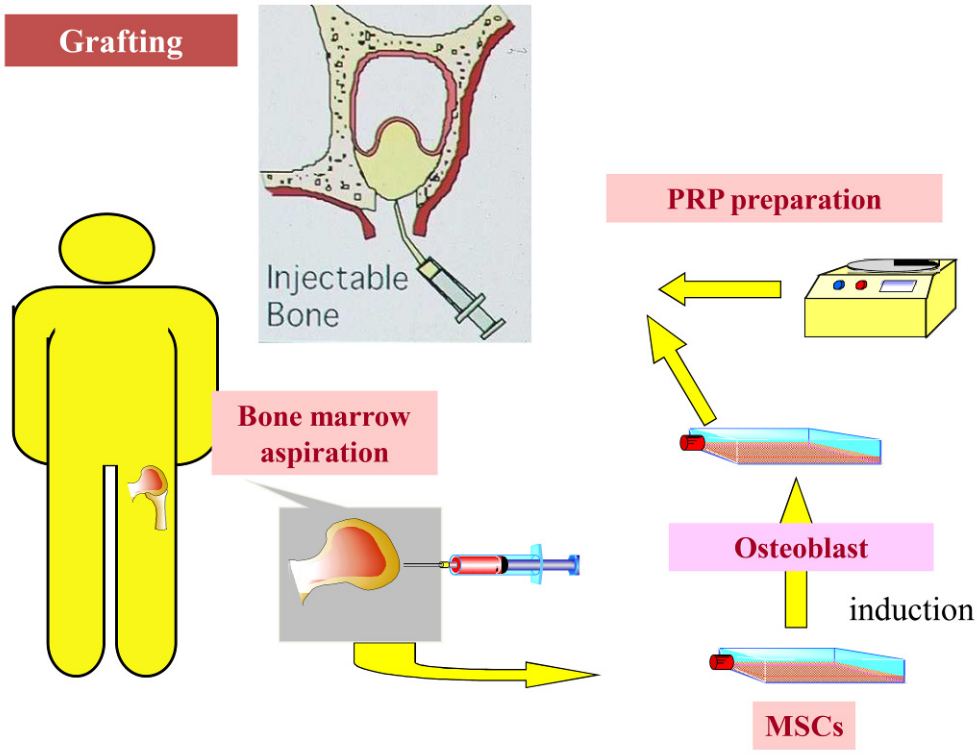

Fig. 1

Because of these circumstances, we attempted to regenerate bone in a significant osseous defect with minimal invasiveness and good plasticity, and to provide a clinical alternative to the previously graft materials. The new technology 'Tissue Engineered Bone (TEB)' that we developed is so called '"injectable bone,"[13,14] and involves the morphogenesis of new tissue using constructs formed from isolated cells with biocompatible scaffolds and growth factors.

We evaluate the clinical results, after functional loading, periimplant tissues of titanium fixtures that had been placed in regions augmented using the injectable bone.

Patient Selection

Sixteen sinus augmentation in 12 patients, partially or totally edentulous patients 44 to 60 years of age (mean age 54 years), were enrolled in this study to undergo maxillary sinus floor of denture retention due to severe posterior alveolar ridge atroply; the average height of their residual sinus floor was <6 mm, for which sinus graft and dental implants would solve the problem (Table 1).

After routine oral and physical examinations, patients who did not desire to undergo any surgery for harvesting of the autogenous bone were selected for injectable tissue bone grafting. They were health and free of any disease that might affect treatment treatment outcome (*e.g.*, diabetes, immunosuppressive chemotherapy, chronic sinus inflammation, rheumatoid arthritis). Each patient was given detailed information about the intervention, including surgical techniquesm types of graft material and dental implants, and uncertainties of conducting a new bone-regenerative procedure.

Surgical Technique

Sinus augmentations was conducted under general anesthesia. The sinus grafting procedure followed Tatum's classical description. Briefly, the mucoperiosteal flap was elevated to create a trap door with a round hollow bur in the lateral wall of the maxillary sinus. After mobilization, the door was reflected inward. The space created by this procedure was filled with 1.8 - 5.4g of injectable tissue-engineered bone to simultaneously place dental implants. The mucoperiosteal flap was repositioned and sutured in the usual manner. After surgery, patients received cepha-losporins (300mg/day; Shinogi, Osaka, Japan) as antibiostics, and loxoporins sodium (180mg/day; Daiichi Sankyo, Tokyo, Japan) as analgesisics for 3 days.

Examination of Regenerated Bone

Orthpantomograms, which are the most frequently used radiograms, were obtained to determine and evaluate the vertical bone height that measured the radiographic distance from the alveolar bone crest to the line of substratum of the maxillary sinus mucosa at the planned implant location (preoperative bone height); the height was confirmed while drikking during the first surgery. After first-stage surgery, and increase in mineralized tissue height was verified by using the length of the place implant as the standard reference. The increase in height of the mineralized tissue content (mineralized tissue height - preoperative bone height) was measured at 3, 6, 12, and 24 months post-operation using orthopantomograms referring to computed tomography (CT) scans (slice thickness: 1 mm). In order to correct dimensional distortion, the apparent dimension of each implant was measured on the radiograph and compared with the real implant length. The radiographic vertical height at the implant site was additionally assessed more accurately on CT scans to confirm regenerated bone height (Table1). Further, the mucoperospsteal flap was reflected to conduct the precise evaluation, and bone biopsies were obtained for histology with a 2-mm diameter trephine bar at the regenerated bone areas during second-stage surgery. Paraffin sections were prepared according to standard protocols, and hematoxylin-eosin staining was performed.

Statistics

Statistical evaluation was performed according to the paired, two-tailed students's-test between BMDSCs and differentiated BMDSCs. A p-value of <0.01 indicated statistical analyses of regenerated bone height with significance set at $P<0.01$.

Clinical and Radiographic Observation

None of the patients had postoperative problems beside normal swelling and inflammation of the surgical site. Perforation of the sinus membrane a rare complication observed during surgery, was documented in four procedures, and provoked only minor postoperative nasal bleeding without severe signs of inflammation of the maxillary region throughout the observation period. Injetable tissue-engineered bine was used to place 1 dental implants. The graft site was observed clinically. Cumulative survival and success

rates of the fixture placed concurrently with injectable tissue-engineered bone were 100%. Postoperative radiographic findings consistently indicated osseointegration between dental implants and regenerated bone. Further, the CT scans obtained after tissue-engneered bpne grafting showed dense mineralized material in the sinus cavities surrounding the implant. In all patients, it was difficult to delineate the borderlines between the original sinus floor and newly formed tissue, and that bo adverse effects and remarkable bone absorption were seen in the 2-6 uear follow-up time. Consecutive images also clearly revealed changes in the graft materials and their incorporation. Together with the absorption of tissue-engineered bone and the simultaneous formation of new absorption of tissue-engineered bone and the simultaneous formation of new bone, the graft was to the bone. Pre and postoperative radiographic evaluations disclosed increases in mineralized tissue, and the value of regenerated bone at 3, 6, 12, and 24 months is 3.8±1.8, 7.4±1.8, 8.9±1.6, 8.8±1.6mm, respectively. The regenerated bone height for 3, 6, 12, and 24 months was found to be significantly higher, ($p<0.01$) than preoperative bone height. During second-stage surgery, which was conducted after the mean healing period of 6.4 months, the mucoperiosteal flap was evaluated relatively widely to observe the graft site. Further, the average follow-up period after first surgery was 3.5 years, and the implants were stable (Table1). None of the patients withdrew from this study.

Two representative patients among those who were enrolled in this study are described below.

	Age (y)	Sex	Location	Operation	Number of Implants
1	51	F	76 67|	Maxillary Sinus Lift	6
2	60	F	567	Maxillary Sinus Lift	3
3	44	F	76	Maxillary Sinus Lift	2
4	54	F	765|567	Maxillary Sinus Lift	6
5	50	F	654|	Maxillary Sinus Lift	3
6	56	F	|567	Maxillary Sinus Lift	3
7	52	F	76 |	Onlay graft	3
8	74	M	7654|	Onlay graft	4
9	54	F	$\overline{76\|}$	Onlay graft	3
10	54	M	$\overline{32\|}$	Onlay graft	2
11	54	F	32|	Onlay graft	2
12	58	F	$\overline{7654\|}$	Onlay graft	4
13	52	F	$\overline{5\text{-}2\|2\text{-}5}$	Onlay graft	8
14	52	F	$\overline{5\text{-}1\|1\text{-}5}$	Onlay graft	8

Table 1. Patient Data

Case 1

A patient was a 58-year-old woman with the edentulous left maxilla (No.10 in Table 1). Physical examination revealed severe maxillary atrophy, and the maxillary bone was insufficient to place dental

implants (Fig. 2A). Afrer maxillary sinus floor augmentation, implants were placed into each alveolar ridge of the maxilla. However, the fixtures remained exposed in the sinus cavity (Fig. 2B). Injectable tissue-engineered bone was injected into the maxillary sinus and the periphery of the fixtures to completely cover the exposed thread (Fig. 2C). Progressive bone regeneration was observed at monts after sugery. (Fig. 2D). Resin prostheses were anchored on dental implants (Fig. 2E). Bone area that had shown insufficient bone mass for implant placement (radiolucency; Fig. 2G) radiopacity was enhanced by tissue-engineered bone-induced bone regeneration (Fig. 2H). Bone regeneration could thus be verified radiographically.

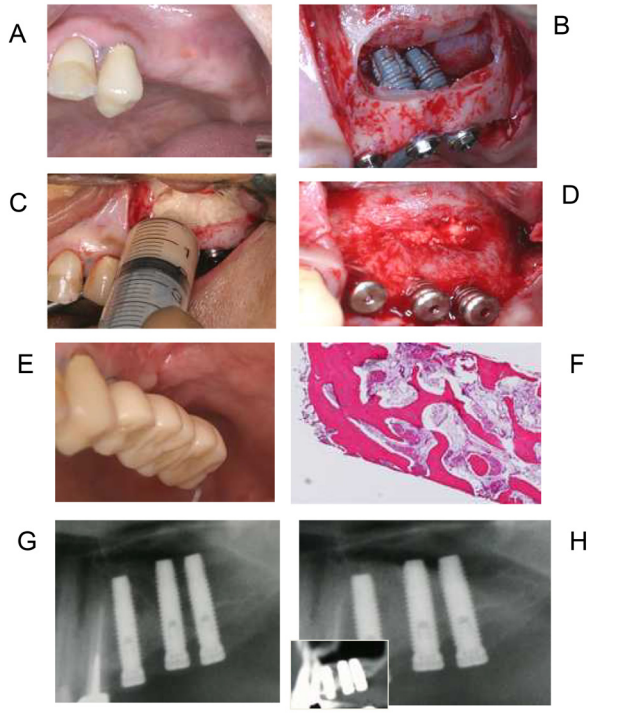

Fig. 2

Case 2

An edentulous 54-year-old woman (No.6 in Table1), showed an insufficient bone height (about 4 mm) of the maxillary sinus floor. A total of 10 dental implant were place into the implantation site (Fig. 3A). The proper position of each implant was confirmed, and tissue-engineered bone was then carefully injected to fill the lateral portion of the maxillary sinus and the exposed area of the implants (Fig. 3B). Second-stage surgery was conducted 8 months later. The exposed thread was surrounded by newly formed bone and successful osseointegration was confirmed clinically (Fig. 3C). A porcelain fused to the metal crown was then fitted (Fig. 3E). The 4.3- year follow-up showed no signs or symptoms of implant failure. Radiographic examination (CT and orthopantomogram) revealed that the exposed thread in the sinus cavity was surrounded by newly formed bone and reached the tip of the fixture gradually (Fig. 3D-J).

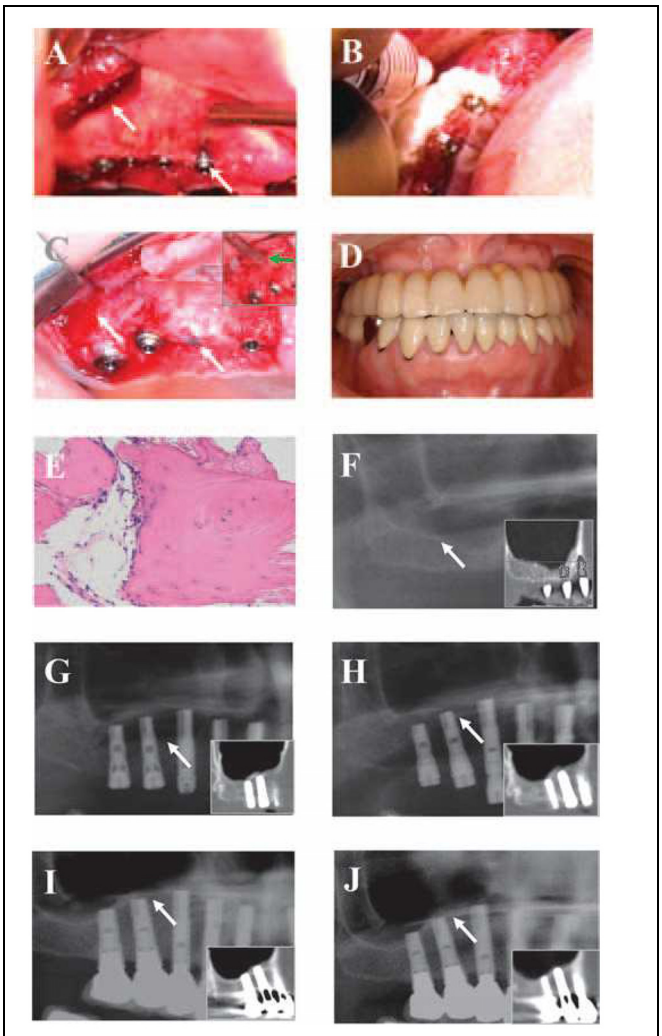

Fig. 3

Tissue Engineered Bone Creats Alveolar Ridge For Implant Installation

This study evaluated the performance of an injectable bone in 1-stage alveolar augmentation with simultaneous implant placement. As a general consensus, the 1-step procedure should be reserved for patients who have at least 5 mm of alveolar bone in the posterior maxilla or mandible to stabilize the implants. If there is less than 5 mm of available host bone, it is

insufficient to mechanically maintain the endosteal implants, and thus the 2-step procedure combined with augmenta-these patients[16-18]. On the other hand, the 1-step procedure offers the advantages of less surgical treatment for the patient and coordinated consolidation of the graft around the implants during healing, thus reducing the surgical and healing times for the patient. Another advantage is that it not only eliminates the need to harvest autogenous bone via its inherent morbidity, but also decreases the surgical recovery time[19]. In this study, all cases of posterior maxilla had more than 5 mm in the sinus floor and in the mandible. The patients underwent the 1-step augmentation procedure with TIE application and simultaneous implant placement. The macro findings showed that TEB induced bone regeneration and the dental implant thread was not exposed.

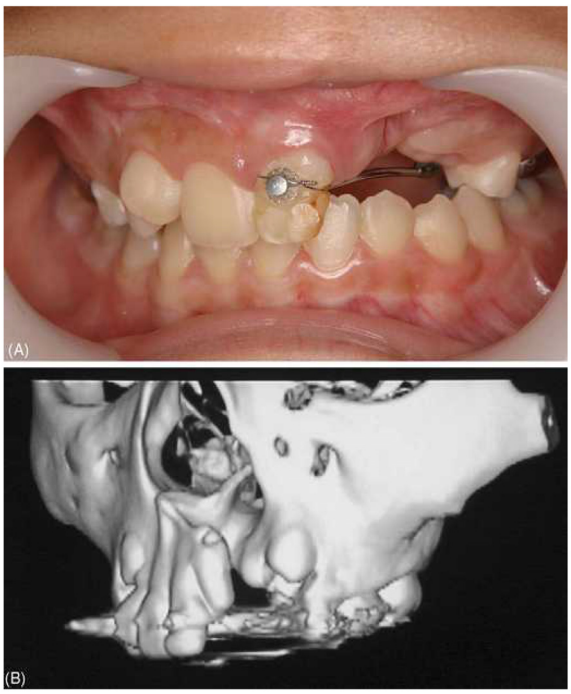

Fig. 4

Thus, these results indicate that ridge augmentation caused by TEB and that simultaneous implantation is possible. The results of this study provide evidence of the safety and technical feasibility of TEB for maxillary sinus floor augmentation and vertical ridge augmentation in agreement with those from earlier animal studies that have indicated that treatment with TEB dose not result in toxicity, significant immunologic reactions, or other serious adverse effects[20-23]. Adverse experiences (*e.g.*, pain, swelling after operation) observed with TEB were consistent with the usual morbidity observed in the maxillary sinus floor augmentation procedure and vertical ridge augmentation. Radiographic assessments indicated that TEB induced new bone growth in the maxillary sinus floor in 100% of the patients treated, and showed 8.7 mm mean increase in mineralized tissue. In the meantime, in clinical human testing, protruding into the sinus cavity stimulated reactive bone regeneration by human bone morphogenetic protein-2 that is limited to 8.51 mm in height[24]. This is almost the same as that regenerated by TEB in this study. Furthermore, in the case of vertical ridge augmentation the mean increase of mineralized tissue was 5 mm, which was affected by the stability of the grafted area. These effects might be dependent on MSCs and PRP. The MSCs in the bone marrow are induced into cells with osteogenic capacity, the MSCs are considered to be more feasible for this tissue engineering because the former proliferates faster because of a lower degree of differentiation. In addition, the PRP contains not only fibrinogen that forms a fibrin network acting as a matrix but also cytokinetic substances such as platelet-derived growth factor, transforming growth factor beta, and fibroblast growth factor. These growth factors contribute to cellular proliferation, matrix formation, collagen synthesis, osteoid production, and other processes that accelerate tissue regeneration.

ALVEOLAR CLEFT OSTEOPLASTY

The reconstruction of alveolar cleft defects is well established, with the most widely accepted approach being secondary alveolar cleft osteoplasty in the mixed dentition phase with autologous bone grafting [25, 26]. The source material for most bone grafts has been particulate marrow harvested from the anterior iliac crest, and this represents the standard material with which other materials from rib, mandible, calvarium, and tibia are compared[25,26,27]. Donorsite morbidity is an important factor in deciding the site for harvesting cancellous bone. Osteoinductive agents such as recombinant human bone morphogenetic protein-[28,29,30] can solve these problems and are expected to be used clinically in the future. As another solution, the use of tissue-engineered bone in bone augmentation procedures as a replacement for autologous bone grafts, offers predictable results with minimal donor-site morbidity[31,32]. Here we report a

technique and case of alveolar cleft osteoplasty using tissue engineered bone.

Case Report

A 3-month-old female patient born with a congenital left unilateral cleft lip and alveolus underwent a cheiloplasty at that had resulted in no remaining oronasal fistula. At 9 years of age, computed tomograms (CTs) revealed that the left maxillary canine, lateral, and supernumerary incisors had formed half of their roots, and that they closely surrounded the alveolar cleft bony defect which was 10 mm wide and 13 mm deep anteroposteriorly (Fig. 4). The left central incisor was orthodontically overcorrected due to previous severe rotation and distal location. When secondary alveolar cleft osteoplasty was indicated, the patient and her parents were informed about the nature of the TEB, and they granted their consent.

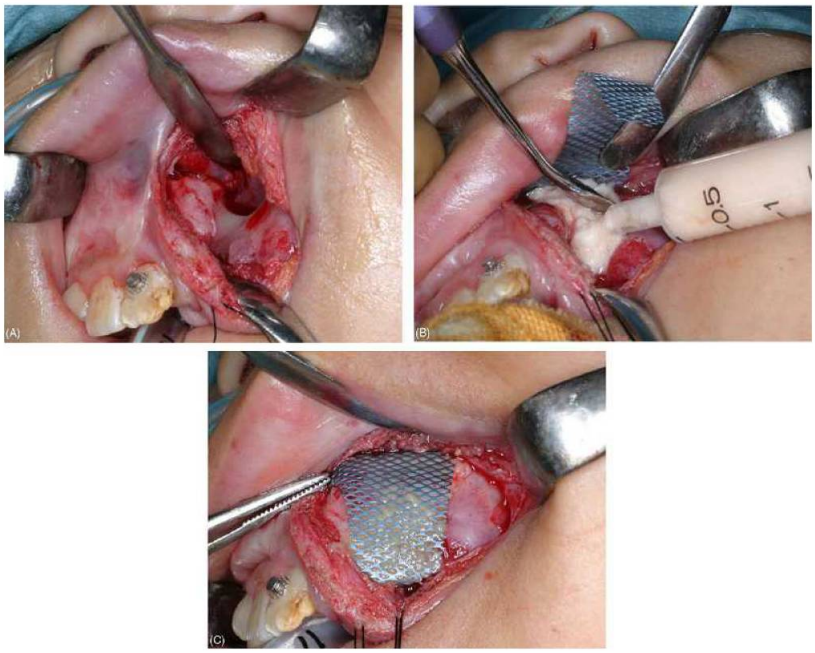

Fig. 5

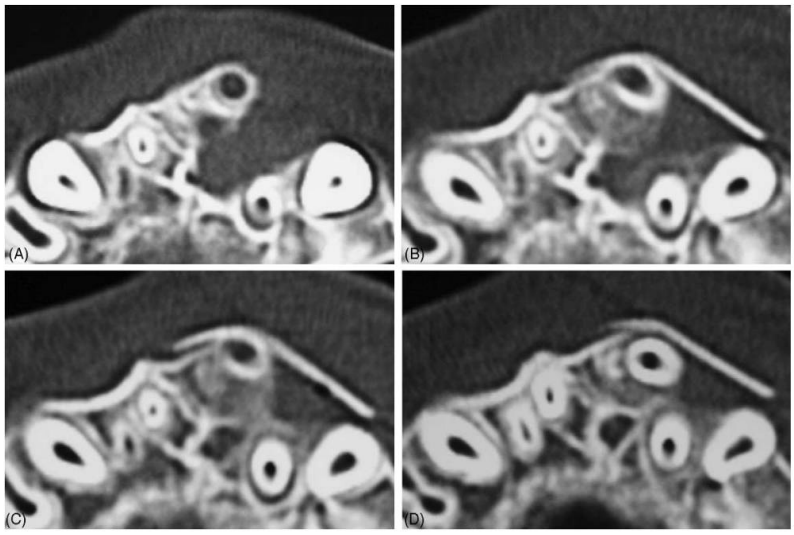

Fig. 6

Following a 3-cm-long mucosal incision at the level of the labiogingival junction, dissections were made in the ingrown scar tissue to reach the bony surface of the cleft walls. The tissue was then elevated in the subperiosteal plane to the levels of the anterior nasal spine anteriorly, whilst taking care not to damage the unerupted teeth and the content of the incisive canal. The flaps of the nasal floor and the oral mucosa formed the ceiling and the floor of the cleft cavity, respectively. The ceiling, floor and front walls of the defect were supported with a 0.1-mm-thick titanium-mesh plate(Stryker, Kalamazoo, MI). The thus-created pouch was filled with all the prepared TEOM through a syringe using a packer (Fig. 5). Following release incisions in the periosteum and the scar tissue of the flaps and to allow them to cover the graft area, the wound was consequently closed without tension.

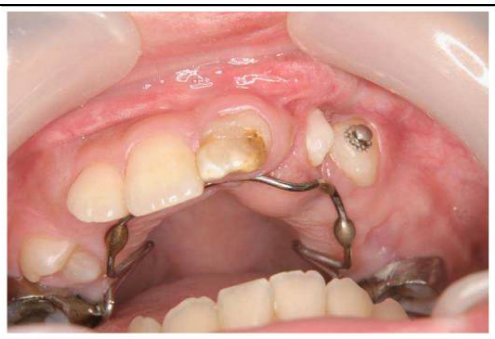

Fig. 7

The patient exhibited an uneventful postoperative course. The radiopacity of serial CTs slicing the middle level of the alveolar cleft in the grafted region increased gradually over the time (Fig. 6). Dome-shaped radiopaque images with 233 Hounsfield units (HU) faced together and extended from the cleft bony walls inside the cavity after 3 months, and were fused together into an image with 324 HU after 6 months. The image increased in radiopacity to 447 HU in 9 months, and at the bony bridge the lateral and supernumerary incisors horizontally approximated from their original positions in the respective major and minor segments.

The incisive canal was reconstructed just medial to the bridge. The erupting canine and lateral incisor pushed the mesh plate vertically, and the mucosa covering the cleft consequently swelled and thinned. A mucosal cut was made in the crest of the alveolar ridge over these teeth, and the part of the plate overlying the teeth was removed under local anesthesia. The canine and the lateral incisor then erupted approximately at the same time (Fig. 7).

Tissue Engineered Bone Solves Donor Site Problems

TEB regenerated the bone in the alveolar cleft defect without donor-site morbidity resulting from the autologous bone graft. Grafted bone remodels new bone due to apposition following resorption, and VAN DER MEIJ et al.[33] reported that 1-year postoperative volumetric rates were approximately 70% for secondary bone grafts before canine eruption. Using their measuring method at 9 months postoperatively the present case showed 79.1% regenerated bone. They also stated that the eruption of the canine generally occurred 2 years after bone graft if the patient was 9-years-old. A high resorbability of the bone in the grafted region may result in the early eruption of canine. In the present case the canine coronally forced the mesh plate at 9 months postoperatively, which was earlier than expected. As the bone regenerated in the cleft defect, the ingrowing bone seemed to accompany the roots of not only the canine but also the lateral and supernumerary incisors, which consequently approximated and erupted. Bone regeneration with the Tissue Engineered Bone may therefore, have helped to induce teeth to reposition properly in the horizontal and vertical planes. The mucoperiosteal flaps require the support in proper reconstruction of alveolar morphology, and hence the TIME technique [34] was indicated for the present simple cleft without palatal defect or oronasal fistula. The titanium mesh plate facilitated a rigid space without disturbing the blood supply from the overlying flaps, but needed to be removed before tooth eruption. Resorbable membranes solve this problem but inhibit the blood supply. The skeletal frame or carriers of biodegradable material such as polylactide polymer or collagen may serve as another solution[35,36]. Distraction of the transport bony segment has been attempted for closing alveolar defects[37]. The defects are actually only reduced and not eliminated, and the teeth in the transport segment also moved unintentionally according to the distraction. Some alteration in teeth positions may be beneficial, but others compromise crown morphology or require its recontouring. The bone transport in repair of the alveolar cleft therefore remains controversial. The Tissue Engineered Bone thus shows promise with further perspectives. Younger patients have more MSCs, and

their harvesting, isolation and cryopreservation allows Tissue Engineered Bone to be supplied repeatedly when needed. This repeatability will facilitate the sequential treatments of cleft patients in the future.

DISTRACTION OSTEOGENESIS ASSISTED BY TISSUE TENGINEERING BONE

Distraction osteogenesis (DO) has become a widely accepted technique for reconstructing bone defects in the maxillofacial region. This technique provides predictable bone formation without gafting procedures but requires a long healing time which includes latent, lengthening, and consolidation periods. To promote bone formation and shorten the consolidation period, some attempts at applying hyperbaric oxygenation or electrical, ultra-sonic, or chemical stimulation have been made[38].

Sev-eral recent studies have shown that injecting cells with osteogenic potential into distracted callus enhances its consolidation[39-42].

Not only animal studies but also clinical trials have demonstrated that tissue engineered bone can effectively regenerate osseous tissue. It was therefore decided to apply the material to DO and present this case of the reconstruction of a mandible with damaged healing potential.

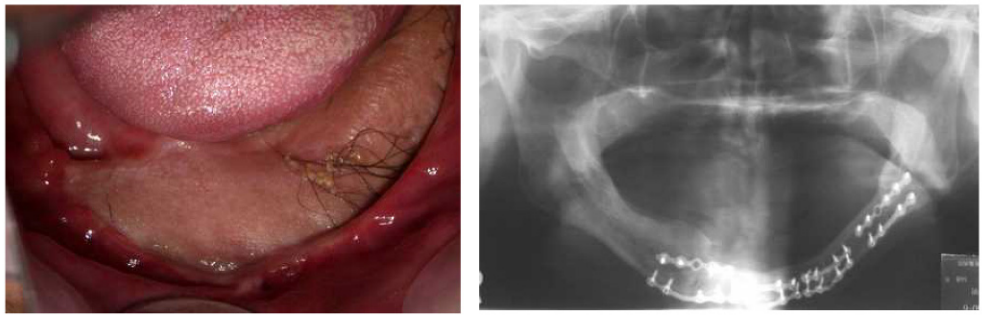

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Fig. 8

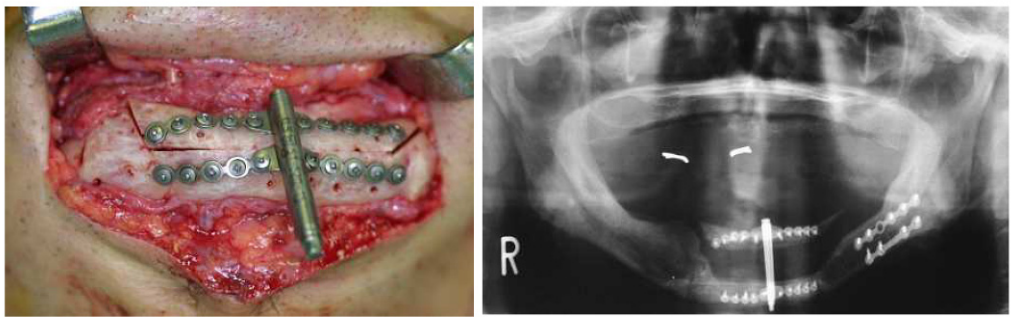

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Fig. 9

Case Report

A 54-year-old male patient was referred to our hospital for rehabilitation of his reconstructed edentulous mandiblyears earlier, the patient had undergone a segmental resection and immediate reconstruction of the mandible in conjunction with the oral floor resultant from squamous cell carcinoma, following chemotherapy and irradiation of 60 Gy. The reconstruction consisted of a 9-cm vascularized fibular graft osteotomized into 3 segments and fixed with 8 miniplates for the mandible and its cutaneous flap for the oral floor (Fig. 8a and 8b). Computed tomograms demonstrated that the grafted fibula had

remodeled into a biangled body of 1 cm in height and width. Vertical DO was planned in the area between the right mental foramen and the left reconstructed segment to allow dental implant placement. From the submandibular approach through the previous scar line under general anesthesia, complete osteotomies were performed with a sagittal saw following the removal of 6 plates and screws. A transport segment, which was 7 cm long, 5 mm high, and attached by a pedicle to the lingual periosteum, was created in the reconstructed mandible with the fibula. A distraction device (TRACK 1.5; Gebruder Martin, Tuttlingen, Germany) was adjusted and fixed with microscrews (Fig. 9a). In closing the wound in layers, the periosteum labial to the horizontal osteotomy line mostly became lacerated and opened because of simultaneous removal of the previous osteosyntheses on this line. After a latent period of 7 days, the distractor was activated at a rate of 0.5 mm twice per day for 15 days (Fig. 9b).

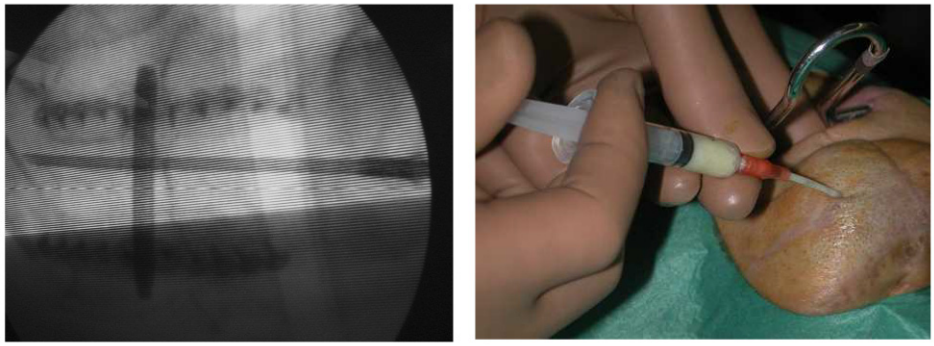

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Fig. 10

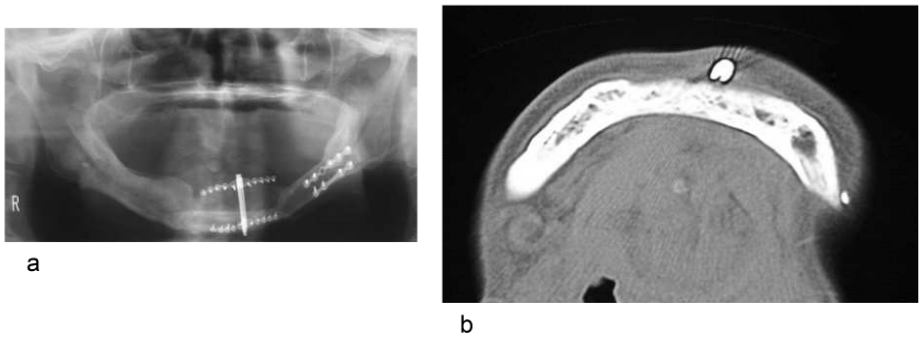

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Fig. 11

The TEB was applied to the distracted tissue at the end of the DO. The 3 mL TEB was prepared and infiltrated for 15 seconds (Fig. 10b). The needle was left in place for an additional minute to allow the gel to increase in viscosity and to prevent the injected material from leaking out of the puncture. No complications were observed during the injection, and the subsequent course was uneventful

A series of monthly panoramic radiographs showed that radiopacity in the distraction gap had begun to appear at 1 month. After 2 to 3 months, during which the transport segment resorbed marginally (Fig. 11a), the area became wholly radiopaque. Computed tomograms at 3 months revealed that newly formed bone in the distraction gap had unclear labial surfaces but clear lingual cortical surfaces. The area in between, which was relatively even with respect to density, scored higher in Hounsfield units than the cancellous bone areas in the neighboring mandibular and fibular bone (Fig. 11b).

The distraction device was removed and 6 titanium screw-type implants, 3.75 mm in diameter and 18 mm in length (Brånemark System, Nobel Biocare, Göteborg, Sweden), were placed under general anesthesia. During the preparation tissue specimens were taken with a trephine (Fig. 12a). The implant furthest to the

right was in native mandible, while the other 5 were in distracted bone. All implants required a torque of 40 Ncm for placement and achieved pri-mary stability. The 2 implants furthest to the right had a shortage of surrounding marginal bone because of a gap in the bone between them (Fig. 12b).

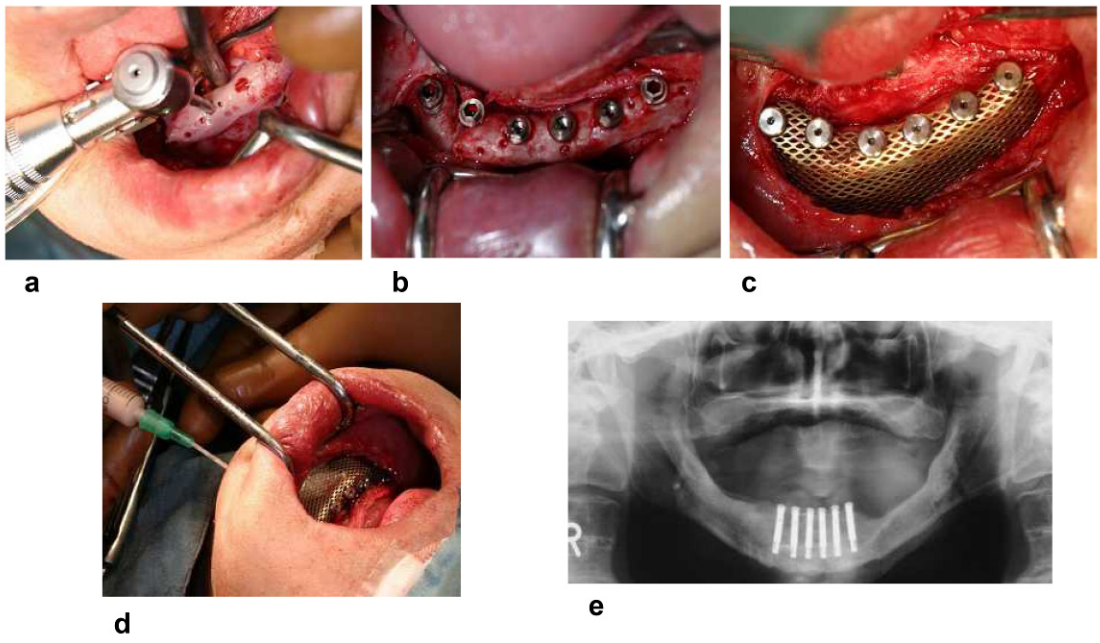

Fig. 12

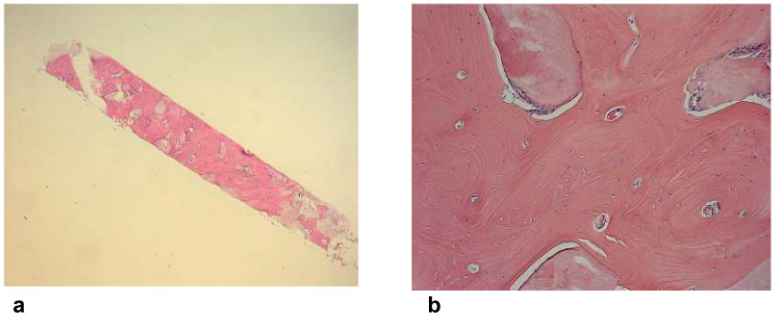

Fig. 13

A 0.1-mm-thick titanium mesh (Micromesh, Stryker, Kalamazoo, MI) was fixed to the platforms of the implants with cover screws, and additional space was created marginally and labially (Fig. 12c). This spacewas filled with 3 mL of injectable bone prepared in the manner already described with 6×10^7 induced MSCs and PRP containing 3.6×10^9 platelets (Fig. 12d).

The postoperative course was uneventful (Fig. 12e). A decalcified section of the histologic specimen showed remodeling lamellar bone with abundant osteocytes in lacunae in the distracted zone (Fig. 13a and 13b).

Three months after implant placement, the implants were uncovered, and the mesh was removed under general anesthesia. All implants had achieved osseointegration, and healing abutments were connected. Under the mesh regenerated hard tissue covered with the periosteumlike membrane was seen (Fig. 14a). On this membrane at the labial and lingual sides of the regenerated ridge, palatal mucosa was transplanted for vestibuloplasty with the uncovered cutaneous flap defatted and positioned lingually and apically. The PRP activated with human thrombin and

calcium chloride were applied to the raw surfaces in the palate and the mandibular ridge. These were covered with a temporary prosthesis and a lyophilized and irradiated porcine skin (Alloask, Taiho Pharmaceutical, Tokyo, Japan) for 5 days (Fig. 14b and 14c).

Three weeks after the uncovering surgery, the donor sites in the palate fully epithelized and a marginal attached mucosa formed around the implants, which were connected to multiunit abutments (Fig. 15a). A maxillary complete denture and a mandibular implant-supported prosthesis were placed and have functioned for a year without problem (Fig. 15b and 15c).

Tissue Engineered Bone Enhance the Calcification Around the Fixture

A vascularized fibular flap is often selected for mandibular reconstruction because it offers adequate length of bone and pedicle, constant geometry, and low donor site morbidity. However, to follow the mandibular arch, the fibula requires multiple osteotomies, which interrupt the medullary vessel and thereby vascular supply since the entire flap depends on the periosteum.[43] The fibular periosteum still supplies the external two thirds of the cortex after revascularization, while its internal third and the medulla have a reduced vascular supply[44].

Preservation of periosteal attachment is therefore considered a critical factor in DO, even if grafted fibular segments have healed and united. Several authors have reported on successful cases of vertical DO of the fibula grafted to reconstruct the mandible[45].

These cases were less complex than the present case, which included a patient with older age, a higher dose of irradiation, a larger transport segment, a longer distance of distraction, and damage to the labial periosteum resultant to simultaneous removal of osteosynthetic plates and screws. These conditions should reflect upon the partial resorption of the superior transport segment. Despite the reflection, the present case demonstrated new bone formation. Not only was the new bone formation less complicated on the labial side of the regenerate, it was also better quality inside, as observed radiographically and histologically, without a longer consolidation period. These favorable results might be attributed to the material injected into the distracted tissue.

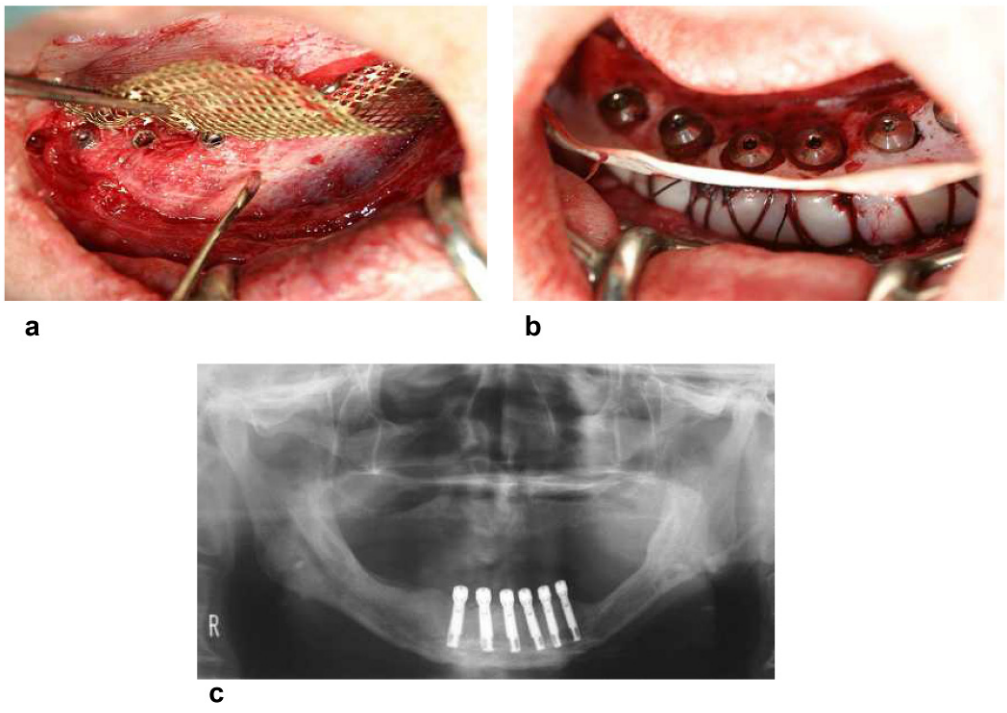

Fig. 14

In applying TEB to DO, they regarded the fibrous tissues in the distracted zone as the scaffold. Several animal studies have shown that the injections of cells with osteogenic potential into distraction gaps enhanced new bone formation with respect to volume and strength and that this enhancement led to short-

ening of the consolidation period[39-42].

The timing of the cell injections was further investigated; it appeared to have no effect on experimental outcome[41].

In this case the 15-mm distraction was considered relatively short, and the injection was administered at the end of the distraction because that is when the number of cells in the distraction gap with osteogenic potenial is the lowest. The injected cells could work before their gradual recruitment via vessel. Growth factors which alpha granules of the platelets secrete can activate cells, including MSCs and osteoblasts, through their membrane receptors[46].

Partial resorption of the transport segment, which left the gap between its neighboring bone, was recovered with TEB. Its gel form allowed the contained cells to contact surface microarchitecture of implants placed simultaneously. For space making with a relatively large shield, a titanium mesh was considered superior to polytetrafluo-roethylene membranes because they restrict new vascularity[47].

The lack of blood supplymight limit bone regeneration with the injectable bone to a certain amount. DO has fewlimitations regarding distraction length but requires longer treatment time than grafting. These innovative methods in combination can allow more effective bone regeneration for adequate implant placement.

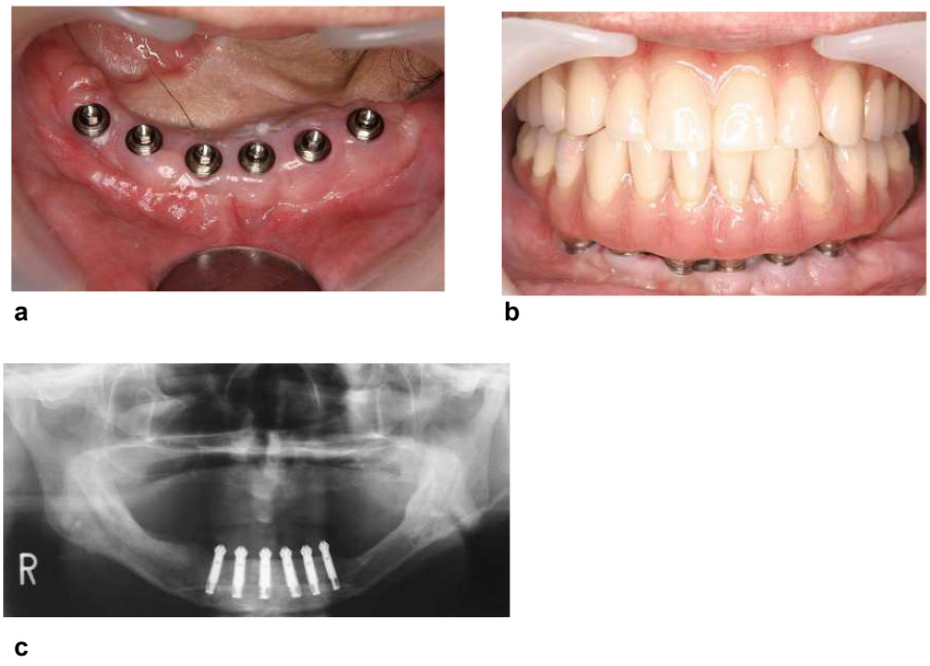

Fig 15.

DISCUSSION

Reconstruction of bone defects secondary to tumors, inflamation and trauma relies on different sources of bone grafts with inherent morbidity. Stem-cell-based tissue engineering is a promising alternative for bone regeneration (Petite et al. 2000; Bianco et al. 2001; Rose and Oreffo, 2002). maxillofacial bone engineering is a fast-moving field with considerable potential clinical applications (Mao et al. 2006; Kaigler et al. 2006: Zhao et al. 2007).

The aim of this article is to summarize current research on bone tissue engineering applied for implant surgery in Nagoya University Hospital and highlight important translational studies that has already been carried out on human subjects.

Unfortunately, only a very small proportion of the above clinical studies except our study make it to the bedside in the form of clinical trials or therapies. Because of practical and ethical reasons, it is sometimes impossible to have proper control groups and therein lies the difficulty of data interpretation. The clinical studies discussed here use a variety of approaches including bone marrow, MSCs and scaffolds, and osteoinductive factorsPRPs) in treating a variety of conditions including implant, tumors defect, alveolar cleft. Our studies are small, observational phase 1-type studies with no control groups and they

have short-term follows. Despite this, they do provide valuable information and we know that the clinical use of autologous bone marrow derived MSCs is relatively safe and does not preclude the use of other techniques in the event of failure.

As described in this paper, reconstruction of alveolar bone defects using tissue-engineered bone has the potential to dramatically improve current methods that rely on sequential bone graftin. The abilities to eliminate donor-site morbidity related to autogenous bone-graft harvest, and to provide comprehensive oral rehabilitation therapies superior to current synthetic implant materials, would make a significant contribution to current dentistry.

The our case reports demonstrate the absence of the reaction of the transplant bed to the transplanted tissue-engineering construct. We observed rapid healing of the operation wound without mucosa ingrowth into the transplant. None patients had microbial inflammatory around fixtures, lost of the implant, or flap necrosis. X-ray examination after transplantation revealed ossified tissue similar to cortical lamina at the boundary of the transplants and oral mucosa. Histological examination of tissue samples from the center of the regenerate revealed the development of young low-mineralized bone tissue.

Thus, the results of our clinical study suggest that transplantation of tissue-engineering bone for alveolar reconstruction is a safe procedure allowing to solve some complex clinical problems in implant surgery.

Taken together, injectable tissue-engineered bone would provide a further option as a graft material for oromaxillofacial bone augmentation, and it might be possible that the use of tissue-engineered bone could decrease healing time. Further, tissue-engineered bone would potentially provide a great benefit to patients in cranio-maxillofacial and plastic surgery and to the bone reconstruction of other parts of the skeleton. Future research will have to address the long-term rates, the stability of tissue-engineered bone, and the application of the therapy to less vascularized environments. We suggest that, based on the present findings, future clinical traials are warranted.

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