FIGURE 5.

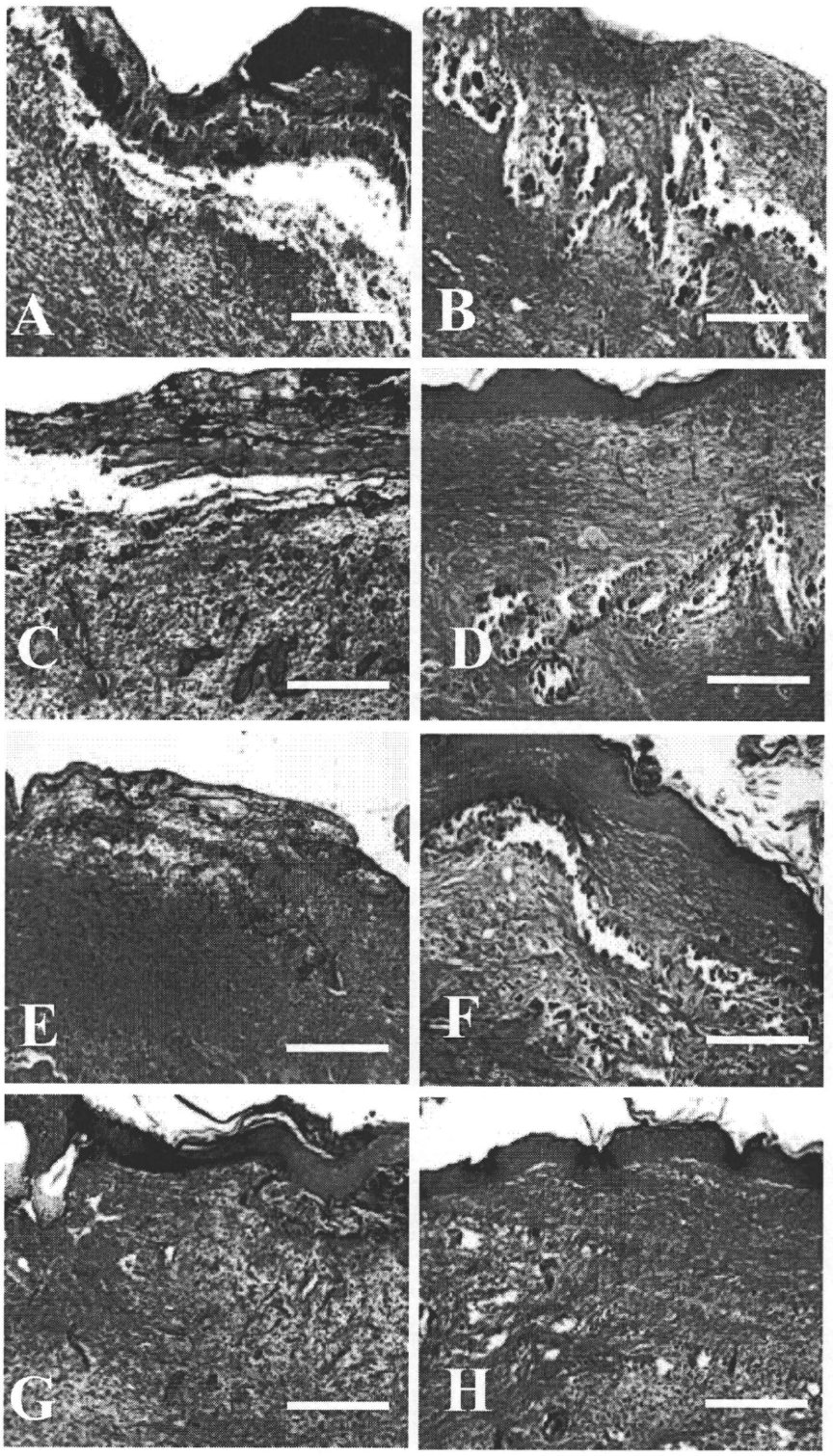
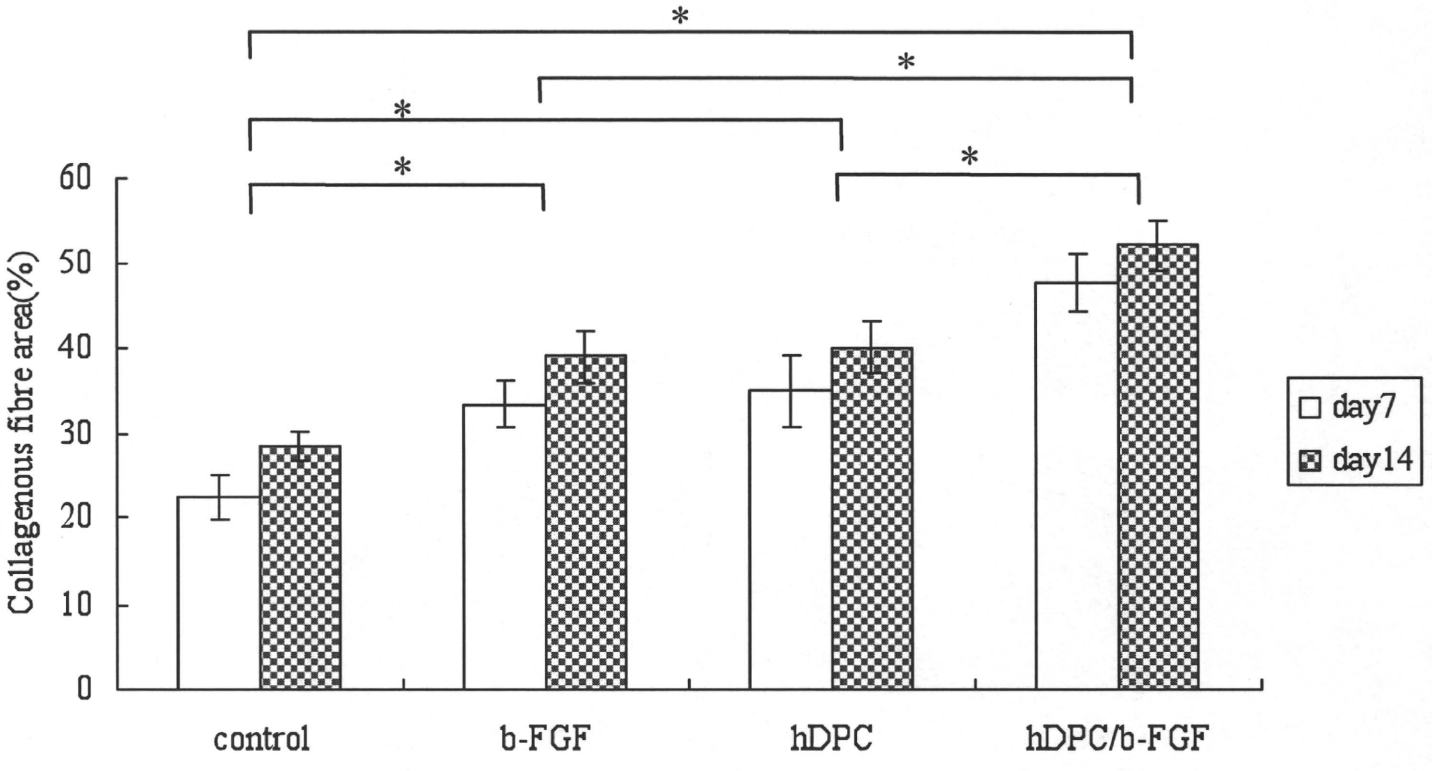


FIGURE 6.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3. Injectable tissue engineered bone -biological requirement and clinical relevant-

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Introduction

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 loss, nerve injury, short- and long-term pain, and functional deficit.

The use of allografts 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

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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 osteogenic differentiation of multipotent mesenchymal stem cells (MSC) of the bone marrow (BM). MSC predifferentiated towards osteogenic lineage are applied on biocompatible materials maintaining osteoinduction and possessing sufficient osteoconductive properties [8] transplanted into the bone defect area.

Creation of bone equivalents is now beyond the scope of experimental numerous experimental study the possibility of effective reconstructed the bone tissue using various biodegradable material and MSC [9-11].

In tissue engineered bone carrying MSC from the BM tissue was performed at the department of Oral Surgery in Nagoya University Hospital in accordance with the research protocol approved by the Nagoya University Ethics committee (Permission No. 172) and in compliance with Helsinki Declaration (2000). Here we present the results of the study.

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. Briefly [12], the basal medium, low-glucose Dulbecco's Modified Eagle's Medium, and growth supplements (50 mL of serum, 10 mL of 200 mM L-glutamine, and 0.5 mL of penicillin–streptomycin mixture containing 25 units of penicillin and 25 g of streptomycin) were purchased from Cambrex Inc. (Walkersville, MD). Three supplements, dexamethasone, sodium 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 $3.1x10^3$ 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 resulting 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 350g. Subsequently, the yellow plasma containing the buffy coat, which contained the platelets and leukocytes, was removed. A second centrifugation at 3500g for 10 minutes was performed to combine the platelets into a single pellet and the plasma supernatant, which was platelet-poor 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 (1.0×10^7 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).

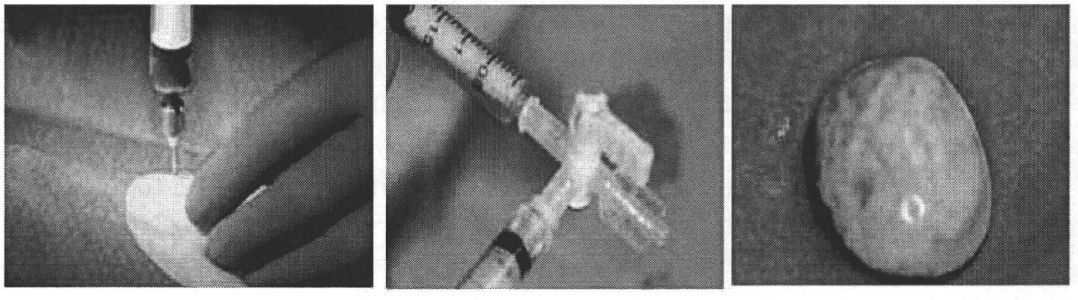


Figure 1

Application for ridge augmentation and dental implant placement

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.

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 previous 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, peri-implant tissues of titanium fixtures that had been placed in regions augmented using the injectable bone.

Patient selection

There were 14 cases aged from 44 to 74 years (mean age 54.6 years). Six patients with partially or totally edentulous ridges were scheduled for sinus floor grafting and 8 patients underwent concurrent onlay plasty. All patients had conventional denture retention problems because of severe anterior or posterior alveolar ridge atrophy. In cases of the maxilla, patients had a residual sinus floor of less than 5 mm in height, to such an extent that the sinus graft and implant would have resolved the problem (Table 1); in the other patients, a large part of the residual alveolar arch was atrophied in the horizontal and sagittal directions (Table 1).

After routine oral and physical examinations, patients were selected and TEB grafting was planned because the patients preferred not to undergo any surgery for harvesting of the autogenous bone. In all cases, the reconstruction included sinus floor grafting and onlay plasty in the anterior or part of the posterior maxilla and mandible with simultaneous implant replacement. All patients were healthy and free from any disease that may have influenced the treatment outcome (e.g., diabetes, immunosuppressive chemotherapy, chronic sinus inflammation, rheumatoid arthritis). The patients were informed

extensively about the procedures, including the surgery, graft material, implants, and uncertainties of using a new bone-regenerative method. They were asked for their cooperation during treatment, and the research protocol was approved by the university ethics committee (Fig. 2).

Table 1

Patient data

	age	sex	location	operation	No. of implants
1	51	F	7 6 | 6 7	Maxillary sinus lift	6
2	60	F	| 5 6 7	Maxillary sinus lift	3
3	44	F	7 6 |	Maxillary sinus lift	2
4	54	F	7 6 5 | 5 6 7	Maxillary sinus lift	6
5	50	F	6 5 4 |	Maxillary sinus lift	3
6	56	F	| 5 6 7	Maxillary sinus lift	3
7	52	F	7 6 |	Onlay graft	3
8	74	M	7 6 5 4 |	Onlay graft	4
9	54	F	7 6 |	Onlay graft	3
10	54	M	2 3 |	Onlay graft	2
11	54	F	2 3 |	Onlay graft	2
12	58	F	7 6 5 4 |	Onlay graft	4
13	52	F	5 - 2 | 2 - 5	Onlay graft	8
14	52	F	5 - 1 | 1 - 5	Onlay graft	8

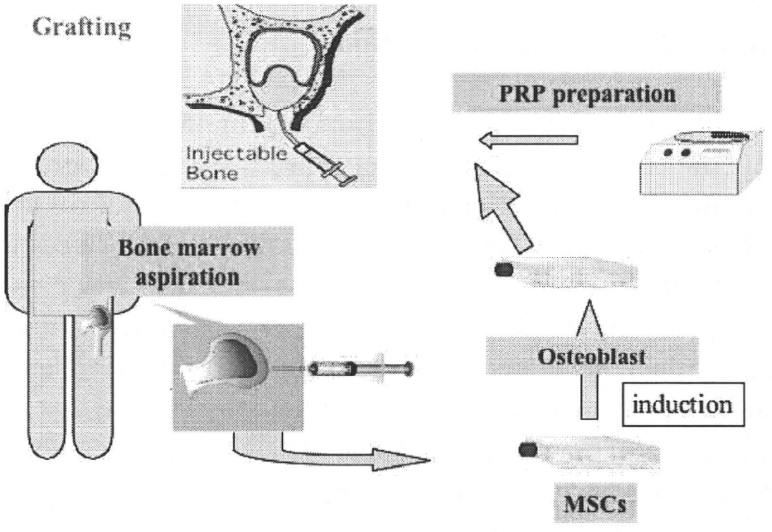


Figure 2

Surgical technique

Sinus augmentations. In all 6 patients, surgery was carried out under general anesthesia. The sinus grafting procedure followed Tatum's classical description [15]. In brief, after the elevation of a mucoperiosteal flap, a door was created with a round hollow bur in the lateral maxillary sinus wall. After mobilization, the door was reflected inward. The space created by this procedure was filled with 1.5 to 5.8 g of tissue-engineered injectable bone, and simultaneous implant placement was performed. Care was taken to keep the inner epithelial lining intact to avoid spilling the grafting material. The mucoperiosteal flap was repositioned and sutured in the usual manner.

Alveolar ride augmentation

The regular titanium fixtures were placed into the atrophied maxilla or mandible at a depth of at least 5 mm, with the coronal part of the fixture exposed. The TEB was applied around the implant to completely cover the exposed threads. After coagulation of the tissue-engineered bone, the grafted area was covered by collagen membrane lined with titanium plate (W.L. Gore & Associates, Inc.,Tokyo) to protect the flap compression.

The membrane was fixed with cover screws, and/or microscrews or pin. Finally, the buccal and labial periosteum was extended in the customary manner, and the wound was closed tension-free. The patients were instructed not to wear any removable prosthesis for 14 days in all cases and not to blow their noses for 7 days in cases of sinus graft. Second stage surgeries were performed approximately 4 to 8 months later.

Clinical and radiographic observations

In case of sinus floor augmentation, evaluation was done from 2 to 5 years after the first surgery. Twenty-three fixtures were installed with injectable bone. The clinical observation was carried out on the grafted area. Cumulative survival and success rates for fixtures placed in conjunction injectable bone were 100%.

Postoperative radiographic findings were consistent with integration between the implant and the regenerated bone (no bone loss or peri-implant radiolucency).

Pre- and postoperative radiographic evaluations showed that the increasing in mineralized tissue was 8.7 mm. Table 1 also describes the vertical ridge augmentation procedure for each patient and the survival data for implants available at re-examination. Also the clinical conditions associated with the 34 remaining fixtures placed in conjunction with ridge augmentation using injectable

bone are presented in the table. At the second surgery, which was performed after a mean healing period of 4.8 months, the mucosal flap was elevated to observe the grafted site. In all cases of vertical ridge augmentation, the spaces around the titanium fixtures were filled with newly formed tissue, which seemed to be calcified tissue. In 2 of 8 cases with wound separation, the bone regeneration was not enough. Average increasing of bone height was 5.0 mm. At 6 months after loading, as tested after removal of the prosthetic reconstruction, all implants maintained stability. Marginal bone resorption at 6 months after loading did not exceed 1.5 mm. None of the patients had postoperative problems besides normal swelling and inflammation at the surgical sites. The main complications during surgery were sinus membrane perforation and wound separation. Perforation of the sinus mucosa was recorded in 4 procedures and resulted in only minor postoperative nasal bleeding without severe inflammatory sign in maxillary region during total observation period.

Case 1

A 44-year-old woman (patient 4 in Table 1) presented with an edentulous right maxilla. She complained of inability to wear her maxillary denture and comfortably chew hard food. Her posterior maxillary soft tissue and severe maxillary atrophy (Fig. 3, A).

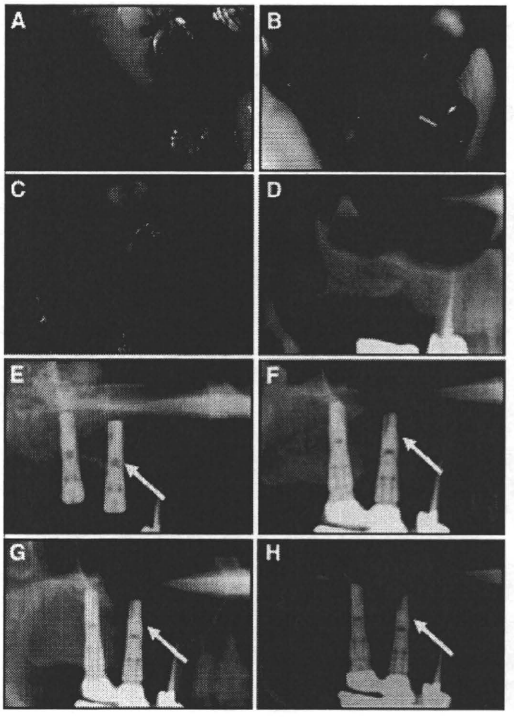


Figure 3

Insufficient bone was present for placement of implants in the maxilla. After the exposure of the maxilla, the door trap was designed by a round bur on the lateral wall of maxilla under water irrigation. The lateral wall of the maxilla was rotated medially with elevation of the sinus membrane. Two implants were placed into each alveolar ridge of the maxilla, however, the fixtures exposed in sinus cavity. The injectable bone (with beta-tricalcium phosphate) was applied in the maxillary sinus and around the fixtures completely to cover the exposed thread (Fig. 3, B). A spark-erosion prosthesis was made over the implants (Fig. 3, C).

The radiographs showed the parallel position of the maxilla and insufficient bone in the maxillary floor (Fig. 3, D, E). However, at 12 months progressive bone regeneration was observed (Fig. 3, F). A radiograph showed a bone filling around the previously exposed threads, reaching the tip of the implants (Fig. 3, F–H). The 3 years follow-up examination showed no signs or symptoms of implant failure (Fig. 3, H).

Case 2

A 52-year-old woman (patient 14 in Table 1), she had only both first molar teeth and lost other teeth with a severely atrophied mandibular alveolar crest. The patient required treatment by 8 implants and ridge augmentation with injectable bone. Occlusal view of mandible before the ridge augmentation procedure showed the narrow ridge and the concave shape of the lateral side of mandible. An incision was made over the crest with vertical release. The mandible has been absorbed vertically and need for the ridge

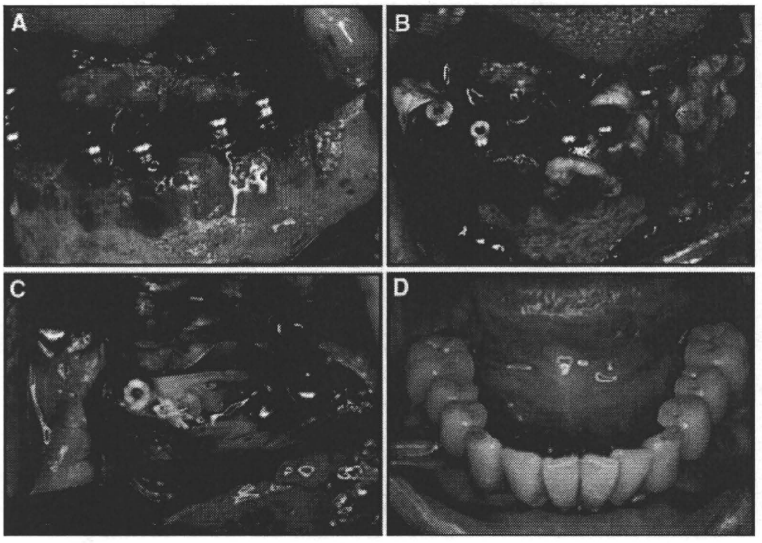


Figure 4

augmentation. Eight fixtures were installed in the crestal part of mandible after flap elevation. She had problems with her denture due to cosmetic reason and wanted it to be improved. The coronal parts of the fixtures protruded from the alveolar crest to the level of the implant neck (Fig. 4, A). The injectable bone was applied to cover the exposed part of fixture (Fig. 4, B). At the stage of second surgery, the grafted area was observed. The fixtures were covered by newly formed bone, and bone increase on the lateral side of the mandible was seen (Fig. 4,C). Final oral implant bridge was achieved (Fig. 4, D) and the patient was pleased. The 2 years follow-up examination showed no signs or symptoms of implant failure.

Usability of tissue engineered bone in implant dentistry

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 augmentation procedures [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 injectable bone application and simultaneous implant placement. The macro findings showed that injectable bone induced bone regeneration and that the dental implant thread was not exposed.

Thus, these results indicate that ridge augmentation caused by injectable bone and that simultaneous implantation is possible. The results of this study provide evidence of the safety and technical feasibility of injectable bone for maxillary sinus floor augmentation and vertical ridge augmentation in agreement with those from earlier animal studies that have indicated that treatment with injectable bone 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 injectable bone were consistent with the usual morbidity observed in the maxillary sinus floor augmentation procedure and vertical ridge augmentation. Radiographic assessments indicated that injectable bone 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 injectable bone 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 MSC and PRP. The MSC in the bone marrow are induced into cells with osteogenic capacity, the MSC 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 [25-27]. Donorsite morbidity is an important factor in deciding the site for harvesting cancellous bone. Osteoinductive agents such as recombinant human bone morphogenetic protein [28-30] 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. 5).

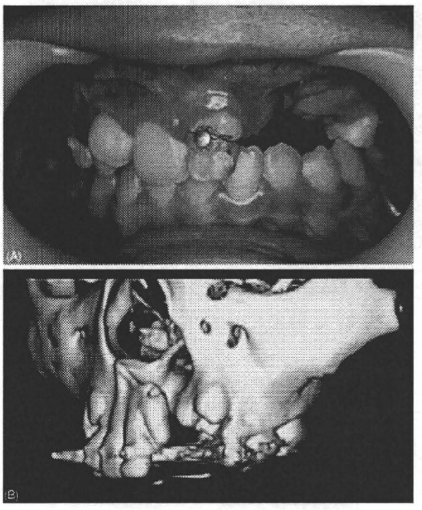


Figure 5

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 TEB, and they granted their consent.

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, the lateral piriform rim superiorly and to the alveolar ridges inferiorly, 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. 6). 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.

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. 7). 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.

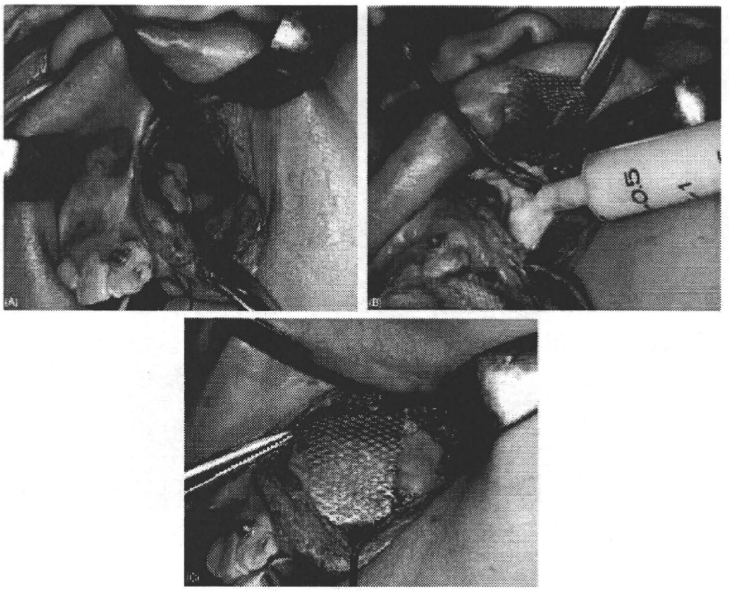


Figure 6

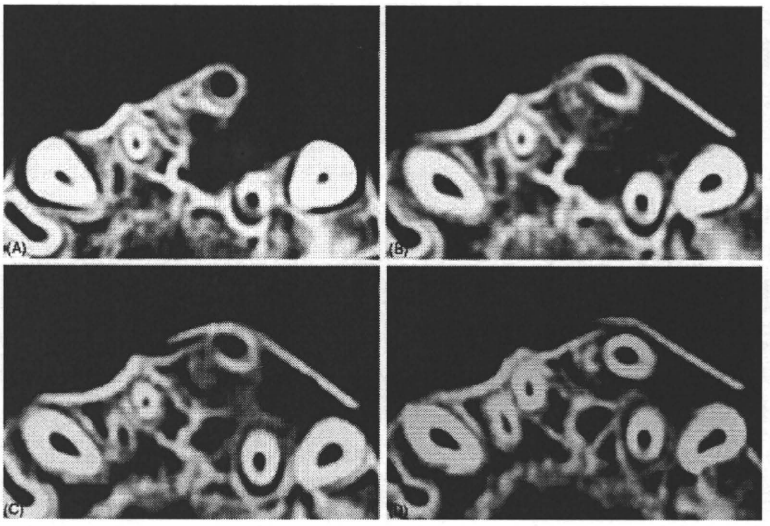


Figure 7

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. 8).

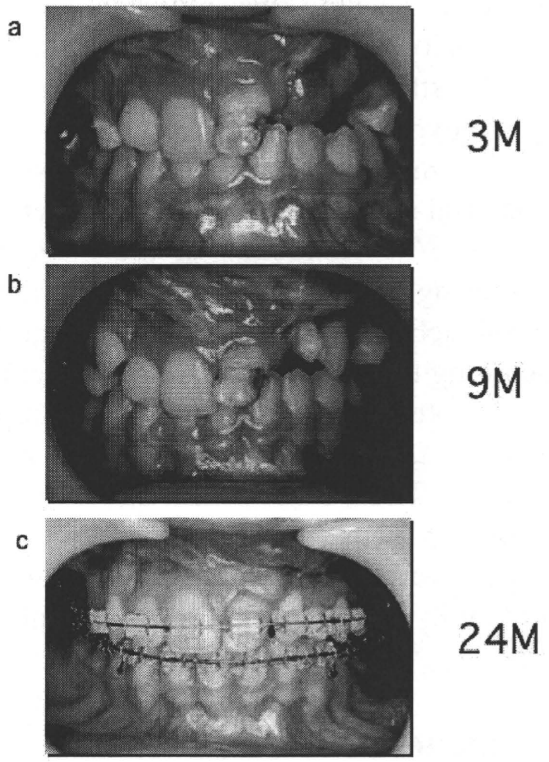


Figure 8

Availability of tissue engineered bone for cleft patient

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 [33] 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 TEB thus shows promise with further perspectives. Younger patients have more MSC, and their harvesting, isolation and cryopreservation allows TEB to be supplied repeatedly when needed. This repeatability will facilitate the sequential treatments of cleft patients in the future.

Distraction osteogenesis assisted by tissue engineering 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 grafting 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, ultrasonic, or chemical stimulation have been made [38].

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

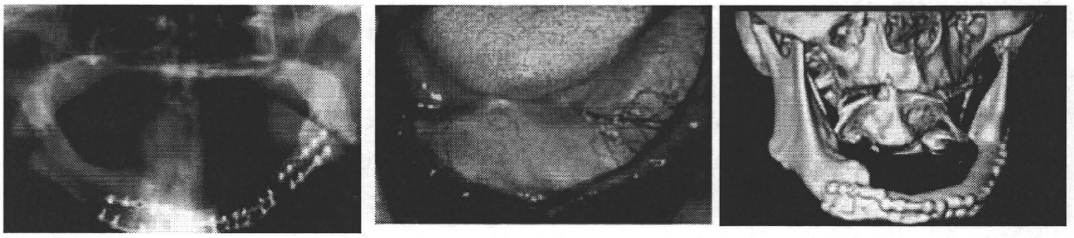
Not only animal studies but also clinical trials have demonstrated that tissue engineered bone can effectively regenerate osseous tissue. It was therefore decided to apply the material to DO and present this case of the reconstruction of a mandible with damaged healing potential.

Case report

A 54-year-old male patient was referred to our hospital for rehabilitation of his reconstructed edentulous mandible. Two years earlier, the patient had undergone a segmental resection and immediate reconstruction of the mandible in conjunction with the oral floor resultant from squamous cell

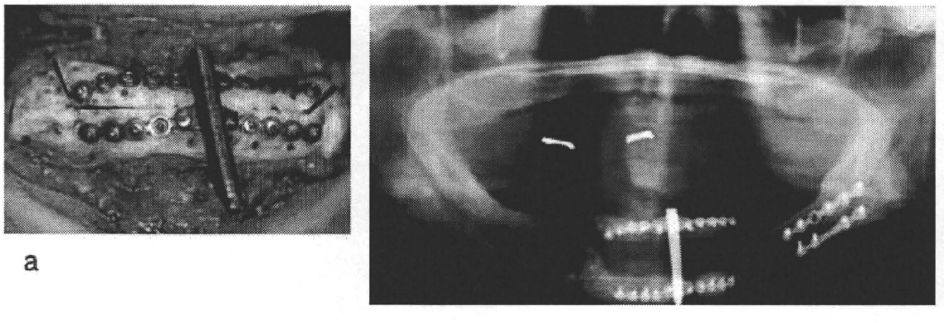
carcinoma, following chemotherapy and irradiation of 60 Gy. The reconstruction consisted of a 9-cm vascularized fibular graft osteotomized into 3 segments and fixed with 8 miniplates for the mandible and its cutaneous flap for the oral floor (Figs 9a and 9b). Computed tomograms demonstrated that the grafted fibula had remodeled into a biangled body of 1 cm in height and width (Fig 9c).

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;Gebrüder Martin, Tuttlingen, Germany) was adjusted and fixed with microscrews (Fig10a). 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 10b).



a b c

Figure 9



a

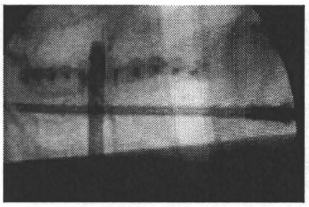
b

Figure 10

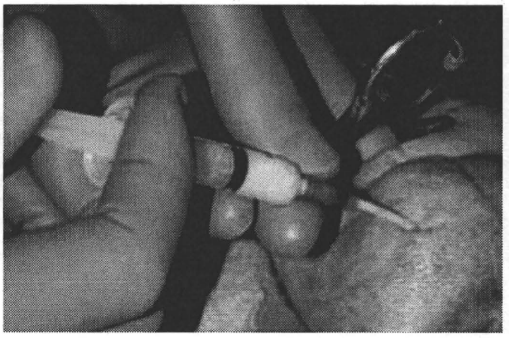
The injectable bone was applied to the distracted tissue at the end of the DO. The MSCs were derived from 10-mL iliac marrow aspirates and expanded in culture to the number of 5×10^7 cells. After induction, they expressed high alkaline phosphatase activity in assay. Twenty milliliters of PRP were isolated from 200 mL of blood; this PRP contained 1.6x 10^9 platelets/mL, a concentration 8.3 times stronger than that of the original whole blood. With a C-arm fluoroscope for guidance, while the patient was under intravenous sedation, a 18-gauge needle was placed into the distraction gap (Fig 11a). The 3 mL of injectable bone was prepared and infiltrated for 15 seconds (Fig 11b). 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 12a), 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 12b).

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 13a). 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 primary 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 13b).

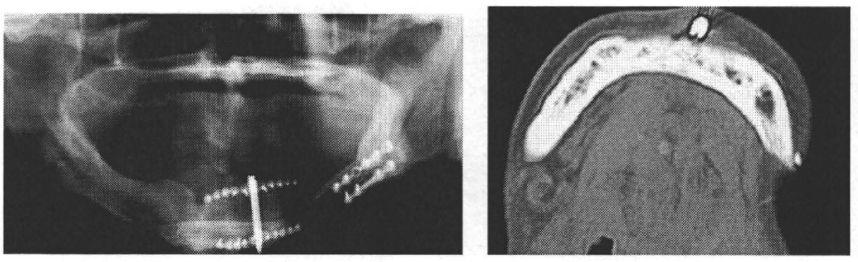


a



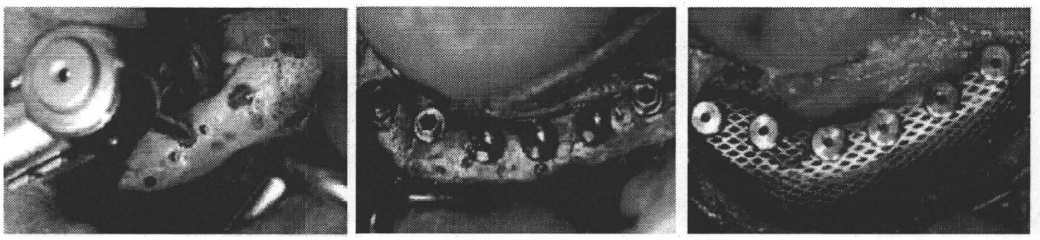
b

Figure 11



a b

Figure 12



a b c

Figure 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 13c). This space was 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 13d). The postoperative course was uneventful (Fig 13e).

A decalcified section of the histologic specimen showed remodeling lamellar bone with abundant osteocytes in lacunae in the distracted zone (Figs 14a and 14b).

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 periosteum-like membrane was seen (Fig 15a). 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 (Figs 15b and 15c).

Three weeks after the uncovering surgery, the donor sites in the palate fully epithelized and a marginal attached mucosa formed around the implants,

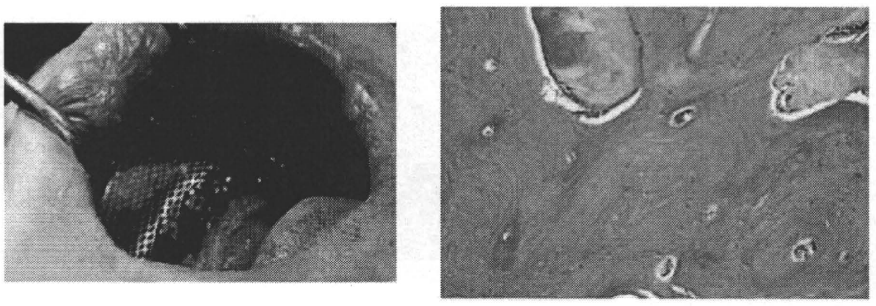


Figure 14

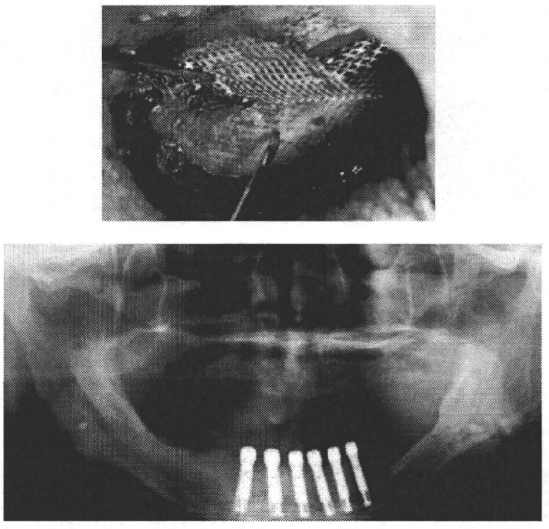


Figure 15

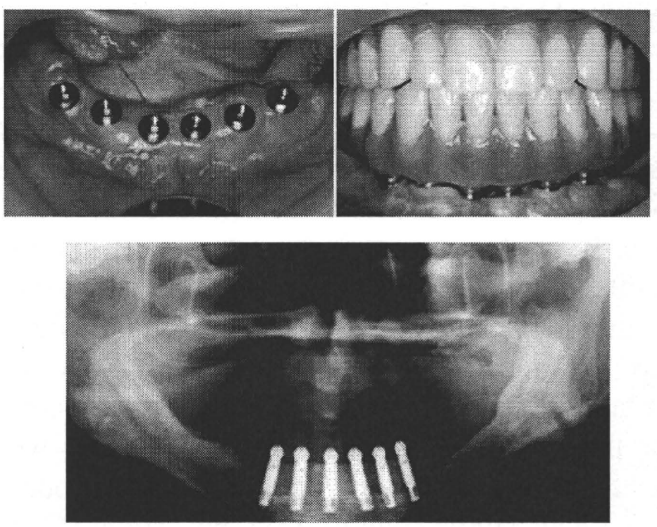


Figure 16