Distraction Osteogenesis Assisted by Tissue Engineering in an Irradiated Mandible: A Case Report

Hideharu Hibi, DDS, PhD[1]/Yoichi Yamada, DDS, PhD[2]/Hideaki Kagami, DDS, PhD[3]/Minoru Ueda, DDS, PhD[4]

Distraction osteogenesis (DO) can provide predictable bone regeneration without grafting procedures but requires long treatment time and forms less bone transverse to the direction of distraction. To promote 3-dimensional bone formation and shorten the consolidation period, tissue-engineered osteogenic material (injectable bone) was applied in a patient who was being treated with vertical DO with an osteocutaneous fibular flap to reconstruct the mandible. The material, which comprised autologous mesenchymal stem cells culture-expanded then induced to be osteogenic in character and platelet-rich plasma (PRP) activated with thrombin and calcium chloride, was infiltrated into the distracted tissue at the end of distraction and injected into a space created labially with a titanium mesh at implant placement. The infiltration contributed to full consolidation of the regenerate for 3 months, and the injection thickened the regenerated ridge and bridged a gap between the native mandible and distracted fibula. The reconstructed mandible was expanded from 10 mm to 25 mm in height despite a lacerated and opened labial periosteum in the distracted area. Six implants 18 mm in length were placed and subsequently achieved osseointegration. The cutaneous flap covering the implants was trimmed, and the palatal mucosa was transplanted to the regenerated ridge for vestibuloplasty. These raw surfaces were covered with PRP; within 3 weeks, they had attained an epithelium. The implants have supported a fixed prosthesis with adequate surrounding bone and attached mucosa. DO was assisted by tissue engineering and became effective in restoring the compromised mandible. Int J Oral Maxillofac Implants 2006;21:141–147

Key words: *distraction osteogenesis, injectable bone, platelet-rich plasma, stem cells, tissue engineering*

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.[1] Several recent studies have shown that injecting cells with osteogenic potential into distracted callus enhances its consolidation.[2–5]

The present authors have recently reported on a tissue-engineered osteogenic material called "injectable bone," which comprises culture-expanded mesenchymal stem cells (MSCs) and platelet-rich plasma (PRP).[6] Not only animal studies but also clinical trials have demonstrated that this material 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.

[1]Associate Professor, Center for Genetic and Regenerative Medicine, Nagoya University School of Medicine, Nagoya, Japan.
[2]Assistant Professor, Center for Genetic and Regenerative Medicine, Nagoya University School of Medicine, Nagoya, Japan.
[3]Associate Professor, Department of Tissue Engineering, Nagoya University School of Medicine, Nagoya, Japan.
[4]Professor, Department of Oral and Maxillofacial Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan.

Correspondence to: Dr Hideharu Hibi, Center for Genetic and Regenerative Medicine, Nagoya University School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550 Japan. Fax: +81 52 744 2352. E-mail: hibihi@med.nagoya-u.ac.jp

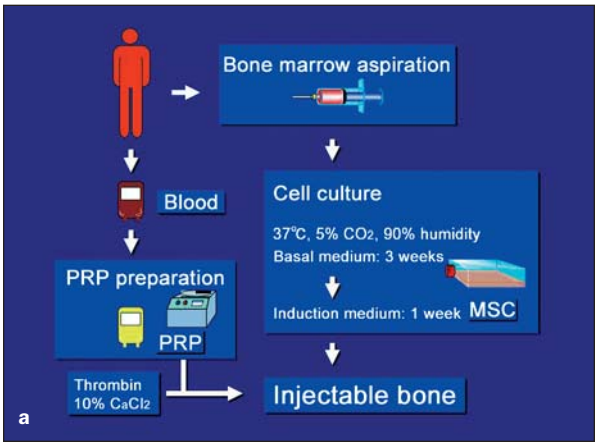


Fig 1a Preparation of MSCs, PRP, and injectable bone.

Fig 1b Aspiration of bone marrow.

Fig 1c Mixing materials of injectable bone. One syringe contains air, calcium chloride, and human thrombin; the other contains PRP and MSCs.

Fig 1d Injectable bone. Mixture keeps gel form for about 20 seconds.

MATERIALS AND METHODS

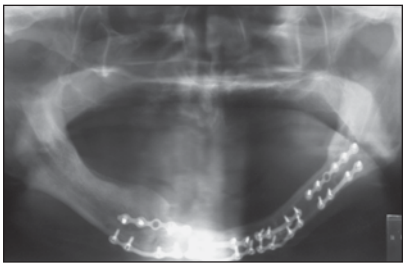
The MSCs and PRP are prepared as described previously.[6] The MSCs are isolated from iliac marrow aspirates, expanded in culture media for 3 weeks, and differentiated in supplemented osteogenesis induction media for another week. The PRP is isolated from autologous blood using density gradient centrifugation and a selective collection technique (Figs 1a and 1b). A 3-way stopcock connects 2 syringes; one contains 1 mL of air, 1 mL of 10% calcium chloride, and 1,000 units of human thrombin; the other contains 6 mL of PRP and all of the induced MSCs. This formula is standard except for the MSCs; the amount of those varies according to need. With the stopcock open, the contents of the 2 syringes are completely mixed for 5 seconds. The injectable bone mixture then maintains its gel form for about 20 seconds (Figs 1c and 1d).

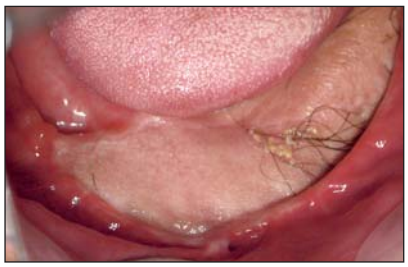
CASE REPORT

A 54-year-old male patient was referred to the authors' 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 2a and 2b). Computed tomograms demonstrated that the grafted fibula had remodeled into a bi-angled body of 1 cm in height and width (Fig 2c).

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 3a). 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 3b).

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





Figs 2a and 2b Reconstructed mandible and oral floor with vascularized osteocutaneous fibular flap.

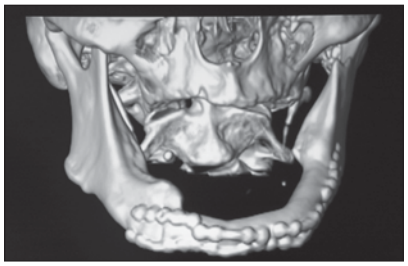


Fig 2c Grafted fibula remodeling into a bi-angled body of 1 cm in height and width.

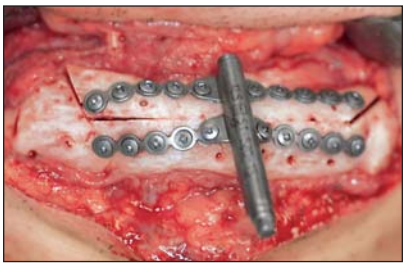


Fig 3a Distraction device. The periosteum lacerated and opened due to simultaneous removal of the previous osteosynthetic plates and screws.

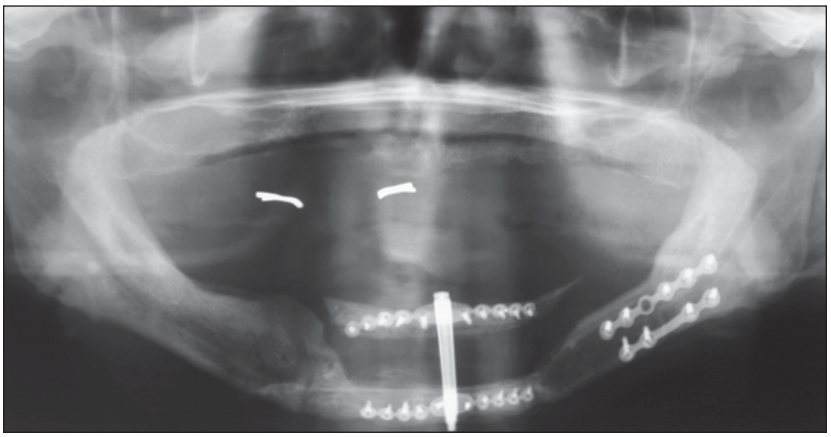
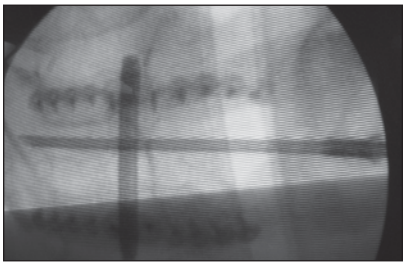
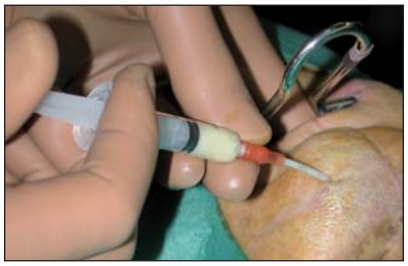


Fig 3b Immediately after distraction. Transport segment was repositioned 15 mm superiorly.

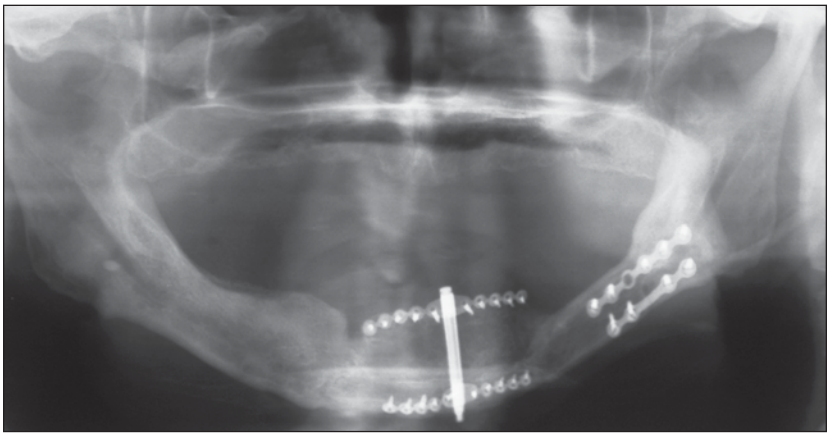
Figs 4a and 4b Application of injectable bone to distracted tissue with fluoroscopic guide.

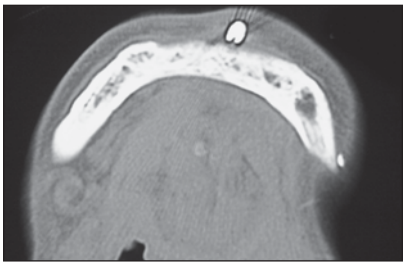




expressed high alkaline phosphatase activity in assay. Twenty milliliters of PRP were isolated from 200 mL of blood; this PRP contained 1.6×10^9 platelets/mL, a concentration 8.3 times stronger than that of the original whole blood. With a C-arm fluoroscope for guidance, while the patient was under intravenous sedation, a 18-gauge needle was placed into the distraction gap (Fig 4a). The 3 mL of injectable bone was prepared and infiltrated for 15 seconds (Fig 4b). 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 5a), the area became wholly radiopaque. Computed tomograms at 3 months revealed that newly formed bone in the distraction gap had





Figs 5a and 5b Three months after distraction. Radiopacity in the distraction gap. Newly formed bone in the distraction gap appeared unclear at the labial aspect but clear on the lingual cortical surface. The area in between, which had a relatively even density, was higher in terms of Hounsfield units than the neighboring cancellous area.

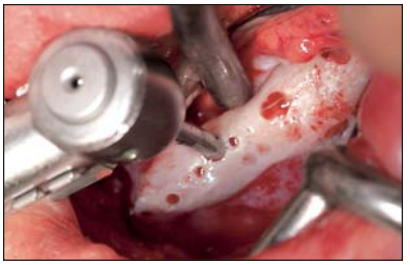


Fig 6a Biopsy sample removal with a trephine bur.

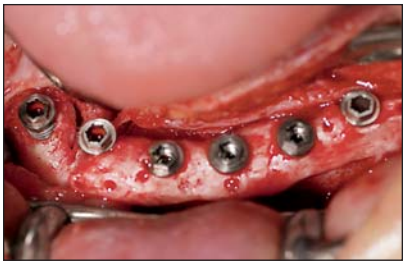


Fig 6b Shortage of marginal bone around the 2 implants furthest to the right.

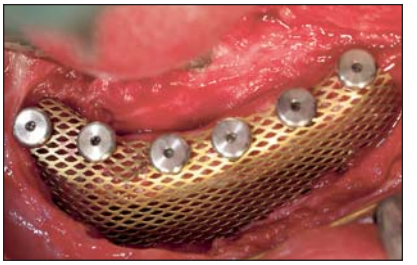


Fig 6c Marginal and labial space created with a titanium mesh.

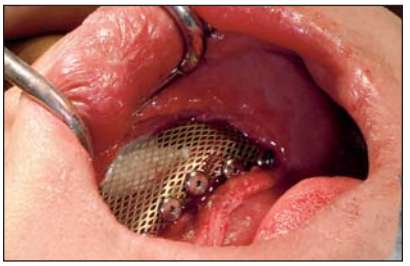


Fig 6d Filling space with injectable bone.

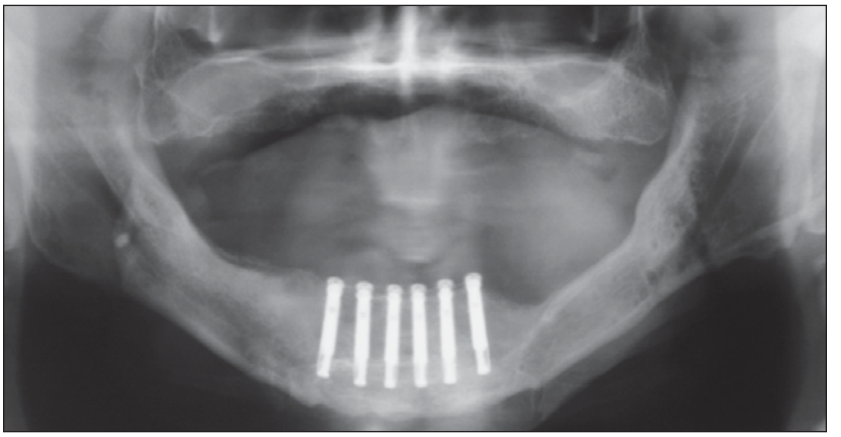
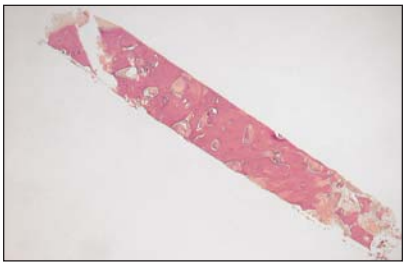


Fig 6e Radiograph obtained immediately after implant placement.

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 5b).

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 6a). 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 6b). 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 6c). 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 6d).



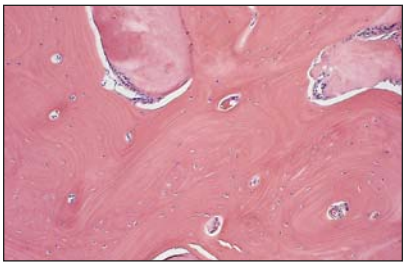
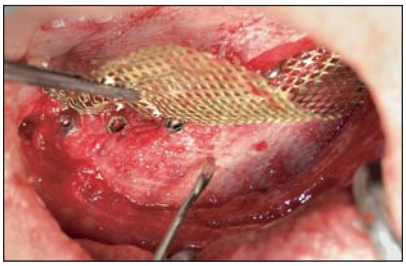


Fig 7a Decalcified section of specimen (hematoxylin and eosin; original magnification ×1.25).

Fig 7b Remodeling lamellar bone with abundant osteocytes in lacunae (hematoxylin and eosin; original magnification ×10).



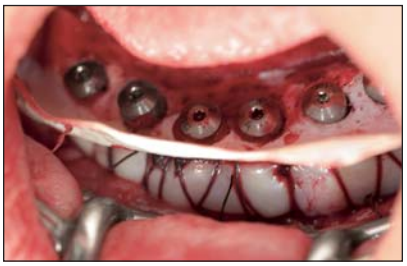


Fig 8a Regenerated hard tissue covered with periosteumlike membrane under a titanium mesh.

Fig 8b Vestibuloplasty of regenerated ridge. Transplanted palatal mucosa and dressing sheet.

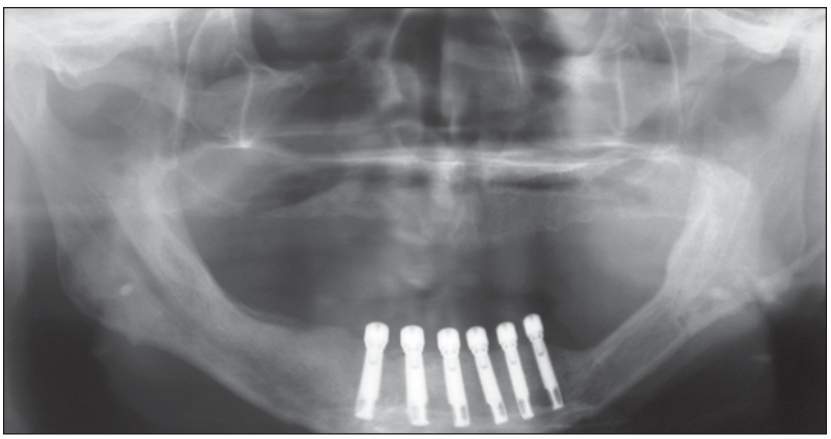


Fig 8c Radiograph obtained immediately after uncovering the implants.

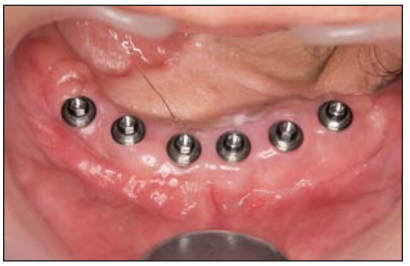
The postoperative course was uneventful (Fig 6e). A decalcified section of the histologic specimen showed remodeling lamellar bone with abundant osteocytes in lacunae in the distracted zone (Figs 7a and 7b).

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 8a). 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 8b and 8c).

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 9a). A maxillary complete denture and a mandibular implant-supported prosthesis were placed and have functioned for a year without problem (Figs 9b and 9c).

DISCUSSION

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



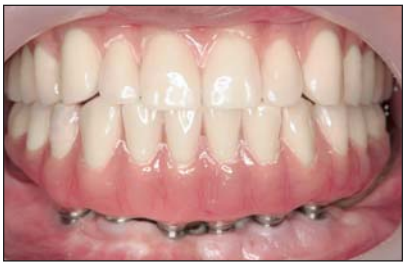


Fig 9a View of the implants 3 weeks after uncovering.

Fig 9b Prosthesis in place.

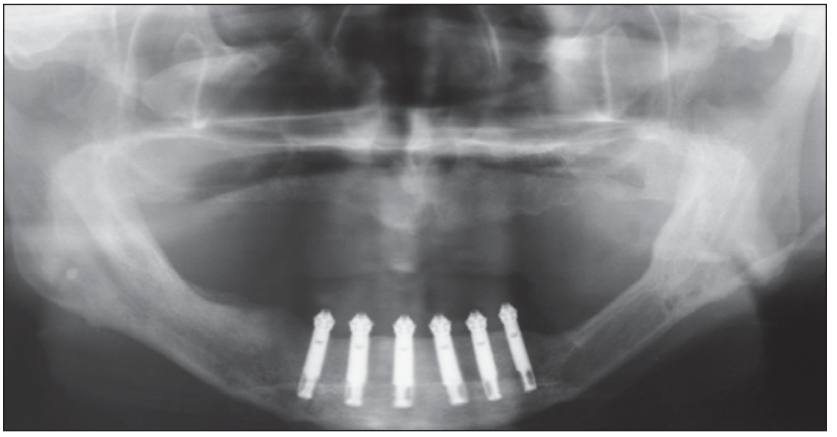


Fig 9c Radiograph obtained 1 year after seating the prosthesis.

osteotomies, which interrupt the medullary vessel and thereby vascular supply since the entire flap depends on the periosteum.[7] 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.[8] 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.[7,9] 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.

Tissue engineering combines 3 key elements: cells, signaling molecules, and scaffolds.[10] For cells, the MSCs were applied; for signaling molecules, there were the growth and transforming factors in the PRP; and for scaffolding, there was the fibrin network of the PRP gel for the injectable bone.[6] In applying injectable bone 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 shortening of the consolidation period.[2–5] The timing of the cell injections was further investigated; it appeared to have no effect on experimental outcome.[4] 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.[11]

Partial resorption of the transport segment, which left the gap between its neighboring bone, was recovered with the injectable bone. 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 polytetrafluoroethylene membranes because they restrict new vascularity.[12] The lack of blood supply might limit bone regeneration with the injectable bone to a certain amount. DO has few limitations 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.

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