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Modern Bone Regeneration Instead of Bone Transplantation: A Combination of Recombinant Human Bone Morphogenetic Protein-2 and Platelet-Rich Plasma for the Vertical Augmentation of the Maxillary Bone—A Single Case Report

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This publication describes the clinical case of a 75-year-old woman. She suffered from total alveolar ridge atrophy due to 20 years of wearing dentures. Bone transplantation, including harvesting of the iliac crest, was rejected by another clinic due to various existing diseases and risk of blood loss on donor side. Moreover, the minimal residual alveolar ridge did not allow bone fixation using screws nor did it allow osteodistraction. Before deciding which bone tissue engineering techniques should best be employed in this surgical treatment, cardiological and internistic consultations and treatments were carried out. In addition, anesthetic preparations were made. The surgical treatment was performed implementing special bridge flap techniques to preserve the periosteum. Tricalcium phosphate blocks soaked with recombinant human bone morphogenetic protein-2 and platelet-rich plasma were implanted on the narrow alveolar ridge. They were attached by tightening the soft tissue, including the periosteum. Four months later, after complication-free wound healing and bone regeneration, six dental implants were inserted into the new alveolar ridge. The histology of all bone samples showed vital lamellar bone. Three months after implantation, a new dental structure was fixed on the implants. The patient's quality of life improved significantly with this new situation.

Introduction and Scientific Background

Wearing dentures in the edentulous maxilla for a long time causes alveolar ridge atrophy in a large group of patients. The consequences are a bad fixation of the dentures in the maxilla, followed by problems of ingestion and a decrease in quality of life. To improve both, a new alveolar ridge, mostly achieved through bone transplantation and harvesting from the iliac crest, is required. Traditionally, the vertical augmentation of alveolar atrophy is carried out using autogenous bone, sometimes in combination with allografts, xenografts, or synthetic biomaterials. The transplantation of autogenous bone, until today, is well known as the gold standard. Each year, more than 2 million autogenous bone transplantations are carried out globally in humans. The osteoinductive and the osteoconductive character of autogenous bone has been demonstrated and led to a number of successful results in the past. However, there are disadvantages:

1. In most cases, two surgical procedures are necessary—one for bone harvesting (e.g., from the iliac crest) and the other for implantation. For some patients this can cause complications associated with the donor side.
2. Regarding the bone transplantation, risks of wound infection, necrosis, and resorption, representing up to 30% of the transplanted material,[1,2] have been observed.

In cases of alveolar ridge atrophy, in addition to bone transplantation, a special technique (called distraction osteogenesis) is employed. In this treatment, the alveolar ridge is split into two parts horizontally. Osteodistraction devices are fixed on both sides. The gap between both parts of the bone is filled with autogenous bone, or this gap regenerates through osteogenesis distracting both sides at a rate of 0.5–1 mm a day. This treatment is carried out for 1–2 weeks followed by consolidation for 2–3 months. Due to this, patients have to wear this osteodistractor for several months, which means a severe impairment of their quality of life.[3–8]

In those cases where the fixation of screws for the stabilization of the transplantation material is impossible and no osteodistraction devices can be fixed, traditional surgical treatments are unable to help. Here, only bone regeneration

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by means of tissue engineering techniques seems to provide a solution for the patients.

Bone Tissue Engineering Using Bone Morphogenetic Proteins

A variety of different techniques have been developed in bone tissue engineering during the last 20 years. In 1965, Urist wrote about bone growth by induction.[9] Meanwhile, several thousand international articles have been published on this innovative treatment. Zheng *et al*.[10] reported at an early stage that recombinant human bone morphogenetic protein (rhBMP-2) induces endochondral ossification. It supports

1. proliferation and differentiation of mesenchymal cells into chondroblasts and osteoblasts,
2. production and maturation of cartilage and bone matrix, and
3. differentiation of circulating osteoclast precursor cells into osteoclasts.

Raida *et al*.[11] proved that rhBMP-2 promotes vascularization.

In the field of oral and maxillofacial surgery, Nevins *et al*.,[12] Boyne,[13] Terheyden *et al*.,[14] Barboza *et al*.,[15] and Ripamonti *et al*.[16] have reported about newly regenerated bone in animal models using growth factors. Except for Terheyden *et al*.,[14] who used rhBMP-7 (OP-1; Stryker), all other authors used rhBMP-2 (INFUSE, US/InductOs, Europe; Wyeth Medicine). Boyne *et al*.,[17,18] Cochran *et al*.,[19] Wikesjö *et al*.,[20] and Fiorellini *et al*.[21] have published the first clinical studies about bone regeneration in humans with rhBMP-2 in dental applications. Jung *et al*.[22] (rhBMP-2) and Warnke *et al*.[23] (rhBMP-7) combined xenogenic deproteinized natural bone mineral with BMPs for jawbone reconstruction in single cases. The other material commonly used is bovine collagen. OP-1 contains a mixture of rhBMP-7 powder and granulated absorbable collagen sponge (ACS). INFUSE (US/InductOs, Europe) is provided as rhBMP-2 powder and a separate collagen sponge (ACS). Only INFUSE (US/InductOs, Europe) enables a complete splitting of both parts. In the presented case, ACS was not used because it is a xenogenic material that causes immunological reactions in 18% of patients.[24] Moreover, ACS is not able to provide suitable structural support for the agglomeration of osteoblasts to reconstruct larger bony defects.[25] We have demonstrated the advantages of using rhBMP-2 in combination with other carrier materials and scaffolds instead of ACS.[25] Whereas Boyne *et al*.[17,18] used 1.77–3.4 mg[17] (1.5 mg/mL[18]) rhBMP-2 in combination with ACS per sinus floor augmentation, Marx[26] reported on cases where he implanted 12–24 mg (1.5 mg/mL) into one maxilla sinus. We achieved comparable results by combining rhBMP-2 with demineralized bone matrix using 1.3–1.5 mg (1.5 mg/mL) rhBMP-2 per sinus floor augmentation.[25]

Negative Aspects of BMPs

Although most of the authors present favorable results using BMPs, the disadvantages have to be mentioned. For example,

1. high cost and
2. BMPs induce the development of osteoblasts and osteoclasts, which means a contrary development to the main target is also initiated.

This negative effect can partly be counteracted by combining the BMPs with platelet-rich plasma (PRP). Cenni *et al*.[27] proved the inhibition of osteoclast activation using PRP.

Platelet-Rich Plasma

The use of PRP therapy was introduced in the late 1990s. There is still controversial discussion regarding the use of PRP and whether or not it favors bone regeneration.[28,29]

However, all authors agree that platelets are the main regulators of the inflammatory phase and play an essential role in the proliferation and differentiation phase (Intini[30]).

Among others, epidermal growth factor, basic fibroblast growth factor (bFGF), insulin-like growth factor-1, and platelet-derived growth factor are very important in bone regeneration. Vascular endothelial growth factor and platelet-derived endothelial cell growth factor play an essential role in angiogenesis.

New publications and overviews indicate the advantages of PRP in bone regeneration. In 1998 Marx *et al*.[31] proposed the use of PRP to enhance the initial phase of the bone wound healing.

Bertoldi *et al*.[32] pointed out the importance of controlled release systems of growth and differentiation factors using biomaterials in combination with PRP, whereas Hu *et al*.[33] highlighted the enhancement of osteogenesis and angiogenesis. This also applies to necrotic bone as Yokota *et al*.[34] stated. Hu *et al*.[33] concluded that PRP possibly starts the process of angiogenesis recruiting the endothelial cells which line blood vessels and begin the initiation of bone regeneration. Park *et al*.[35] presented an *in vitro* study using rhBMP-2 and PRP. As a result they pointed out that PRP with suboptimal doses of rhBMP-2 improves bone formation and enhances bone density.

Cenni *et al*.[27] proved that PRP reduces the osteoclast-mediated bone collagen degradation, which suggests the inhibition of osteoclast activation. Calori *et al*.[36] reported on a randomized clinical study in the treatment of persistent long bone nonunions. They demonstrated that the application of rhBMP-2 as a bone-stimulating agent is superior compared to that of PRP with regard to their clinical and radiological efficacy. Hakimi *et al*.[37] presented an animal trial with minipigs where he showed that only defects, where PRP was added, regenerated entirely. The control group received autologous cancellous bone graft without PRP. This result is supported by Mariano *et al*.[38] Hartmann *et al*.[39] highlighted in their clinical study that the use of PRP provides a faster fusion and higher density of the newly generated bone. Messora *et al*.[40] presented the importance of the activation of PRP using calcium chloride or thromboplastin solution. Lu *et al*.,[41] however, pointed out that good results depend on consistent delivery of PRP-derived growth factors.

Wan *et al*.[42] reported on the interactive regulation between rhBMP-2 and vessels endothelial growth factors (recombinant human vascular endothelial growth factor) signaling pathways during osteoclastogenesis. Wang *et al*.[43] proved that a combination of rhBMP-2 and bFGF significantly increases early bone marrow stromal cell proliferation and

differentiation *in vitro* compared to rhBMP-2 or bFGF alone. When combined, rhBMP-2 and bFGF synergistically promote new bone formation. Due to these facts, the combination of rhBMP-2 and PRP, which releases bFGF, supports each other in bone regeneration and angiogenesis.

In addition, the positive effect of PRP in bone regeneration was reported by Wiltfang *et al.*,[44] Choi *et al.*,[45] Fennis *et al.*,[46] Okuda *et al.*,[47] and Marx.[48] Wiltfang *et al.*[44] demonstrated a significant effect on bone regeneration in mini-pigs using a combination of PRP, tricalcium phosphate (TCP), and autogenous bone. Choi *et al.*[45] stressed the advantage of PRP on bone regeneration in combination with autogenous bone grafts over autogenous bone alone in a canine model. Fennis *et al.*[46] compared autogenous irradiated cortical scaffolds with PRP and autogenous bone graft from the iliac crest versus the original bone alone to bridging the defect. All goats had undergone bone remodeling. Okuda *et al.*[47] presented a comparative controlled clinical study in humans using PRP combined with porous hydroxyapatite grafts for the treatment of intrabony periodontal defects. They compared the results with a control group without PRP. Their results showed significantly better clinical improvement in the test group than in the control group. Graham *et al.*[49] pointed out the relevance of platelet-derived growth factors and the transforming growth factors (TGF-al and TGF-32) for bone regeneration.

Negative Comments on the Use of PRP

The publications with negative results over the last years can be summarized in four groups:

1. The use of PRP in combination with anorganic bovine bone mineral (ABBM). Mooren *et al.*,[50] Döri *et al.*,[51] Pradeep *et al.*,[52] and Camargo *et al.*[53] found no advantage in bone development using PRP in addition to ABBM. Osteoblasts have difficulties in adhering to smooth surfaces, but ABBM has a smooth surface. Due to this, most attempts in creating new bone using ABBM may not be able to provide close contact between bone and the bovine material under reproducible conditions.
2. The second group combined PRP with autogenous bone. Schaaf *et al.*,[54] Nagata *et al.*,[55] and Luaces-Rey *et al.*[56] could not improve the results by adding PRP to the autogenous bone. The background for these results seems to be unclear because there are other authors who increased the bone level by combining autogenous bone and PRP (Hakimi *et al.*[37] and Mariano *et al.*[38]).
3. The third group, Gürbüzer *et al.*[57] and Forriol *et al.*,[58] tried to create new bone using PRP without any scaffold. Without structure-providing matrices the development of newly grown bone seems to be an unobtainable target.
4. The fourth group, representing Piemontese *et al.*,[59] Trombelli and Farina,[60] Powell *et al.*,[61] and Harnack *et al.*,[62] reported on periodontal bone regeneration using the well-known muco-periosteal flap technique. In this surgical procedure the papilla is cut and a muco-periosteal flap is prepared from the alveolar bone. The reunification of the buckle and oral parts of the papilla turns out to be a major difficulty because of the contained situation. Due to this, the risk of wound infection and loss of attachment is high. This might have caused the poor results. Modern bone regeneration in periodontology using PRP or/and BMPs requires a totally different surgical procedure. We presented the bridge flap technique as a new approach to avoid wound healing disturbances and to increase the results of surgical treatments.[63]

Polycaprolactone and TCP in Combination with BMPs and PRP

The combination of polycaprolactone with PRP and 1.2 mg rhBMP-2 (1.5 mg/mL) also showed excellent results.[64]

TCP is a well-known anorganic augmentation material in bone surgery throughout the world.[1,2] The osteoinductive

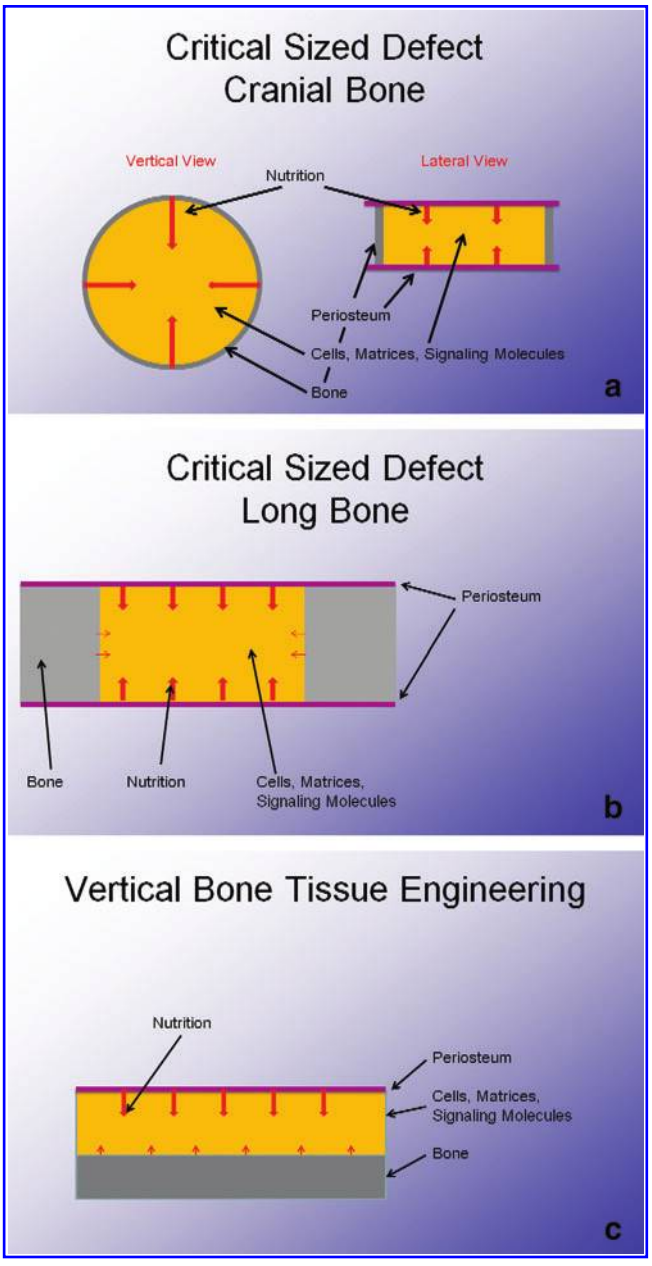

FIG. 1. (**a–c**) Models of bone regeneration. Color images available online at www.liebertonline.com/ten.

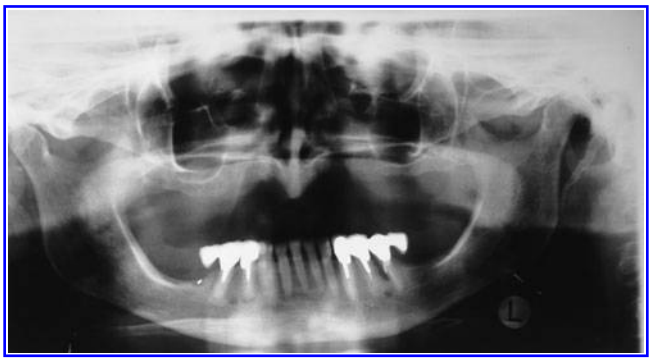

FIG. 2. Preoperative orthopantomography.

potential of hydroxyapatite and injectable biomaterials for regenerating bone was demonstrated by Tsiridis *et al.*[65] and Kretlow *et al.*[66] Basically, the use of PRP in bone regeneration has improved the results in bone surgery. Heliotis *et al.*[67] and Ripamonti *et al.*[68] described in their overviews the potential of growth factors and their relevance for bone induction in clinical use. Wikesjö *et al.*[69–72] and Leknes *et al.*[73,74] highlighted the new method of alveolar ridge augmentation using implants coated with BMPs.

Models of Bone Tissue Engineering

Many animal trials have been carried out to regenerate new bone in critical-sized defect models. Rats are often used. Defects from 8 to 9 mm in diameter are created in their cranium. Under animal model conditions the periosteum must be removed before the implantation of augmentation material. A totally different treatment is required in the regeneration of human cranial bone defects. Spector[75] pointed out the advantage of regenerating tissues *in vivo*. In this context, he stressed the importance of periosteum for nutrition of the augmentation area. Bone surgeons try to preserve the periosteum while treating humans with bony defects. Figure 1a explains bone regeneration in critical-sized defects of the cranial bone in humans. Figure 1b shows the same situation in long bone defects. In both situations, the periosteum is visible surrounding the augmentation material or at least is visible from both sides of the augmentation material. A totally different issue appears in the model for bone tissue engineering in the vertical dimension using onlay technique (Fig. 1c). In this case the periosteum is only visible on top of the augmentation material. Due to this, nutrition is predominantly initiated from one side, which means that a greater number of cells or a greater variety of cells is necessary. In addition, it also signifies that a greater number or a greater

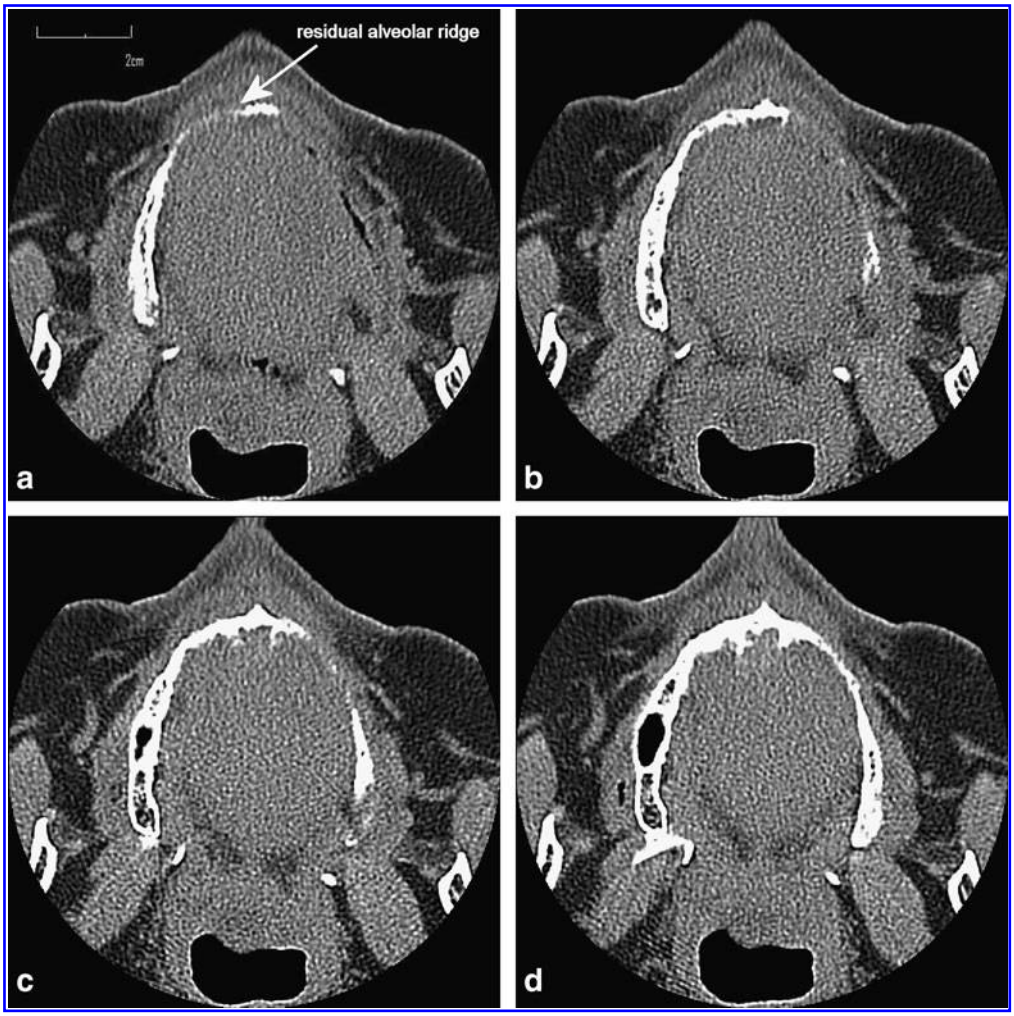

FIG. 3. (**a–d**) Preoperative CT with minimal residual bone in the maxilla (caudal to cranial levels).

FIG. 4. Tricalcium phosphate blocks soaked with platelet-rich plasma and recombinant human bone morphogenetic protein-2. It must be mentioned that the TCP blocks were soaked with PRP and rhBMP-2. Color images available online at www.liebertonline.com/ten.

variety of signaling molecules have to be inserted in the augmentation material to recreate new bone in the required space.

Surgical Case

The 75-year-old woman in this case could receive neither bone transplantation nor osteodistraction to recreate the alveolar ridge in her maxilla. Screws could not be driven in because of the minimal residual bone. Figure 2 represents the preoperative orthopantomography performed by a different clinic, and Figure 3a–d shows a preoperative CT (120 kV, 100 mA) from caudal to cranial. Both X-rays demonstrate the minimal residual bone in the maxilla of this patient. As part of the perioperative process conducted by an anesthetist, 80 mL of venous blood was drawn and centrifugated in two steps (first centrifugation 10 min/2400 rpm: separation of plasma with platelets; second centrifugation 15 min/3600 rpm: separation of the PRP). Calcium chloride (100 mg/mL) was used to activate the PRP. The surgical procedure was carried out under analgosedation with local anesthesia and antibiotic therapy (amoxicillin, 3×1000 mg/day over 2 weeks).

Only transversal cuts through the soft tissue were made. Following this, the complete soft tissue including the periosteum was separated from the bone using the bridge flap technique developed in our institute.

A TCP block of 100×25×5 mm (Vitoss®; Stryker) was cut into smaller pieces and was soaked with activated PRP and rhBMP-2 (12 mg) (1.5 mg/mL) (InductOs, Europe/INFUSE, US; Wyeth Medicine) (Fig. 4). These blocks were implanted endoscopically assisted between the residual bone and the periosteum (Fig. 5a). They were attached by tightening the soft tissue including the periosteum. Figure 5b explains the postoperative situation immediately at the end of the surgical treatment. The amount of augmentation material is visible in Figure 6 (orthopantomography postoperative) (Siemens Orthopantomograph; 75 kV, 15 mA).

Results

One week after the implantation of the TCP scaffold with PRP and rhBMP-2, a complication-free wound healing permitted the removal of the sutures and the patient was able to wear dentures again 2 weeks later. At the same time, a control CT (Fig. 7a–d) was carried out to ensure that enough augmentation material had been implanted in the transversal direction. Controls were made at intervals of every 2–3 weeks. Four months later, six dental implants were placed into the newly grown bone (Fig. 8). Before implant drilling, bone samples were taken at all implant positions for histology,

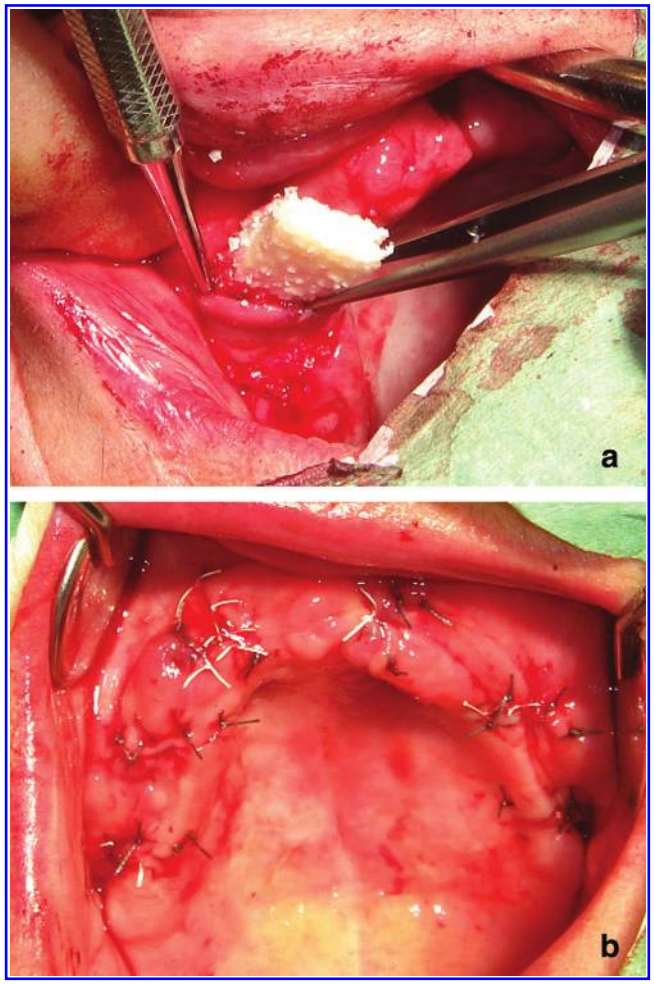

FIG. 5. (**a**) Implantation of soaked tricalcium phosphate blocks between residual bone and bridge flap and (**b**) maxilla situation at the end of surgery. Color images available online at www.liebertonline.com/ten.

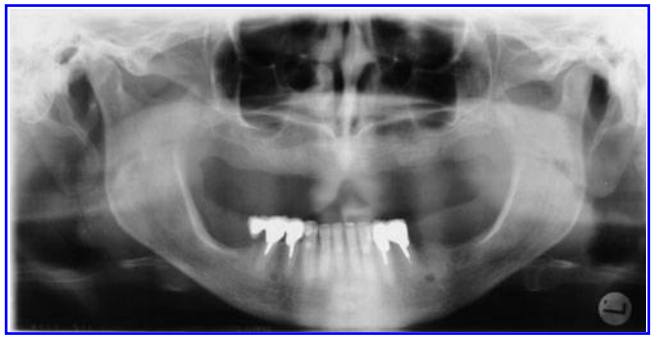

FIG. 6. Postoperative orthopantomography.

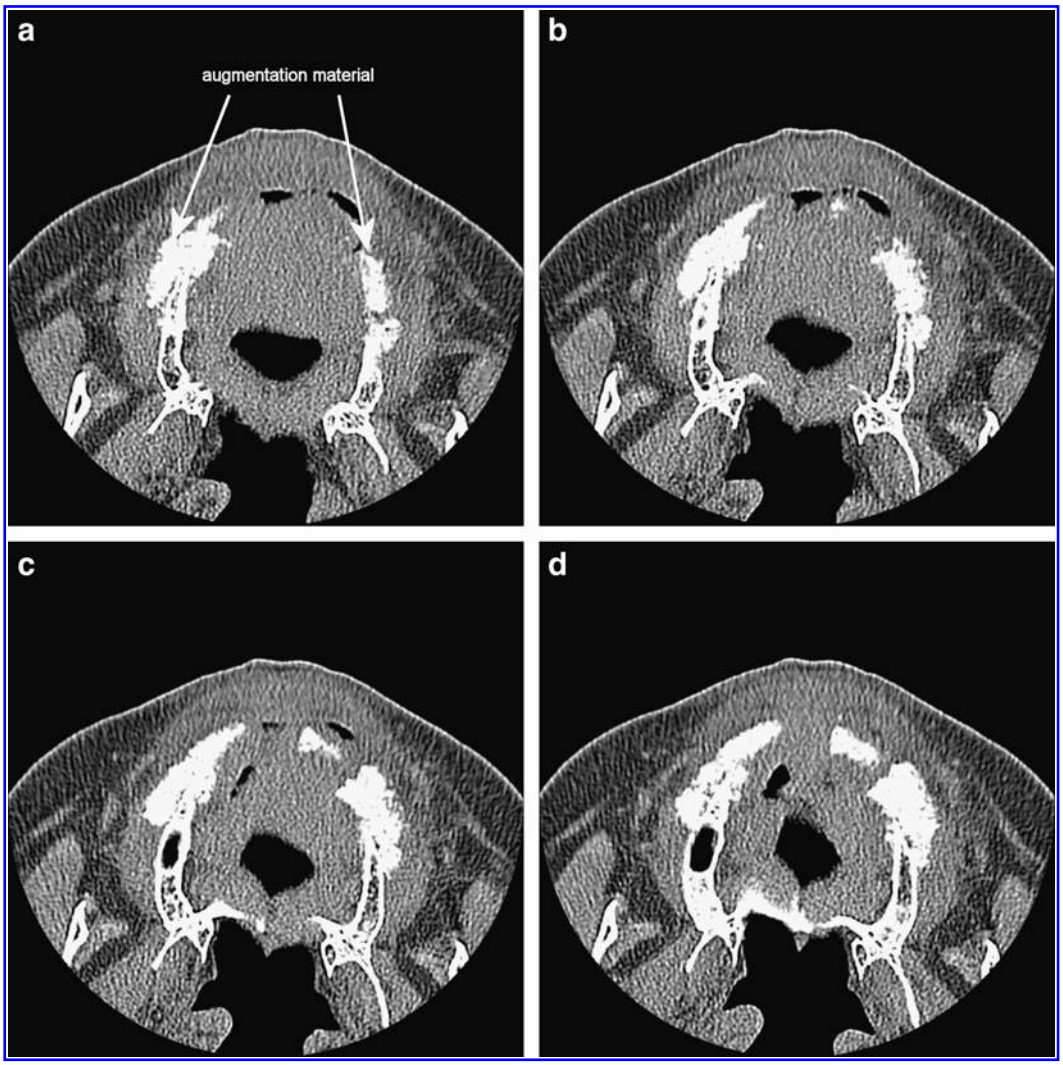

FIG. 7. (**a–d**) CT 2 weeks postoperative with augmentation material (caudal to cranial levels).

using a trephine drill (diameter 2.5 mm). All bone samples from the maxilla demonstrate vital lamellar bone with small parts of soft tissue in histological specimens (stained elastic van Gieson), (Fig. 9a–c right side; Fig. 10b,c left side) except for the one from the anterior position left side (Fig. 10a). The histology from this sample presents capillary-rich connective tissue with a small fragment of lamellar bone and slightly arranged osteocytes. Figure 11 shows the 1-year follow-up orthopantomography with a stable dental structure fixed on the implants (12 months after augmentation, 8 months after implantation) and Figure 12 the situation in the patient's maxilla.

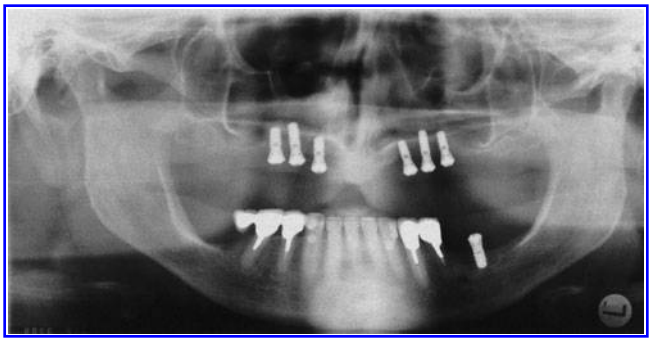

FIG. 8. Orthopantomography control after implantation.

Discussion

The results of this single case are comparable to those of other authors who augmented the alveolar ridge in the maxilla of patients using autogenous bone grafts or autogenous bone grafts in combination with other materials. The major difference is the initial situation where surgeons are unable to carry out the well-known techniques using screws for fixation of graft materials or osteodistraction devices.

Demarosi *et al.*[76] presented their results using a special osteotome technique that widens the space between the two cortical bone parts. This can only be carried out if the residual alveolar ridge is wide enough to split and screw in implants. In their clinical study they presented the results of 23 patients with 36 implants and reported of little morbidity and good results. Sbordone *et al.*[77] presented a review of the treatment of 14 patients with 32 iliac crest or chin grafts in

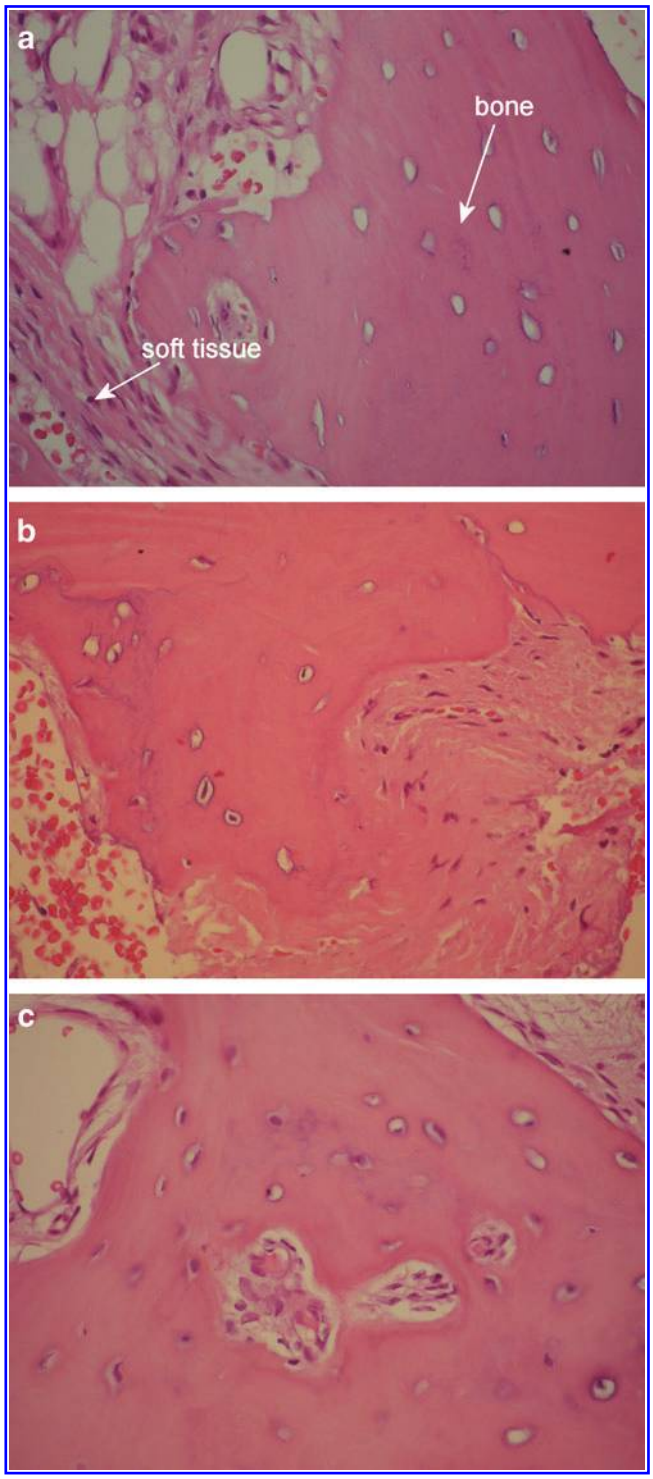

FIG. 9. (**a–c**) Bone histologies from maxilla right side (newly grown bone). Color images available online at www.liebertonline.com/ten.

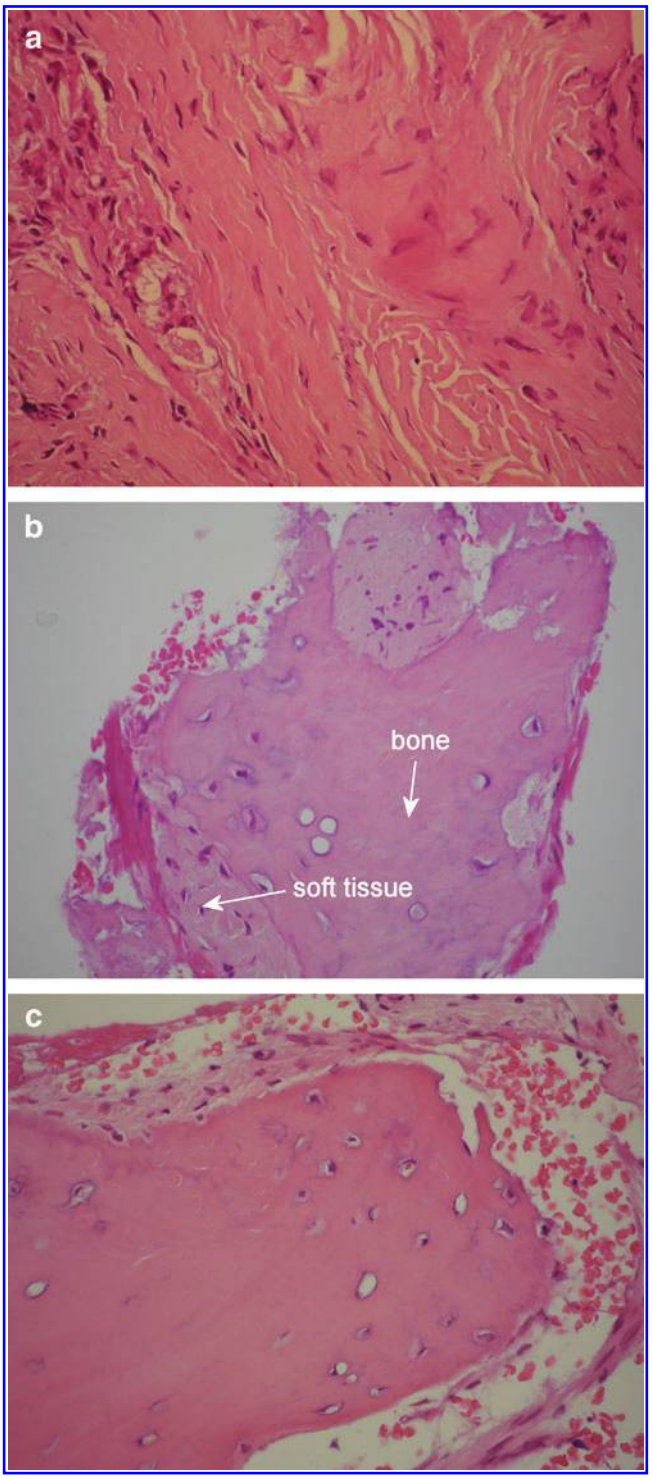

FIG. 10. Histology from maxilla left side: (**a**) anterior position; (**b, c**) newly grown bone. Color images available online at www.liebertonline.com/ten.

onlay position. After 1 year they realized an average resorption rate of 42% in the anterior maxilla. Positioning implants at an earlier stage could perhaps have avoided such an enormous resorption rate. In a second article, Sbordone *et al.*[78] presented the treatment of 40 patients with 48 onlay grafts in the maxilla. They realized a mean resorption in the maxilla in the buccal side of 4.6 ± 0.9 mm and 3.8 ± 0.8 mm in the palatinal side, using chin grafts and 3.4 ± 1.7 mm buccally and 2.6 ± 1.4 mm palatally using iliac grafts. These resorption rates appear to be very high. Contar *et al.*[79] reconstructed the maxilla alveolar ridge using fresh frozen tibia bone chips as allografts in 15 patients. The re-entry with setting implants after 9 months proved a well-incorporated and vascularized bone situation. They concluded that bone

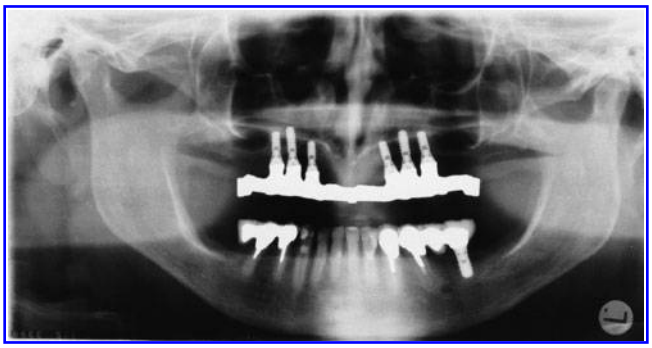

FIG. 11. Orthopantomography—1 year after surgery.

allografts can be successfully used as graft material for the treatment of maxilla ridge defects. If adequate surgical techniques are adopted, this type of bone graft can be safely used in regions of implant placement as a suitable alternative to autogenous grafts. This article does not contain any information regarding resorption of the augmentation material. Barone and Covani[80] presented a clinical study including 56 patients with iliac crest grafts for maxilla alveolar ridge augmentation. Dental implants were screwed in 4–5 months after augmentation. Three out of the 129 grafts needed to be removed due to early exposure occurring with the bone grafts. Corinaldesi *et al*.[81] combined autogenous bone with bovine porous bone mineral (BPBM) for alveolar bone augmentation in 12 patients. In addition, they used a titanium micromesh. At the time of implant insertion, 8–9 months after grafting, bone biopsies were taken. They reported that the BPBM particles were surrounded completely by newly formed bone without signs of resorption. It can therefore be concluded that the BPBM particles were not biodegraded. Valentini *et al*.[82] published a retrospective analysis on 233 patients with facial bone defects having been reconstructed with autogenous bone grafts. In 215 cases they found adequate integration of the graft material. Complications occurred in 7 of 72 patients in which bone grafts had been fixed with wire fixation, and in 11 of 161 patients in which bone grafts had been fixed with rigid internal fixation.

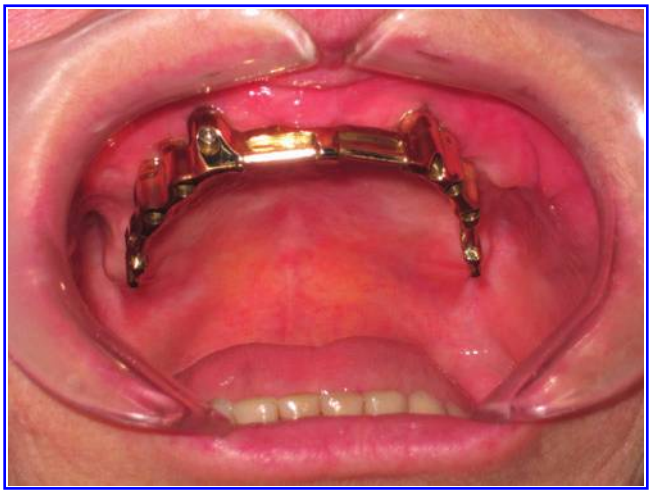

FIG. 12. Prosthetic construction on implants. Color images available online at www.liebertonline.com/ten.

Sjöström *et al*.[83] reported on 29 patients with iliac graft augmentation of the alveolar ridge in the maxilla. Implants were screwed in 6 months after augmentation. They presented an implant survival rate of 90% at the 3-year follow-up. From Zerbo *et al*.[84] and Acocella *et al*.,[85] we know that the majority of the osteocytes of the monocortical bone that were transplanted did not survive grafting. They also reported on new vascularization of nonvital grafted bone. Their results explain that the remodeling process takes up to 7 months after grafting. Krenkel and Grunert[3] inserted a special osteodistraction device, the so-called endodistractor. They treated 18 patients in the anterior mandible and presented a mean distraction amplitude of 11.3 mm (8–14 mm). The mean retention time was 186.6 days (37–309 days). They realized two mandibular fractures occurring 6 weeks after placing the endodistractor. Robiony *et al*.[4] treated 12 patients with osteodistraction osteogenesis using autogenous bone graft and PRP. They reported on a distraction rate of 0.5 mm/day and removed the distraction device after a 60-day period of consolidation. They concluded that long-term results confirm the combination of autogenous bone–platelet gel with alveolar distraction osteogenesis as an effective and predictable procedure in restoration of severe atrophic mandible. Rachmiel *et al*.[5] used a distraction rate of 0.8 mm/day for 10–16 days, also followed by a consolidation period of 60 days. They achieved good results without adding autogenous bone. Marchetti *et al*.[6] reported on 10 patients, where osteodistraction was carried out in 2 patients in the maxilla and in 8 patients in the mandible. They used a distraction rate of 1 mm/day and proved well-organized lamellar bone in all bone samples. Raghoebar *et al*.[7] used vertical distraction osteogenesis in edentulous mandibles of 10 patients. Two months after the final day of distraction, a bone biopsy was taken with a trephine and afterward both distraction screws were replaced by endosseous implants. Radiographical and histomorphological examination of the bone biopsies revealed the presence of two cortical zones, one at each end of the biopsies and a poorly mineralized, fibrous interzone in the middle of the distraction gap.

Advantages of Bone Tissue Engineering

Comparing the abovementioned results of different authors using the transplantation of autogenous bone with or without other materials and/or osteodistraction to those we achieved with modern bone regeneration using rhBMP-2, PRP, and TCP, we conclude that there seem to be advantages of bone tissue engineering.

1. No bone harvesting was carried out; as a result, none of the negative effects of bone harvesting occurred.
2. No resorption of newly grown bone was visible because of loading with implants at an early stage.
3. The total procedure of bone tissue engineering prevented an impairment of the patient as far as it was possible. The perioperative stress, the surgical risk, and the perioperative risk for the patient were reduced.

From the first publication on bone induction via growth factors reported on by Urist in 1965,[9] a number of animal models and clinical studies on bone regeneration with BMPs have been carried out. Since 2002, rhBMP-2 and rhBMP-7 have been available as therapeutics for use in humans. The

number of patients having received bone tissue engineering treatments meanwhile amounts to more than 100,000 world wide.

The development from a tiny residual part of the alveolar ridge to a size that enables the surgeon to set six implants into the newly grown bone is a special challenge in modern bone regeneration in oral and maxillofacial surgery. The demonstrated surgical procedure, including the bridge flap technique and the intensive perioperative precautions to avoid swelling and wound infection, opens more options in oral and maxillofacial surgery than ever before.

It is important to take into account that this is only the presentation of one single case. In addition, expert knowledge and special equipment are necessary to treat such patients. From our present point of view, clinical studies concerning this technique have to follow to assess whether this technique is effective in a wide range of patients or whether it requires further development.

Acknowledgments

This single clinical case was performed with the knowledge of the ethics authorities. Unlike clinical studies, in the run-up to these single cases, no written application or final acceptance is required. The authors wish to thank Ms. Birgit Gemmeke, Prof. Lang, and Mr. Jochen Eichholtz for their technical support and Ms. Conny Helbling for her linguistic review as a native speaker.

Disclosure Statement

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References

1. Betz, R.R. Limitations of autograft and allograft: new synthetic solutions. Orthopedics **25,** 561, 2002.
2. Horch, H.-H., and Pautke, C. Regeneration instead of reparation. Mund Kiefer Gesichtschir **10,** 213, 2006.
3. Krenkel, C., and Grunert, I. The endo-distractor for preimplant mandibular regeneration. Rev Stomatol Chir Maxillofac **110,** 17, 2009.
4. Robiony, M., Zorzan, E., Polini, F., Sembronio, S., Toro, C., and Politi, M. Osteogenesis distraction and platelet-rich plasma: combined use in restoration of severe atrophic mandible. Long-term results. Clin Oral Implants Res **19,** 1202, 2008.
5. Rachmiel, A., Srouji, S., and Peled, M. Alveolar ridge augmentation by distraction osteogenesis. Int J Oral Maxillofac Surg **30,** 510, 2001.
6. Marchetti, C., Coriinaldesi, G., Pieri, F., Degidi, M., and Piattelli, A. Alveolar distraction osteogenesis for bone augmentation of severely atrophic ridges in 10 consecutive cases: a histologic and histomorphometric study. J Periodontol **78,** 360, 2007.
7. Raghoebar, G.M., Liem, R.S., and Vissink, A. Vertical distraction of the severely resorbed edentulous mandible: a clinical, histological and electron microscopic study of 10 treated cases. Clin Oral Implants Res **13,** 558, 2002.
8. Merli, M., Merli, M., Triaca, A., and Esposito, M. Segmental distraction osteogenesis of the anterior mandible improving facial esthetics. Preliminary results. World J Orthod **8,** 19, 2007.
9. Urist, M.R. Bone: formation by autoinduction. Science **150,** 893, 1965.
10. Zheng, M.H., Wood, DJ., Wysocki, S., Papadimitriou, J.M., and Wang, E.A. Recombinant human bone morphogenetic protein-2 enhances expression of interleukin-6 and transforming growth factor-beta 1 genes in normal human osteoblast-like cells. J Cell Physiol **159,** 76, 1994.
11. Raida, M., Heymann, A.C., Günther, C., and Niederweiser, D. Role of bone morphogenetic protein 2 in the crosstalk between endothelial progenitor cells and mesenchymal stem cells. Int J Mol Med **18,** 735, 2006.
12. Nevins, M., Kirker-Head, C., Nevins, M., Wozney, J.A., Palmer, R., and Graham, D. Bone formation in the goat maxillary sinus induced by absorbable collagen sponge implants impregnated with rhBMP-2. Int J Periodontics Restorative Dent **16,** 8, 1996.
13. Boyne, P.J. Animal studies of application of rhBMP-2 in maxillofacial reconstruction. Bone **19,** 83, 1996.
14. Terheyden, H., Jepsen, S., Moeller, B., Tucker, M.M., and Rueger, D.C. Sinus floor augmentation with simultaneous placement of dental implants using a combination of deproteinated bone xenografts and recombinant human osteogenic protein-I. Clin Oral Implants Res **10,** 510, 1999.
15. Barboza, E.P., Duarte, M.E., Geolas, L., Sorensen, R.G., and Wikesjö, U.M. Ridge augmentation following implantation of rhBMP-2 in the dog. J Periodontol **71,** 488, 2000.
16. Ripamonti, U., Crooks, J., Petit, J.C., and Rueger, D.C. Periodontal tissue regeneration by combined applications of recombinant human osteogenic protein-1 and bone morphogenetic protein-2. A pilot study in Chacma baboons (*Papio ursinus*). Eur J Oral Sci **109,** 241, 2001.
17. Boyne, P.J., Marx, R.E., Nevins, M., Triplett, G., Lazaro, E., Lilly, L.C., Alder, M., and Nummikoski, P. A feasibility study evaluating rhBMP-2/absorbable collagen sponge for maxillary sinus floor augmentation. Int J Periodontics Restorative Dent **17,** 11, 1997.
18. Boyne, P.J., Lilly, L.C., Marx, R.E., Moy, P.K., Nevins, M., and Spagnoli, D. *De novo* bone induction by recombinant human bone morphogenetic protein-2 (rhBMP-2) in maxillary sinus floor augmentation. J Oral Maxillofac Surg **63,** 1693, 2005.
19. Cochran, D.L., Jones, A.A., Lilly, L.C., Fiorellini J.P., and Howell, H. Evaluation of recombinant human bone morphogenetic protein-2 in oral applications including the use of endosseous implants: 3-year results of a pilot study in humans. J Periodontol **71,** 1241, 2000.
20. Wikesjö, U.M., Qahash, M., Thomson, R.C., Cook, A.D., Rohrer, M.D., Wozney, J.M., and Hardwick, W.R. rhBMP-2 significantly enhances guided bone regeneration. Clin Oral Implants Res **15,** 194, 2004.
21. Fiorellini, J., Howell, T., Cochran, D., Malmquist, J., Lilly, L.C., Spagnoli, D., Toljanic, J., Jones, A., and Nevins, M. Randomized study evaluating rhBMP-2 for extraction socket augmentation. J Periodontol **76,** 605, 2005.
22. Jung, R.E., Glauser, R., Scharer, P., Hammerle, C.H., Sailer, H.F., and Weber, F.E. Effect of rhBMP-2 on guided bone regeneration in humans. Clin Oral Implants Res **14,** 556, 2003.
23. Warnke, P.H., Springer, I.N., Wiltfang, J., Acil, Y., Eufinger, H., Wehmöller, M., Russo, P.A., Bolte, H., Scherry, E., Behrens, E., and Terheyden, H. Growth and transplantation of a custom vascularised bone graft in a man. Lancet **364,** 766, 2004.

24. Medtronic Sofamor Danek, Inc. Summary of Safety and Effectiveness Data, INFUSE™ Bone Graft, Memphis, TN, July 2, 2002.
25. Schuckert, K.H., Jopp, S., and Müller, U. Bone tissue engineering with BMPs—first experiences and results in oral surgery by using rhBMP-2 in humans. Int J Oral Maxillofac Surg **34,** 68, 2005.
26. Marx, R.E. Lecture during 17th International Conference on Oral & Maxillofacial Surgery, 29 August–02 September, 2005, Vienna, Austria.
27. Cenni, E., Avnet, S., Fotia, C., Salerno, M., and Baldrini, N. Platelet-rich plasma impairs osteoclast generation from human precursors of peripheral blood. J Orthop Res 2010 (Epub ahead of print).
28. Plachokova, A.S., Nikolidakis, D., Mulder, J., Jansen, J.A., and Creugers, N.H. Effect of platelet-rich plasma on bone regeneration in dentistry: a systematic review. Clin Oral Implants Res **19,** 539, 2008.
29. Nikolidakis, D., and Jansen, J.A. The biology of platelet-rich plasma and its application in oral surgery: literature review. Tissue Eng Part B Rev **14,** 249, 2008.
30. Intini, G. The use of platelet-rich plasma in bone reconstruction therapy. Biomaterials **30,** 4956, 2009.
31. Marx, R.E., Carlson, E.R., Eichstaedt, R.M., Schimmele, S.R., Strauss, J.E., and Georgeff, K.R. Platelet-rich plasma: growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod **85,** 638, 1998.
32. Bertoldi, C., Pinti, M., Zaffe, D., Cossarizza, A., Consolo, U., and Ceccherelli, G.B. Morphologic, histochemical, and functional analysis of platelet-rich plasma activity on skeletal cultured cells. Transfusion 2009 (Epub ahead of print).
33. Hu, Z.M., Peel, S.A., Ho, S.K., Sándor, G.K., and Clokie, C.M. Comparison of platelet-rich plasma, bovine BMP, and rhBMP-4 on bone matrix protein expression *in vitro*. Growth Factors **27,** 280, 2009.
34. Yokota, K., Ishida, O., Sunagawa, T., Suzuki, O., Nakamae, A., and Ochi, M. Platelet-rich plasma accelerated surgical angio-genesis in vascular-implanted necrotic bone: an experimental study in rabbits. Acta Orthop **79,** 106, 2008.
35. Park, E.J., Kim, E.S., Weber, H.P., Wright, R.F., and Mooney, D.J. Improved bone healing by angiogenic factor-enriched platelet-rich plasma and its synergistic enhancement by bone morphogenetic protein-2. Int J Oral Maxillofac Implants **23,** 818, 2008.
36. Calori, G.M., Tagliabue, L., Gala, L., d'Imporzano, M., Peretti, G., and Albisetti, W. Application of rhBMP-7 and platelet-rich plasma in the treatment of long bone nonunions: a prospective randomised clinical study on 120 patients. Injury **39,** 1391, 2008.
37. Hakimi, M., Jungbluth, P., Sager, M., Betsch, M., Herten, M., Becker, J., Windolf, J., and Wild, M. Combined use of platelet-rich plasma and autologous bone grafts in the treatment of long bone defects in mini-pigs. Injury 2010 (Epub ahead of print).
38. Mariano, R., Messora, M., de Morais, A., Nagata, M., Furlaneto, F., Avelino, C., Paula, F., Ferreira, S., Pinheiro, M., and de Sene, J.P. Bone healing in critical-sized defects treated with platelet-rich plasma: a histologic and histometric study in the calvaria of diabetic rat. Oral Surg Oral Med Oral Pathol Oral Radiol Endod **109,** 72, 2010.
39. Hartmann, E.K., Heintel, T., Morrison, R.H., and Weckbach, A. Influence of platelet-rich plasma on the anterior fusion in spinal injuries: a qualitative and quantitative analysis using computer tomography. Arch Orthop Trauma Surg 2009 (Epub ahead of print).
40. Messora, M.R., Nagata, M.J., Dornelles, R.C., Bomfim, S.R., Furlaneto, F.A., de Melo, L.G., Deliberador, T.M., Bosco, A.F., Garcia, V.G., and Fucini, S.E. Bone healing in critical-size defects treated with platelet-rich plasma activated by two different methods. A histologic and histometric study in rat calvaria. J Periodontal Res **43,** 723, 2008.
41. Lu, H.H., Vo, J.M., Chin, H.S., Lin, J., Cozin, M., Tsay, R., Eisig, S., and Landesberg, R. Controlled delivery of platelet-rich plasma-derived growth factors for bone formation. J Biomed Mater Res A **86,** 1128, 2008.
42. Wan, C., He, Q., and Li, G. Osteoclastogenesis in the nonadherent cell population of human bone marrow is inhibited by rhBMP-2 alone or together with rhVEGF. J Orthop Res **24,** 29, 2006.
43. Wang, L., Huang, Y., Pan, K., Jiang, X., and Liu, C. Osteogenic responses to different concentrations/ratios of BMP-2 and bFGF in bone formation. Ann Biomed Eng **38,** 77, 2010.
44. Wiltfang, J., Kloss, F.R., Kessler, P., Nkenke, E., Schultze-Mosgau, S., Zimmermann, R., and Schlegel, K.A. Effects of platelet-rich plasma on bone healing in combination with autogenous bone and bone substitutes in critical size defects. Clin Oral Implants Res **15,** 187, 2004.
45. Choi, B.H., Im, C.J., Huh, J.Y., Suh, J.J., and Lee, S.H. Effect of platelet rich plasma on bone regeneration in autogenous bone graft. Int J Oral Maxillofac Surg **33,** 56, 2004.
46. Fennis, J.P.M., Stoelinga, P.J.W., and Jansen, J.A. Reconstruction of the mandible with an autogenous irradiated cortical scaffold, autogenous corticocancellous bone-graft and autogenous platelet-rich plasma: an animal experiment. Int J Oral Maxillofac Surg **34,** 158, 2005.
47. Okuda, K., Tai, H., Tanabe, K., Suzuki, H., Sato, H., Kawase, T., Saito, Y., Wolff, L.F., and Yoshiex, H. Platelet-rich plasma combined with a porous hydroxyapatite graft for the treatment of intrabony periodontal defects in humans: a comparative controlled clinical study. J Periodontal **76,** 890, 2005.
48. Marx, R.E. Platelet rich plasma: evidence to support its use. J Oral Maxillofac Surg **62,** 489, 2004.
49. Graham, S., Leonidou, A., Lester, M., Heliotis, M., Mantalaris, A., and Tsiridis, E. Investigating the role of PDGF as a potential drug therapy in bone formation and fracture healing. Expert Opin Investig Drugs **18,** 1633, 2009.
50. Mooren, R.E., Dankers, A.C., Merkx, M.A., Bronkhorst, E.M., Jansen, J.A., and Stoelinga, P.J. The effect of platelet-rich plasma on early and late bone healing using a mixture of particulate autogenous cancellous bone and Bio-Oss((R)): an experimental study in goats. Int J Oral Maxillofac Surg 2010 (Epub ahead of print).
51. Döri, F., Kovács, V., Arweiler, N.B., Huszár, T., Gera, I., Nikolidakis, D., and Sculean, A. Effect of platelet-rich plasma on the healing of intrabony defects treated with an anorganic bovine mineral: a pilot study. J Periodontol **80,** 1599, 2009.
52. Pradeep, A.R., Pai, S., Garg, G., Devi, P., and Shetty, S.K. A randomized clinical trial of autologous platelet-rich plasma in the treatment of mandibular degree II furcation defects. J Clin Periodontol **36,** 581, 2009.
53. Camargo, P.M., Lekovic, V., Weinlaender, M., Divnic-Resnik, T., Pavlovic, M., and Kenney, E.B. A surgical reentry study on the influence of platelet-rich plasma in enhancing the regenerative effects of bovine porous bone mineral and guided tissue regeneration in the treatment of intrabony defects in humans. J Periodontol **80,** 915, 2009.
54. Schaaf, H., Streckbein, P., Lendeckel, S., Heidinger, K.S., Rehmann, P., Boedecker, R.H., and Howaldt, H.P. Sinus lift

augmentation using autogenous bone grafts and platelet-rich plasma: radiographic results. Oral Surg Oral Med Oral Pathol Oral Radiol Endod **106,** 673, 2008.
55. Nagata, M.J., Messora, M., Pola, N., Campos, N., Vieira, R., Esper, L.A., Sbrana, M., Fucini, S., Garcia, V., and Bosco, A. Influence of the ratio of particulate autogenous bone graft/platelet-rich plasma on bone healing in critical-size defects: a histologic and histometric study in rat calvaria. J Orthop Res **28,** 468, 2010.
56. Luaces-Rey, R., Arenaz, J., Lopez-Cedrún, J.L., Herrero, S., Sironvalle, S., Igelsias, E., and Pombo, M. Is PRP useful in alveolar cleft reconstruction? Platelet-rich plasma in secondary alveoloplasty. Med Oral Patol Oral Cir Bucal 2009 (Epub ahead of print).
57. Gürbüzer, B., Pikdöken, L., Urhan, M., Süer, B.T., and Narin, Y. Scintigraphic evaluation of early osteoblastic activity in extraction sockets treated with platelet-rich plasma. J Oral Maxillofac Surg **66,** 2454, 2008.
58. Forriol, F., Longo, U.G., Concejo, C., Ripalda, P., Maffulli, N., and Denaro, V. Platelet-rich plasma, rhOP-1 (rhBMP-7) and frozen rib allograft for the reconstruction of bony mandibular defects in sheep. A pilot experimental study. Injury **40,** S44, 2009.
59. Piemontese, M., Aspriello, S.D., Rubini, C., Ferrante, L., and Procaccini, M. Treatment of periodontal intrabony defects with demineralized freeze-dried bone allograft in combination with platelet-rich plasma: a comparative clinical trial. J Periodontol **79,** 802, 2008.
60. Trombelli, L., and Farina, R. Clinical outcomes with bioactive agents alone or in combination with grafting or guided tissue regeneration. J Clin Periodontol **35,** 117, 2008.
61. Powell, C.A., Bannister, S.R., Mackey, S.A., Maller, S.C., McDonnell, H.T., and Deas, D.E. Periodontal wound healing with and without platelet-rich plasma: histologic observations and assessment of flap tensile strength. J Periodontol **80,** 985, 2009.
62. Harnack, L., Boedecker, R.H., Kurtulus, I., Boehm, S., Gonzales, J., and Meyle, J. Use of platelet-rich plasma in periodontal surgery—a prospective randomised double blind clinical trial. Clin Oral Investig **13,** 179, 2009.
63. Schuckert, K.H., Jopp, S., and Müller, U. Use of rhBMP-2 in periodontal surgery—micro surgical reconstruction and flap. Dtsch Zahnärztl Z **5,** 259, 2006.
64. Schuckert, K.H., Jopp, S., and Teoh, S.H. Mandibular defect reconstruction using three-dimensional polycaprolactone scaffold in combination with platelet-rich plasma and recombinant human bone morphogenetic protein-2: *de novo* synthesis of bone in a single case. Tissue Eng **15,** 493, 2009.
65. Tsiridis, E., Bhalla, A., Ali, Z., Gurav, N., Heliotis, M., Deb, S., and DiSilvio, L. Enhancing the osteoinductive properties of hydroxyapatite by the addition of human mesenchymal stem cells, and recombinant human osteogenic protein-1 (BMP-7) *in vitro.* Injury **37,** 25, 2006.
66. Kretlow, J.D., Young, S., Klouda, L, Wong, M., and Mikos, A.G. Injectable biomaterials for regenerating complex craniofacial tissues. Adv Mater Deerfield **21,** 3368, 2009.
67. Heliotis, M., Ripamonti, U., Feretti, C., Kerawala, C., Mantalaris, A., and Tsiridis, E. The basic science of bone induction. Br J Oral Maxillofac Surg **47,** 511, 2009.
68. Ripamonti, U., Heliotis, M., and Ferretti, C. Bone morphogenetic proteins and the induction of bone formation: from laboratory to patients. Oral Maxillofac Surg Clin North Am **19,** 575, 2007.
69. Wikesjö, U.M., Qahash, M., Huang, Y.H., Xiropaidis, A., Polimeni, G., and Susin, C. Bone morphogenetic proteins for periodontal and alveolar indications; biological observations—clinical implications. Orthod Craniofac Res **12,** 263, 2009.
70. Wikesjö, U.M., Qahash, M., Polimeni, G., Susin, C., Shanaman, R.H., Rohrer, M.D., Wozney, J.M., and Hall, J. Alveolar ridge augmentation using implants coated with recombinant human bone morphogenetic protein-2: histologic observations. J Clin Periodontol **35,** 1001, 2008.
71. Wikesjö, U.M., Xiropaidis, A.V., Qahash, M., Lim, W.H., Sorensen, R.G., Rohrer, M.D., Wozney, J.M., and Hall, J. Bone formation at recombinant human bone morphogenetic protein-2-coated titanium implants in the posterior mandible (type II bone) in dogs. J Clin Periondontol **35,** 985, 2008.
72. Wikesjö, U.M., Huang, Y.H., Polimeni, G., and Qahash, M. Bone morphogenetic proteins: a realistic alternative to bone grafting for alveolar reconstruction. Oral Maxillofac Surg Clin North Am **19,** 535, 2007.
73. Leknes, K.N., Yang, J., Qahash, M., Polimeni, G., Susin, C., and Wikesjö, U.M. Alveolar ridge augmentation using implants coated with recombinant human bone morphogenetic protein-2: radiographic observations. Clin Oral Implants Res **19,** 1027, 2008.
74. Leknes, K.N., Yang, J., Qahash, M., Polimeni, G., Susin, C., and Wikesjö, U.M. Alveolar ridge augmentation using implants coated with recombinant human bone morphogenetic protein-7 (rhBMP-2/rhOP-1): radiographic observations. J Clin Periodontol **35,** 914, 2008.
75. Spector, M. Biomaterials-based tissue engineering and regenerative medicine solutions to musculoskeletal problems. Swiss Med Wkly **137,** 157, 2007.
76. Demarosi, F., Leghissa, G.C., Sardella, A., Lodi, G., and Carrassi, A. Localised maxillary ridge expansion with simultaneous implant placement: a case series. Br J Oral Maxillogac Surg **47,** 535, 2009.
77. Sbordone, L., Toti, P., Menchini-Fabris, G.B., Sbordone, C., Piombino, P., and Guidetti, F. Volume changes of autogenous bone grafts after alveolar ridge augmentation of atrophic maxillae and mandibles. Int J Oral Maxillofac Implants **38,** 1059, 2009.
78. Sbordone, L., Toti, P., Menchini-Fabris, G.B., Sbordone, C., and Guidetti, F. Implant survival in maxillary and mandibular osseous onlay grafts and native bone: a 3-year clinical and computerized tomographic follow-up. Int J Oral Maxillofac Implants **24,** 695, 2009.
79. Contar, C.M., Sarot, J.R., Bordini, J., Jr., Galvão, G.H., Nicolau, G.V., and Machado, M.A. Maxillary ridge augmentation with fresh-frozen bone allografts. J Oral Maxillofac Surg **67,** 1280, 2009.
80. Barone, A., and Covani, U. Maxillary alveolar ridge reconstruction with nonvascularized autogenous block bone: clinical results. J Oral Maxillofac Surg **65,** 2039, 2007.
81. Corinaldesi, G., Pieri, F., Marchetti, C., Fini, M., Aldini, N.N., and Giardino, R. Histologic and histomorphometric evaluation of alveolar ridge augmentation using bone grafts and titanium micromesh in humans. J Periodontol **78,** 1477, 2007.
82. Valentini, V., Cassoni, A., Marianetti, T.M., Romano, F., Terenzi, V., and Iannetti, G. Reconstruction of craniofacial bony defects using autogenous bone grafts: a retrospective study on 233 patients. J Craniofac Surg **18,** 953, 2007.

83. Sjöström, M., Sennerby, L., Nilson, H., and Lundgren, S. Reconstruction of the atrophic edentulous maxilla with free iliac crest grafts and implants: a 3-year report of a prospective clinical study. Clin Implant Dent Relat Res **9,** 46, 2007.
84. Zerbo, I.R., de Lange, G.L., Joldersma, M., Bronckers, A.L., and Burger, E.H. Fate of monocortical bone blocks grafted in the human maxilla: a histological and histomorphometric study. Clin Oral Implants Res **14,** 759, 2003.
85. Acocella, A., Bertolai, R., Colafranceschi, M., and Sacco, R. Clinical, histological and histomorphometric evaluation of the healing of mandibular ramus bone block grafts for alveolar ridge augmentation before implant placement. J Craniomaxillofac Surg **38,** 222, 2010.

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