Clinical and Histologic Evaluation of Allogeneic Bone Matrix Versus Autogenous Bone Chips Associated with Titanium-Reinforced e-PTFE Membrane for Vertical Ridge Augmentation: A Prospective Pilot Study

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Purpose: *To compare clinically and histologically an allogeneic bone matrix to autogenous bone chips in the vertical ridge augmentation technique using titanium-reinforced e-PTFE membranes.* ***Materials and Methods:*** *The study protocol was designed to include patients with bilateral posterior mandibular partial edentulism. Patients were treated with a split-mouth design approach: each side was randomly assigned to the test group (titanium-reinforced e-PTFE membrane and allogeneic bone matrix) or to the control group (titanium-reinforced e-PTFE membrane and autogenous bone chips). Different clinical parameters including the amount of vertically regenerated bone (DSB) and biologic complications were recorded. Histomorphometric analysis and the bone-implant contact percentage were performed.* ***Results:*** *Five female patients were enrolled in the study. Ten edentulous sites were vertically augmented and 25 implants were inserted (13 test group, 12 control group) with a staged approach. In the test group no membrane was exposed. The mean bone regeneration was 4.70 mm (SD 0.48 mm). All 13 implants appeared clinically stable. In the control group, 1 membrane was exposed after 2 months. The mean crestal bone regeneration was 4.10 mm (SD 0.88 mm). All 12 implants were stable at the abutment connection. Nine biopsy specimens from the regenerated areas were evaluated. Vertical bone regeneration was evident in both groups since all the samples demonstrated trabecular bone with different degrees of maturation and mineralization in the regenerated area.* ***Conclusion:*** *Within the limits of this study based on 5 patients, it appears that the behavior of the allogeneic bone matrix is similar to that of autogenous bone chips when used for vertical ridge augmentation by means of guided bone regeneration techniques. Both grafts demonstrated analogous histologic characteristics. Nevertheless, long-term clinical studies are needed to confirm these preliminary results.* Int J Oral Maxillofac Implants 2008;23:1003–1012

Key words: *allogeneic bone matrix, autogenous bone, barrier membranes, demineralized bone matrix, guided bone regeneration, osseointegration, vertical ridge augmentation*

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During the last 10 years vertical ridge augmentation techniques have been progressively modified to increase their predictability and versatility. For this purpose, the clinical application of guided bone regeneration has been shown to be predictable in short- and long-term studies.[1,2]

In 1994 a report of 6 cases by Simion et al[1] first demonstrated the possibility of vertically augmenting the bone in atrophic edentulous ridges in humans using a titanium reinforced expanded polytetrafluoroethylene (e-PTFE) membrane. The space under the membrane was filled with blood clot. In the following years different studies[2,4–6] showed that the use of a particulated autogenous bone graft, in

association with the membrane, increases the potential for vertical bone regeneration. Autogenous bone is still considered the "gold standard" for vertical ridge augmentation with GBR techniques: It is biocompatible, it has osteogenic and osteoinductive properties, it is capable of maintaining adequate space under the membrane for a sufficient period of time, and it can be eventually resorbed and replaced with new bone. Nevertheless, the harvesting procedure is considered technically demanding and somewhat invasive.

In the early 1990s Mellonig et al[7,8] proposed the use of demineralized freeze-dried bone allograft (DFDBA) for bone regeneration around dental implants. In 1998 Simion et al[2] evaluated the addition of DFDBA or autogenous bone chips to a membrane technique for vertical ridge augmentation. Clinical and histologic results indicated a beneficial effect when a bone graft filled the space under the membrane. Similar conclusions were drawn by the same investigators in a retrospective clinical study in 2001.[3]

Nevertheless, the controversy about the osteoinductive properties of DFDBA is still open, since, as widely reported, results with such materials have been highly variable.[9–13]

A malleable allogeneic bone matrix (Regenaform, Regeneration Technologies, Alachua, FL, USA) has been proposed to overcome the problem of inconsistency. To ensure tissue quality, every lot of Regenaform is tested for osteoinductivity. This allograft is a combination of assayed demineralized bone matrix, also known as DFDBA, and cortico-cancellous chips uniformly dispersed in a thermoplastic biological carrier. The carrier is a collagen gel not soluble in aqueous environments that confers to the allograft matrix its thermoplasticity. Its consistency is a resilient solid at body and room temperature, but it becomes soft and moldable when warmed at 43°C to 49°C.

The aim of this prospective study was to compare, clinically and histologically, an allogeneic bone matrix to autogenous bone graft in the vertical ridge augmentation technique using titanium-reinforced e-PTFE membranes.

MATERIALS AND METHODS

The study protocol was designed to include patients with bilateral posterior mandibular partial edentulism (Applegate-Kennedy Class I) in whom vertical bone augmentation was needed for esthetic or functional reasons prior to implant placement.

Patients were recruited in the Department of Periodontology of the University of Milan using the following inclusion criteria:

- Good general health
- Bilateral posterior mandibular partial edentulism (Applegate-Kennedy Class I)
- Vertical bone defect greater than 3 mm as measured on a panoramic radiograph as the distance between the deeper area of the edentulous ridge and the distal bone peak of adjacent tooth in reference to the virtual occlusal plane
- Interarch distance > 10 mm
- Age > 18 years
- No relevant medical conditions
- No medical history of head and neck radiation therapy
- No daily intake of the following drugs: anticoagulants, antiplatelets, diphosphonates, glucocorticoids
- For female patients, no pregnancy or lactation
- Smoking < 10 cigarettes/d
- No heavy bruxism
- No tooth extraction involving the surgical sites in the preceding 2 months
- Full Mouth Plaque Score and Full Mouth Bleeding Score < 25% at 4 sites per tooth[14]
- No active periodontal disease

All patients received an exhaustive explanation of the surgical procedure, the possible risks, and the alternative prosthetic solutions. Written informed consent was given by all participants. No ethical committee permission was necessary for this study, in that both the test and the control sites were treated with institutionally approved materials and methods.

All patients were treated with a split-mouth design approach: at the time of the first surgery one side was randomly assigned to either the test group (Regenaform allogeneic bone matrix; Regeneration Technologies) or the control group (autogenous bone chips) by opening an envelope with the group assignment sealed inside. In both groups, an e-PTFE membrane was used.

Each patient was treated according to the surgical protocol previously described for 2-stage vertical ridge augmentation.[1,2,4] The surgery was performed under sterile conditions in the same operating room by the same surgeon.

Clinical Procedure

The performance of vertical ridge augmentation by means of guided bone regeneration was considered "time 0" (Figs 1 to 8). Periapical radiographs (made using a paralleling technique personalized bite blocks), panoramic radiographs, and in some cases, computed tomographic scans were used to assess the morphology of the alveolar ridge.

Presurgical medication of the patients consisted of a chlorhexidine digluconate 0.2% mouthrinse

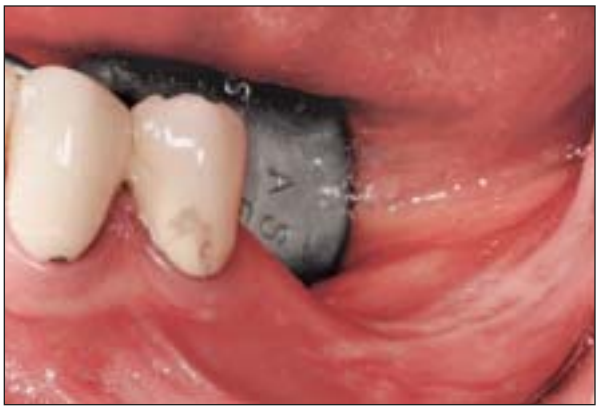


Fig 1 Intraoral view of the defect in the posterior left mandible that required a vertical ridge augmentation (patient 4A).

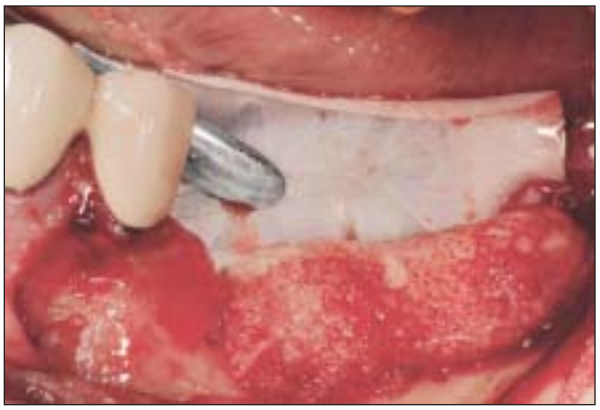


Fig 2 After mucoperiostal flap elevation, a titanium-reinforced e-PTFE membrane is fixed lingually with miniscrews. The allogeneic bone matrix is packed into the defect.

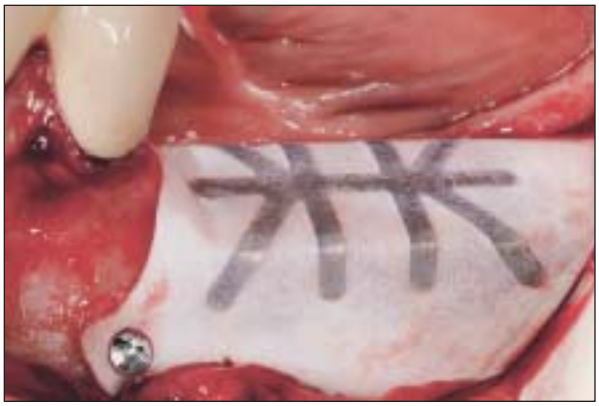


Fig 3 The membrane is gently pulled and fixed buccally with two miniscrews.

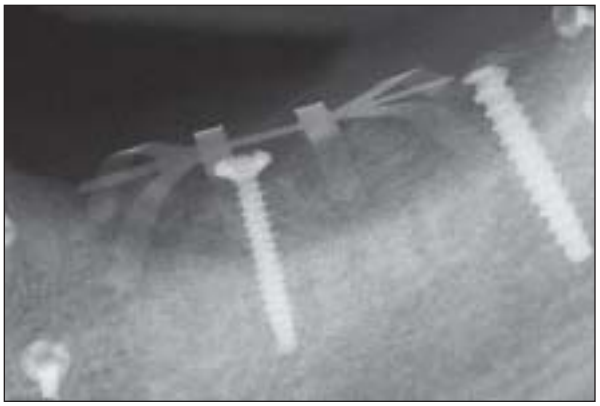


Fig 4 Periapical radiograph showing the vertical bone augmentation performed in the left mandible. Note the presence of a tending screw mesially and the IPI implant distally.

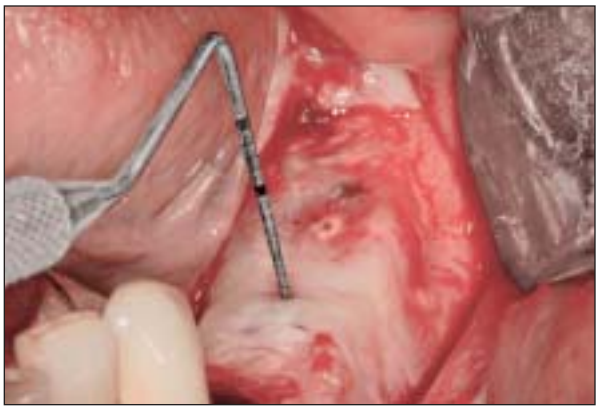


Fig 5 Probing of the soft tissue layer under the membrane.

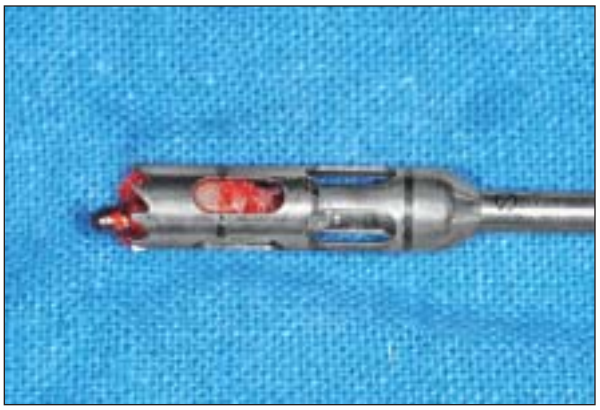


Fig 6 Sampling of a 4-mm trephine bonenblock with inserted IPI.

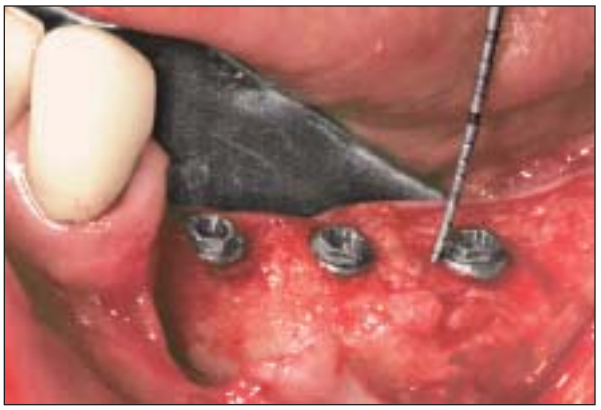


Fig 7 Implant placement. The DIB was assessed at 4 aspects of each implant to value marginal bone loss.

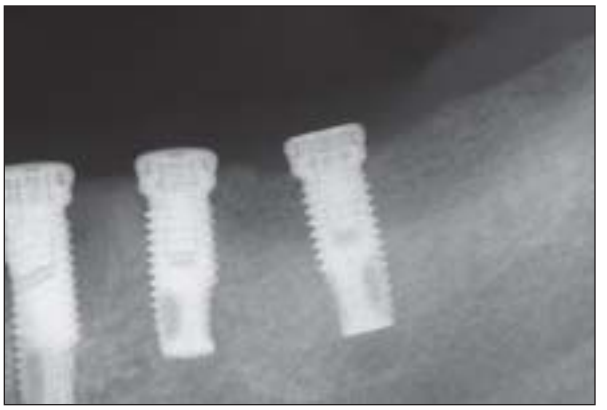


Fig 8 Periapical radiograph demonstrating implant insertion in the vertically augmented area.

(Corsodyl; GlaxoSmithKline, London, UK) for 2 minutes and extraoral scrub with povidone-iodine solution (Betadine; Viatris, Milano, Italy). Sedative premedication with diazepam (20–30 gtt; Valium-2, Roche, Basel, Switzerland) and with ottatroprina metilbromuro and diazepam (2 cpr, Valpinax 20; Crinos, Milano, Italy) were administered 30 minutes before the surgery. Local anesthesia consisted of articaine 4% and epinephrine 1:100,000 (Citocartin 100, Molteni Dental, Milano, Italy).

The surgical technique adopted for all patients has been described in several articles.[1,2,4,5,15] The surgery started with a full-thickness crestal incision within the keratinized mucosa of the edentulous ridge. The incision extended intrasulcularly to 1 or 2 distally and mesially adjacent teeth. Two vertical releasing incisions were made buccally at the distal and mesial ends of the crestal incision. The buccal and palatal full-thickness flaps were elevated to gain a wide access for membrane and eventual implant placement. A continuous releasing periosteal incision at the base of the buccal flap was made, which connected the mesial and distal vertical incisions to obtain, at the end of the surgery, a completely tension-free suture. To vertically augment the bone crest with the guided bone regeneration technique a titanium-reinforced e-PTFE membrane (Gore-Tex, WL Gore, Flagstaff, AZ, USA) was necessary. The authors suggest the use of a TR9W or a TR6Y type, depending on the amount of bone to regenerate. The membrane was bent and trimmed to adapt to the ridge and to predetermine the width and the height of the area to rebuild. To avoid any interference during the healing process, the membrane should not reach the periodontal ligament of the adjacent teeth and should overlap the residual crestal bone by at least 3 to 4 mm. Two devices were used as "poles" to support the membrane; they were positioned in the residual bone and left to protrude for the required height. One of the poles was a stainless steel mini-screw (6 to 12 mm in length; Ace Dental Implant System, Brockton, MA, USA), and the other was an immediate provisional implant (IPI; Sterio-Oss, Nobel Biocare, Sweden). This micro-implant (2 mm in diameter and 10 mm in length) was removed during the second surgery with a 4-mm trephine bur for the histologic analysis. Several drill holes were made on the cortical bone to ensure bleeding necessary to promote bone formation. Once positioned in the recipient site, the membrane was lingually stabilized with fixation mini-screws in the mandible.

In each patient one side was randomly assigned to receive autogenous bone chips or allogeneic bone matrix (Regenaform). Autogenous bone was harvested from the retromolar region with trephine burs and subsequently particulated with a bone mill.

The allograft was prepared in accordance with the directives provided by the company. After the outer pouch had been carefully opened, the inner foil pouch containing the paste was passed to the sterile field. This pouch must not be opened but must be used to warm the paste, because direct exposure of the carrier to water warmer than 38°C can cause the paste to dissolve. The product must be warmed for at least 5 minutes in a water bath at 43°C to 49°C prior to surgery.

The bone graft (autogenous or allogeneic) was then placed on the bone crest under the partially fixated membrane and covered with it. The membrane was gently pulled buccally and affixed at the mesial and distal buccal borders of the membrane to achieve optimal adaptation. Horizontal mattress sutures (CV 5 Gore-Tex suture; WL Gore) with U stitches were applied first to ensure proper flap apposition with the connective tissue surfaces facing each other at least 3 mm. Subsequently, interrupted

sutures were used between the horizontal mattress and to close the vertical incisions. All patients underwent antibiotic prophylactic treatment (amoxicillin/clavulanic acid; Augmentin, GlaxoSmithKline, London UK) starting 1 hour before surgery (2 tablets) and then 3 times a day for 1 week. An anti-inflammatory agent (Ketoprofen; Orudis 50 mg, Aventis Pharma, Paris, France) was prescribed: 1 tablet 1 hour before surgery and 1 tablet 3 times a day for 4 days. Patients were also instructed to rinse twice daily for 7 to 10 days with a 0.2% chlorhexidine solution (Corsodyl, GlaxoSmithKline, London, UK). Postoperative instructions were to use a cold pack, keep to a soft food diet, avoid hot food or drinks, avoid demanding physical work or exercise, and no prostheses on the treated area. Sutures were removed 14 days after surgery. After 6 months of submerged healing all patients underwent the second surgery in order to remove the e-PTFE membrane and the space-maintaining mini-screws, to take the bone sample with inserted IPI with a 4-mm trephine, and to place implants. Cylindric screw-shaped Branemark implants (MK III; Nobel Biocare, Göteborg, Sweden) were inserted in the vertically augmented bone. All implants had a TiUnite rough surface, a 3.75-mm diameter (RP = Regular Platform), and a length of 8.5 to 13 mm, depending on the anatomic limitations. Bone quality[16] (Lekholm and Zarb) and insertion torque were assessed for each implant. After 5 months healing abutments were placed and implants were tested for stability.

Patient Records

At the first visit and throughout the follow-up period, all the information was recorded on the patient form by a single clinical examiner to avoid any involvement between the surgeon and the patient in both the pre- and postoperative assessments.

The presence of edema, hematoma, suppuration, flap dehiscence, flap necrosis, anesthesia, paresthesia, and dysesthesia were evaluated and, if applicable, reported on the patient form.

Two intrasurgical measurements were also recorded:

1. The distance between the top of the screw head and the first visible bone-screw contact (DSB): At first surgery and at second surgery the DSB was assessed at the mesial and distal aspect of each mini-screw with a 15-mm periodontal probe to evaluate the amount of vertical bone regeneration gained.
2. The distance between the top of implant head shoulder and the first visible bone-implant contact (DIB)[17,18]: At implant insertion and at the abutment connection the DIB was recorded with a 15-mm periodontal probe (4 aspects for each implant) to evaluate bone resorption around the implants.

Periapical and panoramic radiographs were obtained at baseline, after first surgery, after implant insertion, after healing abutment connection, and once a year after delivery of the prosthetic restoration.

Histologic Analysis

The implant and the surrounding tissues were immediately stored in 10% buffered formalin and processed to obtain thin ground sections with the Precise 1 Automated System (Assing, Rome, Italy). The specimen was dehydrated in an ascending series of alcohol rinses and embedded in a glycolmetha crylate resin (Technovit 7200 VLC; Kulzer, Wehrheim, Germany). After polymerization the specimen was sectioned longitudinally along the major axis of the implant with a high-precision diamond disk at about 150 µm and ground down to about 30 µm. Three slides were obtained. The slides were stained with basic fuchsin and toluidine blue.

Histomorphometric analysis and the bone-implant contact (BIC) percentage were carried out using a light microscope (Laborlux S; Leitz, Wetzlar, Germany) connected to a high-resolution video camera (3CCD, JVC KY-F55B Yokohama, Japan) and interfaced to a monitor and PC (Intel Pentium III 1200 MMX). This optical system was associated with a digitizing pad (Matrix Vision, Oppenweiler, Germany) and a histometry software package with image-capturing capabilities (Image-Pro Plus 4.5, Media Cybernetics; Immagini & Computer Snc, Milano, Italy). Histometric measurements of the tissue fractions (mineralized bone, medullary spaces, connective tissue, autologous bone particles or cortico-cancellous chips) and the BIC were performed only in the augmented area.

RESULTS

Five female patients referred to the Department of Periodontology, University of Milan, were considered eligible and were consecutively enrolled in the prospective study. They ranged in age from 47 to 66 years (average, 55 years). Patients were recruited and subjected to the surgical procedures from October 2003 to September 2005.

All patients were treated with a split-mouth approach: one side was randomly assigned to the test group (titanium-reinforced e-PTFE membrane and allogeneic bone matrix) and the other site to the control group (titanium-reinforced e-PTFE membrane and autogenous bone chips). Therefore a total

Table 1 Vertical Bone Gain in the Test and Control Groups

	DSB		
	First surgery	Second surgery	ΔDSB
Test group			
1A	5.00	1.00	4.00
2A	5.50	0.50	5.00
3A	5.00	0.50	4.50
4A	5.50	0.25	5.25
5A	4.75	0.00	4.75
Mean	5.15	0.45	4.70
SD	0.34	0.37	0.48
Control group			
1B	5.50	2.50	3.00
2B	5.25	0.00	5.25
3B	5.25	0.75	4.50
4B	5.25	1.00	4.25
5B	3.25	-0.25	3.50
Mean	4.90	0.80	4.10
SD	0.93	1.08	0.88

Table 2 Histomorphometric Analysis of 5 Biopsy Specimens in the Test Group and 4 Biopsy Specimens in the Control Group

	Cortico-cancellous chips (%)	Mineralized bone (%)	Connective tissue (%)	Medullary space (%)
Test group				
1A	4.10	25.73	10.07	60.10
2A	3.00	42.20	0.00	54.80
3A	2.20	25.19	15.97	56.64
4A	5.20	41.30	0.00	53.50
5A	1.50	30.50	0.00	68.00
Mean	3.20	32.98	5.21	58.61
SD	1.48	8.27	7.43	5.81
Control group				
1B	9.20	37.45	19.19	34.16
2B	5.90	47.51	0.00	46.59
3B	11.90	19.82	20.70	47.58
4B	10.40	31.75	25.71	32.14
5B	-	-	-	-
Mean	9.35	34.13	16.40	40.12
SD	2.55	11.55	11.28	8.10

- no data.

Table 3 BIC in the Test and Control Groups

	BIC (%)
Test group	
1A	59.00
2A	25.00
3A	30.00
4A	12.00
5A	38.00
Mean	32.80
SD	17.43
Control group	
1B	30.00
2B	15.00
3B	30.00
4B	26.00
5B	-
Mean	25.25
SD	7.09

- no data.

of 10 surgical sites were treated. No dropouts or exclusions occurred until the definitive prosthetic restoration.

The reinforced e-PTFE membrane remained covered for 24 to 32 weeks of submerged healing (average 28 weeks in the test group and 26 weeks in the control group). At the second surgery, the patients received a total of 25 Brånemark implants (Nobel Biocare, Göteborg, Sweden) in the vertically augmented bone according to the protocol. Thirteen implants were inserted in the test group, 12 in the control group.

After the definitive prosthetic restoration all the patients underwent a maintenance program. The prosthetic and radiologic follow-up was between 1 and 3 years. All patients of both groups reported satisfactory function, without any foreign body sensation, pain, or dysesthesia. The intraoral examination revealed healthy peri-implant mucosa. No evidence of serious adverse systemic side effects was observed in any patient throughout the study.

Due to the limited number of samples in both of the 2 groups, only a descriptive statistical analysis was performed. For the same reason, the findings from the 2 groups (test and control) could not be statistically compared. Means and standard deviation have been calculated in Tables 1, 2, and 3.

Test Group

The soft tissue healing was uneventful in all 5 surgical sites. After the first surgery 1 patient (1A) reported paresthesia that resolved spontaneously in less than 2 months.

At second surgery, a regenerated tissue clinically similar to bone visibly extended until the top of the screw. In all the sites, a thin soft tissue layer was present between the membrane and the regenerated bone-like tissue. The thickness of the soft tissue layer was probed and, for each site, the maximum and the average probing depth were reported. The test group showed a maximum probing depth of 3 mm and an average of 0.75 mm (range, 0.5 to 3 mm). The tenting screws were left to protrude 3 to 6 mm from the crestal bone to achieve vertical ridge augmenta-

tion. A comparison of the mean DSB between the first surgery (5.15 ± 0.34 mm) and second surgery (0.45 ± 0.37 mm) demonstrated a mean crestal bone regeneration (ΔDSB) of 4.70 ± 048 mm (Table 1). At abutment connection all 13 implants appeared clinically stable and were used for provisional and definitive prosthetic restoration.

In the test group a mean marginal bone loss around implants (ΔDIB) of 1.26 mm (SD 1.18 mm) was reported. Implants were inserted with the shoulder almost at the level of the regenerated bone (DIB 0.01 ± 0.64 mm), but at the abutment connection the mean DIB value was 1.24 mm (SD 1.24 mm). A very high ΔDIB value (7.5 mm) was observed in the buccal aspect of implant 45 in patient 1A. A dehiscence at the buccal aspect of this implant (DIB 8 mm, ΔDIB 7.5 mm) was observed without any presence of infection or flap dehiscence. The dehiscence was surgically treated with an autogenous bone graft harvested with a bone scraper and a resorbable membrane (Bio-Gide; Geistlich, Wolhusen, Switzerland).

Control Group

The healing period after vertical ridge augmentation was uneventful in 4 surgical sites, in which the membrane was maintained in site for a 24- to 30-week period (average, 26 weeks).

One surgical site (patient 1B) showed an infection without membrane exposure 2 months after the first surgery. The membrane was gently removed, and the infection appeared limited to a small area. The infected tissue was removed, but most of the regenerating tissue showing healthy conditions was left in place to allow vertical ridge augmentation. There was no evidence of infection in the area around the micro implant. In this site the second surgery (including the bone biopsy with the IPI implant) was performed following the standard protocol (after 6 months) and without any further complications. One patient (3B) reported paraesthesia for 4 weeks after the vertical augmentation procedure.

At membrane removal, the regenerated tissue appeared clinically similar to bone. The average thickness of the soft tissues underlying the membrane was 0.25 mm (range, 0 to 1 mm).

The mean crestal bone regeneration (ΔDSB) was 4.10 mm (SD ± 0.88 mm). The miniscrews, which acted as tentposts to support the membrane, were left to protrude 4 to 6 mm. The DSB changed from a mean of 4.90 mm (SD ± 0.93 mm) at first surgery to a mean of 0.80 mm (SD ± 1.08 mm) at second surgery (Table 1).

At abutment connection, all 12 implants appeared clinically stable and were used for provisional and definitive prosthetic restorations.

In the control group the mean ΔDIB was 0.84 mm (SD 0.92 mm). The DIB varied from a mean value of 0.27 mm (SD 0.70 mm) at implant insertion to a mean value of 1.03 mm (SD 0.88 mm).

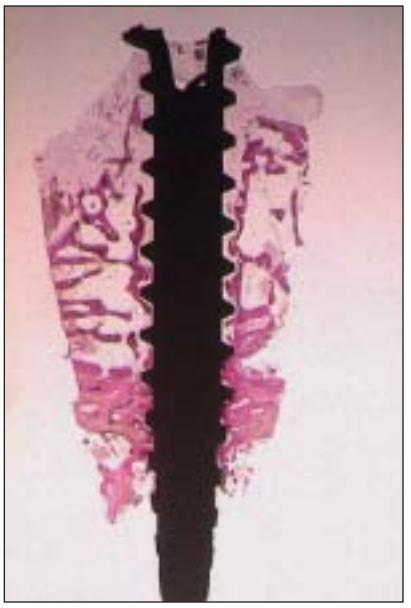
Histologic and Histomorphometric Observations

Of 10 sites, 1 cylindric bone biopsy specimen was deeply damaged during explantation (patient 5B) and could not be used for histological analysis. Therefore, a total of 9 specimens were analyzed.

Vertical bone regeneration was evident both in the test group and in the control group, since all the samples demonstrated trabecular bone with different degrees of maturation and mineralization in the regenerated area. In the apical portion, native lamellar bone was present in direct continuity with the overlying regenerated bone (Figs 9 and 10). Osteoblastic activity could be identified adjacent to newly formed bone, demonstrating ongoing deposition of osteoid matrix in augmented areas of both groups (Figs 11 and 12). A connective tissue layer was present in the most coronal part of some biopsy specimens (Figs 9 and 10).

In the test group some biopsy specimens showed the presence of residual corticocancellous chips. These grafted particles, originally embedded in the allogeneic bone matrix, were generally surrounded by a thin layer of newly formed bone (Fig 12). In most of these specimens only a small amount of newly formed bone appeared in contact with the implant surface. The mean BIC in the test group was 32.80% (17.43%; range, 12.00% to 59.00%; Table 3).

In the control group biopsy specimens a clear differentiation between autologous bone graft and newly formed bone was not always detectable. The regenerated area showed different steps of regeneration. In the apical third, close to the native bone, mature lamellar bone was present, whereas in the middle and coronal thirds woven bone, osteoid and bone marrow (including blood vessels) were more frequently observed. In some specimens the newly formed bone was clearly distinguishable from the autograft particles because of different staining affinity to the fuchsin. In some instances autologous bone particles appeared embedded and surrounded by newly formed bone. Intense osteoblastic activity was always present (Fig 11). In the control group the BIC varied from 15.00% to 30.00% (mean ± SD 25.25% ± 7.09%; Table 3).



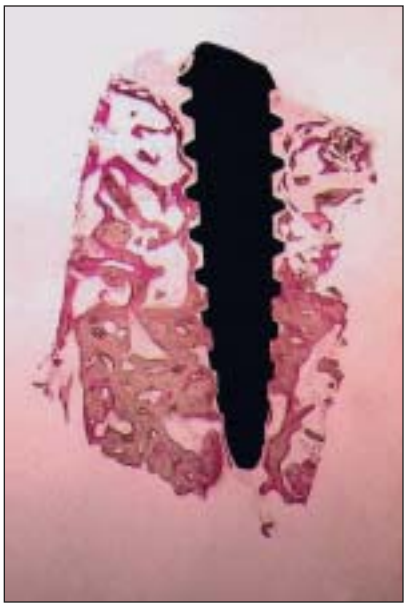


Fig 9 *(Left)* Cylindric bone biopsy showing the IPI implant inserted in the native bone (NB) and the overlying regenerated area (RB) with the allogeneic bone matrix. Some connective tissue (CT) is present in the coronal part of the biopsy. Patient 3A; test group (basic fuchsin and toluidine blue; original magnification ×8).

Fig 10 *(Right)* Cylindric bone biopsy with the inserted IPI implant in the control group. In the apical portion, native lamellar bone (NB) is evident in direct continuity with the adjacent area (RB) regenerated with autogenous bone graft. Connective tissue (CT) is evident on the top of the augmented area. Patient 3B; control group (basic fuchsin and toluidine blue; original magnification ×8).

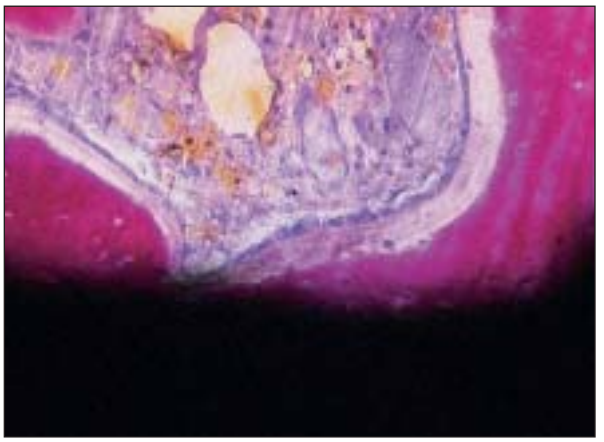


Fig 11 Newly formed bone (NFB) in close contact with the implant surface. Note a long layer of osteoblasts (yellow arrows) depositing osteoid matrix (blue arrows). No cell infiltrate is present. A wide marrow space is evident near the implant surface (I). Patient 4B; control group (basic fucsin and toluidine blue; original magnification ×100).

Fig 12 A corticocancellous chip (RC) of the allogeneic bone matrix embedded in newly formed bone (NFB). Presence of connective tissue (CT) close to the implant surface. No inflammatory cell infiltrate is present in the CT. Patient 1A; test group (basic fucsin and toluidine blue; original magnification ×40).

DISCUSSION

The purpose of this human randomized prospective pilot study was to compare clinically and histologically an allogeneic bone matrix to an autogenous bone graft as a bone filler in vertical ridge augmentation by means of a guided bone regeneration technique.

Even though the School of Dentistry of the University of Milan is considered a reference center for the treatment of advanced alveolar bone deficiencies, only a small number of patients requiring bilateral vertical ridge augmentation was found during the recruitment period.

Five patients were enrolled in this study. Each edentulous site was randomly assigned to the test group (allogeneic bone matrix + titanium-reinforced e-PTFE membrane) or to the control group (autogenous bone graft + titanium-reinforced e-PTFE membrane).

There is scientific evidence[2–6] demonstrating that the use of a bone graft to fill the space under the e-PTFE membrane increases the potential and the predictability of vertical bone regeneration and bone-implant contact. Autogenous bone grafts are generally considered the "gold standard" for bone regeneration due to their osteoinductive and osteoconductive properties.[2,4–6] However, the use of other biomaterials, such as DFDBA, has been recently suggested[7,8] to overcome the drawbacks associated with autogenous bone harvesting. In addition to its space-making abilities, DFDBA has been shown to induce host mesenchymal cells' differentiation into osteoblasts.[9]

Its osteogenic activity has also been described by Harakas[10] and Urist et al.[11] These authors have reported the presence of osteoinductive proteins termed *bone morphogenic proteins* (BMPs).

Even though the aforementioned studies support the efficacy of allografts to enhance bone regeneration, some studies have questioned the bone-inducing properties of DFDBA.[12,13] Becker et al[19] reported the use of this bone substitute in a histologic study investigating its effects on the healing of extraction sockets in humans. Sites grafted with DFDBA revealed the presence of particles of allograft with no evidence of bone formation and no evidence of osteoclastic resorption of the bone particles. Shigeyama et al[20] hypothesized that the bone inductive proteins were present in insufficient quantities or that they lacked osteoinductive activity. Another explanation could be that the improper recruitment and storage methods of harvested bone play a significant role in the insufficient activity of DFDBA preparation.[20–22]

Regenaform is an allogeneic bone matrix recently made available on the market. Its osteoinductive properties are proven by implanting a sample of each lot into an intramuscular site in a rat model. After 4 weeks, ex-novo bone formation is measured through a radiological, histological, and analytical analysis.

The results from the present study suggested that allogeneic bone matrix associated with a titanium reinforced e-PTFE membrane could be as effective as autogenous bone chips for GBR procedures in vertical ridge augmentation of severely atrophic ridges. The material demonstrated great manageability: when warmed at 43°C to 49°C it becomes softer and malleable, and it hardens at body temperature. The advantage of using this bone substitute was to perform vertical ridge augmentation without harvesting autogenous bone, reducing the invasiveness of the surgery and donor site morbidity.

The association of both graft materials and the titanium-reinforced e-PTFE membrane resulted in vertical regeneration of the atrophic crest. Only one site exhibited a dehiscence of 7.5 mm at the buccal aspect (implant 45 in patient 1A, test group) even though the healing period was uneventful. A possible explanation could be that the regenerated area was too narrow, with a consequent resorption of the buccal plate after implant insertion.

The DSB measurements from the test group showed a mean vertical bone regeneration (ΔDSB) of 4.70 mm (SD 0.48 mm; range, 4 to 5.25 mm). Even if no statistical comparison was possible due to the limited number of patients, these data compare favorably with the control group, where a mean crestal bone regeneration (ΔDSB) of 4.10 mm (SD 0.88 mm) was achieved. Moreover, these clinical findings were in accordance with previous clinical and histological studies[2,4] on vertical bone regeneration by means of GBR. Simion et al[2] in 1998 demonstrated a mean vertical bone gain of 3.1 mm and of 5.02 mm, respectively, in sites vertically augmented with DFDBA and autogenous bone graft.

From the available scientific literature, complications of GBR procedure by means of nonresorbable membrane happen with an incidence varying from 9% to 18%.[3,23–25] In the present study, one patient in the control group showed an infection without membrane exposure 2 months after the first surgery. Different factors might be involved with this complication: *(1)* bacterial contamination of the e-PTFE during membrane handling; *(2)* intraoral harvesting of the autogenous bone graft (associated with an increased risk of infective complications as reported by a Cochrane review[26]); and *(3)* improper suture removal.

A soft tissue layer between the membrane and the regenerated bone was detected in most of the surgical sites of both groups. An average soft tissue thickness of 0.25 mm (range, 0 to 1 mm) in the control group and 0.75 mm (range, 0.50 to 3 mm) in the test group was observed. This can be explained by the following: *(1)* the possible contraction of the allograft during the cooling phase generating empty spaces under the membrane. As reported in the literature[1,27,28] the connective tissue layer has shown to be particularly evident when the space under the membrane is not completely filled. *(2)* Different bone-forming activities of the 2 graft materials leading to different thicknesses of the soft tissue layer.

Histologic observations from the retrieved IPIs confirmed the findings from previous studies[1,2] demonstrating the possibility of supracrestal bone regeneration in direct contact with the titanium surface. The mean percentage of BIC was 32.80% (SD 17.43%) in the test group and 25.25% (SD 7.09%) in the control group. These data are within the normal range for machined-surface implants inserted in native bone after 6 months of unloaded healing.[2,29]

The histomorphometric analysis revealed a mean percentage of mineralized bone of 32.98% (SD 8.27%) in the test group and of 34.13% (SD 11.55%) in the control group. These data are in accordance with the percentage of 36.6% (SD 11.89%) showed by Simion et al[2] on vertical ridge augmentation by means of autogenous bone and titanium-reinforced e-PTFE membrane.

Both the corticocancellous chips of the allogeneic bone matrix and the autogenous bone particles were still visible after a submerged healing period varying from 24 to 32 weeks in the test group and from 24 to 30 weeks in the control group. They appeared embedded and surrounded by newly formed bone, and an intense osteoblastic activity was always present.

CONCLUSIONS

Within the limits of this study based on 5 patients, it appears that the behavior of the allogeneic bone matrix is similar to that of autogenous bone chips when used for vertical ridge augmentation by means of guided bone regeneration techniques. Both grafts demonstrated analogous histologic characteristics. After a healing period of at least 6 months, the vertically regenerated bone allowed proper implant placement. Bone-implant contact of these implants could be observed. Long-term clinical studies are needed to confirm these preliminary results.

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