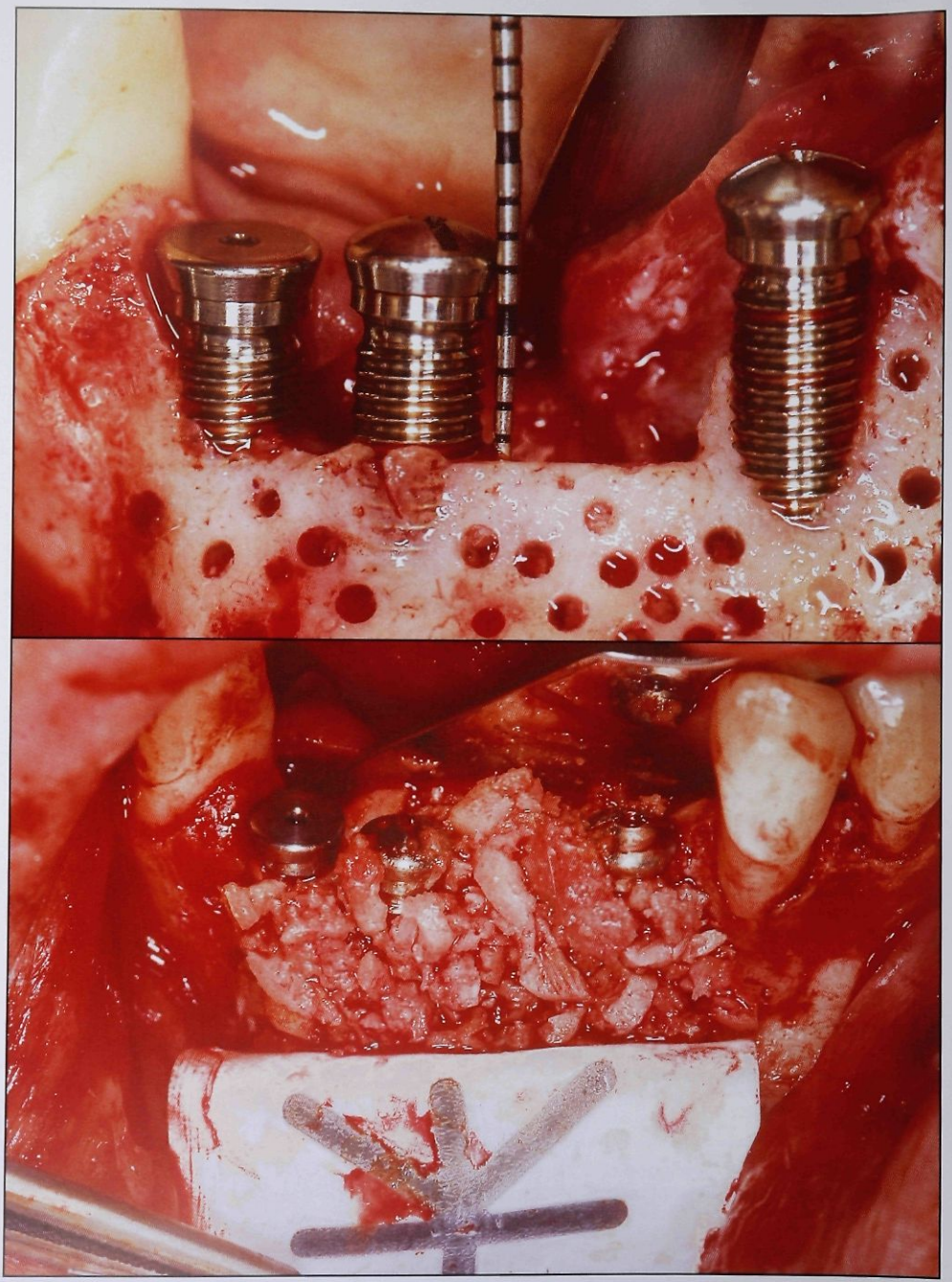

Vertical Ridge Augmentation Around Dental Implants Using a Membrane Technique and Autogenous Bone or Allografts in Humans

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This study investigated the effect on vertical bone regeneration of the addition of demineralized freeze-dried bone allograft or autogenous bone chips to a membrane technique. Twenty partially edentulous patients with vertical jawbone deficiencies were selected for this study. The patients were divided into two groups of 10 individuals. The 10 patients of Group A received 26 Brånemark implants in 10 surgical sites. The 10 patients of Group B received 32 implants in 12 surgical sites. Fifty-two out of 58 implants (22 in Group A and 30 in Group B) extended 1.5 to 7.5 mm superior to the bone crest. Titanium-reinforced expanded polytetrafluoroethylene membranes were used to cover the implants and, before complete membrane fixation, demineralized freeze-dried bone allograft particles were condensed under the membrane in Group A, and autogenous bone chips were used in Group B. At the reentry after 7 to 11 months the membranes were removed and a small biopsy was collected from 11 sites comprehending the miniscrews. The clinical measurements from Group A demonstrated a mean vertical bone gain of 3.1 mm (SD = 0.9 mm, range 1 to 5 mm) with a mean percentage of bone gain of 124% (SD = 46.6%). The measurements from Group B showed a mean vertical bone gain of 5.02 mm (SD = 2.3 mm, range 1 to 8.5 mm) with a mean percentage of bone gain of 95% (SD = 26.8%). Histomorphometric analysis of the present study clearly demonstrated a direct correlation between the density of the pre-existing bone and the density of the regenerated bone. The mean percentage of new bone-titanium contact was from 39.1% to 63.2%, depending on the quality of the pre-existing bone. Both the clinical and histologic results indicate a beneficial effect of the addition of demineralized freeze-dried bone allograft or autogenous bone particles to vertical ridge augmentation procedures in humans.
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Vertical bone loss in edentulous ridges of partially dentate patients constitutes one of the major surgical challenges in implant dentistry. In fact, the presence of structures such as the nasal cavity, the maxillary sinus, and the alveolar nerve limits the bone height available for proper implant placement and stabilization. Moreover, a large interarch space, consequent to vertical bone loss, alters coronal length and form and produces an unfavorable crown-to-root ratio in the final prosthetic reconstruction.[1]

Sinus elevation techniques have been successfully used to augment the vertical dimension of the maxilla,[2–4] but only in an apical direction, and these procedures cannot reduce the interarch space when necessary. Therefore, their application should be considered only in clinical situations in which crestal bone resorption in an apical direction is limited and the vertical bone deficiency is mainly a result of sinus pneumatization.

Alveolar nerve transposition has been proposed to allow dental implants to be placed in atrophic posterior regions of the mandible[5,6]; however, this technique maintains a large interarch space and presents the risk of mandibular nerve disorders.

Onlay bone grafts, harvested from the hip, constitute a valid alternative to the abovementioned techniques.[7] They result in an increase in height of available bone for implant stabilization and reduce the interarch space. On the other hand, this technique has demonstrated a high rate of resorption during the first 6 to 12 months of healing[8] and is an invasive surgical approach.

Recent technical developments in barrier membrane materials allow improved space maintenance in guided bone regeneration (GBR) and result in vertical ridge augmentation. A protected space is created with a barrier membrane over the area to be augmented to stabilize the blood clot and to exclude soft tissue penetration. The protected space can thus be populated by slow-migrating osteogenic cells, resulting in new bone formation.[9]

The external soft tissue pressure and consequent collapse of the barrier membrane is considered to be a major reason for failure of the regenerative procedure in nonspace-making alveolar bone sites.[10–14] Recently, Simion et al,[15] in a clinical and histologic study in humans, and Jovanovic et al,[16] in an experimental study in dogs, demonstrated the use of titanium-reinforced expanded polytetrafluoroethylene (e-PTFE) membranes to promote a significant amount of vertical bone formation without the addition of grafting materials. Both studies demonstrated a gain of vertically regenerated bone varying from 2.5 to 4 mm after a submerged healing period of 9 months in humans and 6 months in dogs. However, bone regeneration under the membrane was incomplete, and histologic examination showed a layer of loose connective tissue between the newly formed bone and inner surface of the membrane. The mean thickness of the soft tissue was 2.1 mm in the human study and 0.9 mm in the dog study. The possible explanations for the incomplete bone regeneration included micromovement of the membranes, incomplete blood clot stabilization, and empty space under the membranes. Tinti et al[17] has shown improved results by adding autogenous bone chips to the membrane technique. The use of filling material for blood clot and membrane stabilization was postulated to be of some benefit in providing increased and more predictable bone formation.

Autogenous bone chips and demineralized freeze-dried bone allografts (DFDBA) have been demonstrated to be effective as grafting materials in association with barrier membranes.[13,18,19] The rationale for the use of autografts or allografts is that they are biocompatible and provide a stable framework under the barrier membrane for population with osteoprogenitor cells. The bone particles are also thought to improve bone formation by means of osteoconductive activity and by transferring stimulating factors incorporated into the bone matrix.

The aim of this clinical and histologic study was to investigate the beneficial effect on vertical bone formation of the addition of DFDBA or autogenous bone chips to the reinforced membrane technique.

Method and materials

Patients

Twenty partially edentulous patients, between 34 and 66 years of age (mean 51 years) and with vertical jawbone deficiencies, were selected for study. All patients showed excellent general health and no detectable contraindications to implant surgery. Clinical intraoral examination, orthopantomograms, and, in some cases, computerized axial tomographic examination with reconstruction of bone profiles, were used to

assess the morphology of the alveolar ridges to be augmented.

The criteria chosen for surgical site selection were in accordance with the indications for vertical bone regeneration described in a previous article.[15] Four patients presented vertical ridge deficiencies combined with implant dehiscences and infrabony defects. However, only the vertical components of the defects were considered in this study. The patients were informed that a new surgical technique was going to be applied and were provided with detailed information relative to the risks and benefits of the regenerative therapy.

Surgical technique

Patients were divided into two groups of 10 individuals each. The surgical protocol was the same as that described previously,[15] except that DFDBA (Group A) and autogenous bone particles (Group B) were used as grafting material under the membranes (Figs 1 to 4). The 10 patients from Group A received a total of 26 Brånemark implants (Nobel Biocare) in 10 surgical sites. The 10 patients from Group B received a total of 32 implants, out of which 30 were placed into regenerated bone in 12 surgical sites.

The implant head was positioned in an ideal vertical position located about 3 mm apically from the cementoenamel junction of the adjacent teeth. This resulted in 22 implants extending 1.5 to 4.5 mm (mean 2.68 mm, SD = 0.78) superior to the original bone crest in Group A. In Group B, 15 implants were positioned simultaneously with the regenerative procedure, and 15 were inserted after vertical bone regeneration was achieved. The resulting positions of the 30 implants in Group B were 2 to 7.5 mm superior to the original bone crest.

In five surgical sites of each group a pure titanium miniscrew (1.3 × 10 mm) was allowed to protrude 2 to 7 mm from the bone level to improve the support of the barrier membrane and to provide a biopsy for histologic examination of the regenerated bone and of the bone-implant interface at the second stage of implant surgery. The most protruding portions of the implants and the miniscrews were measured with a calibrated periodontal probe. Titanium reinforced (TR) expanded polytetrafluoroethylene (e-PTFE) membranes (GTRM, WL Gore) were used to cover the defects. After a partial fixation of the membranes to the distal or palatal aspect of the surgical site with tags or screws, rehydrated DFDBA particles (for Group A) or autogenous bone chips (for Group B) were condensed into the space between the membrane and the alveolar bone. The fixation was completed at the mesial and buccal edges of the membrane to achieve optimum stabilization, and the flaps were sutured to obtain an absolute tension-free adaptation. Reentry surgery was performed after 7 to 11 months of submerged membrane healing. After membrane removal, the thickness of the soft tissue layer was measured with a periodontal probe, and the maximum score was recorded. The percentage of new bone formation was calculated; 100% regeneration would indicate a complete regeneration from the top of the bone crest to the top of the implant head. Before implant abutment connection, 11 biopsies that included the 10 miniscrews were collected with a small trephine.

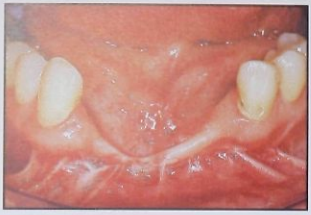


Fig 1a *Partially edentulous ridge in the anterior mandible: the incisors and surrounding alveolar bone were lost in a car accident.*

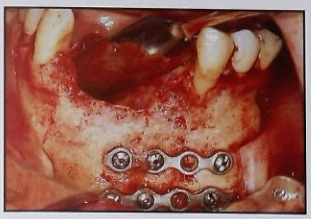


Fig 1b *Remote flap is elevated and bone defect is exposed and cleaned. Titanium osteosynthesis plates that had been placed to treat the mandibular fracture, are still present.*

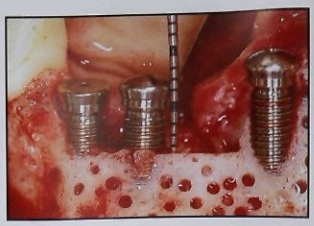


Fig 1c *Osteosynthesis plates are removed and three Brånemark implants are placed in the ideal prosthetic position. The periodontal probe indicates that the implant head in the site of the mandibular right central incisor is 5.5 mm coronal to the bone crest.*

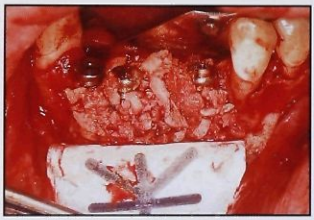


Fig 1d *Autogenous bone was harvested from the chin with a trephine bur and particulated with a Quetin Bone Mill. The bone particles were positioned around the exposed implants.*

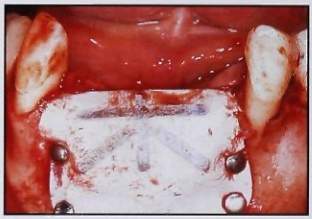


Fig 1e *Gore-Tex reinforced membrane is fixed with tags to protect the underlying bone graft and blood clot.*

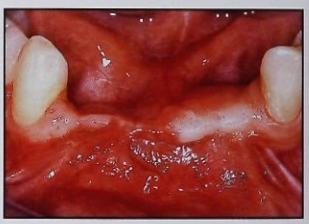


Fig 1f *Clinical aspect of the ridge after 7 months of uneventful healing.*

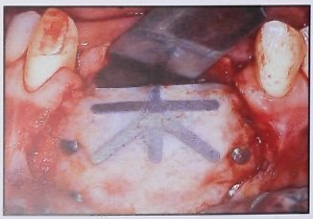
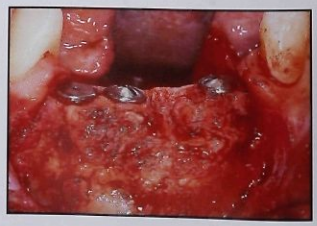


Fig 1g (left) *At second-stage surgery the membrane appears adherent to the underlying tissues without clinical signs of inflammation.*

Fig 1h (right) *After membrane removal, a thin layer of soft tissue is curetted, demonstrating the new ridge height with regenerated bone.*



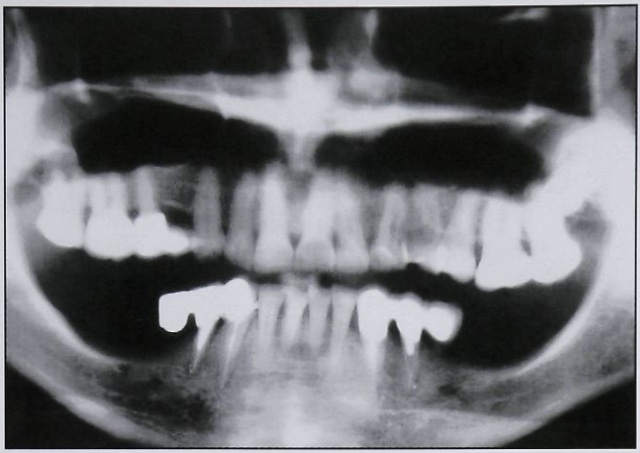


Fig 2a *Orthopantomogram demonstrating a bilateral atrophic edentulous ridge in the posterior mandible. The inferior alveolar nerve is located 4 to 5 mm from the crest of the ridge, preventing implant stabilization.*

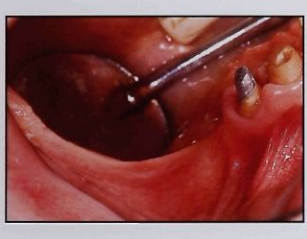


Fig 2b *Preoperative clinical status of the right resorbed edentulous ridge.*

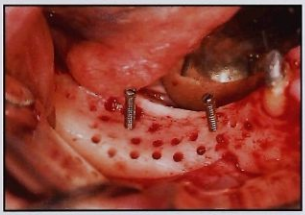


Fig 2c *Staged surgical approach was chosen. Two commercially pure titanium pins are placed 7 mm above the bone crest to support the reinforced membrane. Multiple perforations are made in the cortical bone plate to favor bleeding and stimulate bone repair.*

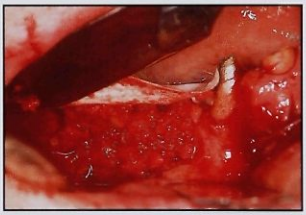


Fig 2d *Autogenous bone particles, collected from the chin, are grafted into the space under the titanium membrane.*

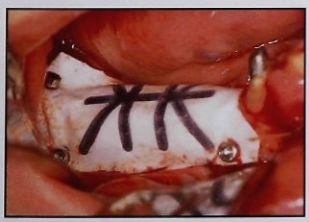
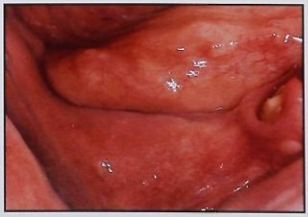


Fig 2e (left) *Titanium membrane is trimmed, formed, and stabilized with Memfix screws.*

Fig 2f (right) *Clinical status after a 7-month healing period. Note the newly established vertical height.*



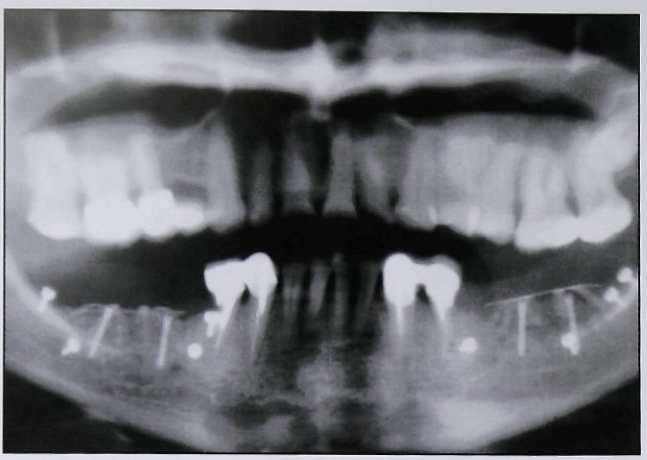


Fig 2g Orthopantomogram illustrating the bilateral treatment effect after 6 months of healing.

Fig 2h At second-stage surgery after 7 months, the membrane demonstrates a good maintenance of space and ridge contour.

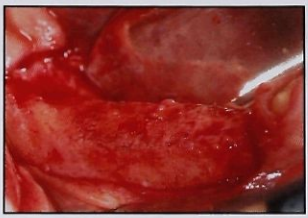


Fig 2i After membrane removal, the regenerated bone is visible. The titanium pins are completely embedded in new bone.

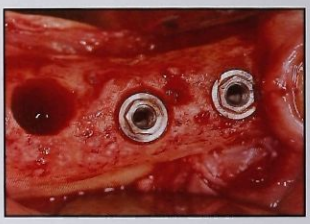


Fig 2j Two Brånemark implants have been positioned bilaterally. One of the pins was removed and analyzed for histomorphometric evaluation.

Histologic processing

The bone biopsies were rinsed in saline solution, fixed in 10% neutral buffered formalin, and processed to obtain thin ground sections. After fixation, the specimens were dehydrated in an ascending series of alcohol rinses and embedded in glycol-methacrylate resin. Sections (200 to 250 µm thick) were obtained with a wafering high-speed rotating blade microtome and were subsequently reduced to about 40 to 50 µm with a grinding machine. After polishing, the histologic slides were stained with basic fuchsin, toluidine blue, hematoxylin and eosin, light green, and von Kossa for light-microscopic observation.

Histomorphometric analysis was performed by counting, in a grid eyepiece, the area occupied by bone trabeculae over the bone-plus-marrow spaces. These measurements were performed with a 10× lens in the fields of all the specimens. Finally, the results were

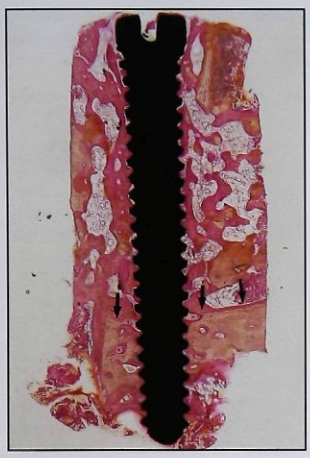
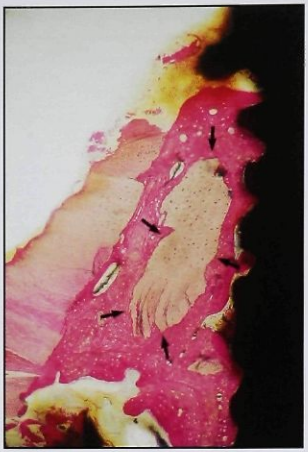


Fig 2k (left) *Histologic section of the bone biopsy from the site in Fig 2j treated with autogenous bone chips. The pre-existing cortical bone plate* (arrows) *is still distinguishable at 3 mm from the apex of the titanium pin. The regenerated bone extends for 7 mm to the top of the pin and shows a high rate of direct contact with the titanium surface (Original magnification × 10; basic fuchsin stain.)*

Fig 3 (right) *Higher magnification of histologic section of a bone biopsy from a site treated with autogenous bone chips. The transplanted cortical bone particles* (arrows) *are still distinguishable and are well integrated with the newly formed bone (Original magnification × 50; basic fuchsin.)*



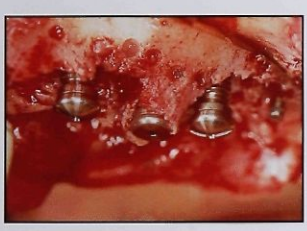


Fig 4a *Three Brånemark implants have been positioned supracrestally in an atrophic edentulous ridge in the maxilla.*

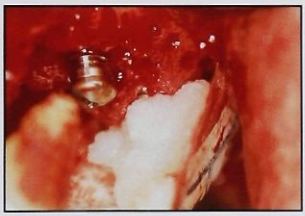


Fig 4b *Demineralized freeze-dried bone allograft is used to fill the space under the titanium membrane.*

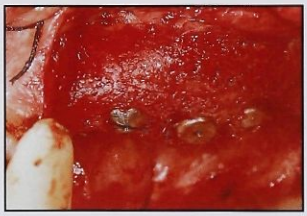


Fig 4c *After 9 months of healing the membrane and the underlying soft tissue are removed and the newly formed bone is exposed.*

expressed as a percentage of the area of bone trabeculae over the total bone area. The same method was applied to evaluate the percentage of direct bone contact with the titanium surface of the mini-screws.

Results

Clinical results

The clinical results of the present study are summarized in Tables 1 and 2. Two patients (RJ and SH) from Group A and 1 patient (CF) from Group B demonstrated membrane exposure after 1 to 4 months of healing. The membranes were immediately removed to prevent possible infection of the underlying regenerating tissues. At removal, the membranes appeared still to be integrated with the surrounding tissues, and under the membrane a whitish, translucent, and scarcely bleeding soft tissue was present. Demineralized freeze-dried bone

Table 1 Supracrestal bone gains resulting from augmentation in Group A (treated with DFDBA)

		Implant		Implant exposure		Bone gain		Healing	
Implant no.	Patient	Site (FDI)	Length (mm)	Baseline (mm)	Reentry (mm)	Height (mm)	Percentage	Time (mos)	Comments
1	CG	35	10	−2.5	+1.0	3.5	140	10	Uneventful
2	CG	36	10	−2.0	+1.5	3.5	175	10	Uneventful
3	MP	24	15	−2.5	+1.0	3.5	140	10	Uneventful
4	MP	26	13	−3.0	0.0	3.0	100	10	Uneventful
5	MN	16	13	−4.0	+1.0	5.0	125	10	Uneventful
6	NL	26	13	−2.5	+1.5	4.0	160	9	Uneventful
7	NL	27	13	−2.5	+1.5	4.0	160	9	Uneventful
8	PM	15	13	−2.5	+1.0	3.5	140	9	Uneventful
9	RJ	25	10	−2.0	0.0	2.0	100	10	Exposure
10	RJ	26	13	−4.5	−1.0	3.5	77	10	Exposure
11	RJ	27	10	−4.0	0.0	4.0	100	10	Exposure
12	VG	32	13	−1.5	+1.5	3.0	200	8	Uneventful
13	VG	33	13	−1.5	+2.0	3.5	233	8	Uneventful
14	AD	36	10	−2.0	+1.0	3.0	150	7	Uneventful
15	AD	37	10	−3.0	0.0	3.0	100	7	Uneventful
16	WV	35	10	−2.0	+1.0	3.0	150	9	Uneventful
17	WV	36	10	−3.0	+1.0	4.0	133	9	Uneventful
18	WV	37	8.5	−3.0	0.0	3.0	100	9	Uneventful
19	SH	12	10	−3.0	−1.0	2.0	66	11	Exposure
20	SH	11	10	−3.0	−1.0	2.0	66	11	Exposure
21	SH	21	10	−2.0	−1.0	1.0	50	11	Exposure
22	SH	22	10	−3.0	−1.0	2.0	66	11	Exposure
Mean				−2.68	0.45	3.14	124		
SD				0.78	0.98	0.90	46.6		

allograft particles or bone chips were detectable under the translucent surface of the tissue. Another patient (OC) from Group B showed an abscess after 1 month of healing. The patient received antibiotic therapy and the membrane was immediately removed. The tissue under the membrane appeared immature and inflamed. The flaps were sutured back and left to heal until second-stage implant surgery. The overall percentage of healing complications was 18%.

In the remaining 18 surgical sites, the membrane was removed after 7 to 11 months of uneventful healing. At removal there was no evidence of inflammatory reaction in the tissues surrounding the membranes. The central portion of the membranes, where the titanium mesh was located, detached easily from the underlying newly formed tissue. The newly regenerated tissue beneath the TR membranes resembled bone tissue covered with a thin and incomplete soft tissue layer. The maximum thickness of the soft layer appeared to be less than 1 mm. The site was curetted until a hard bone surface was detectable. The clinical appearance of the newly formed hard tissue was identical to bone, but DFDBA particles or bone chip remnants appeared macroscopically to be incorporated into the context of the bone tissue.

The clinical measurements from Group A (initial mean defects = 2.68 mm, SD = 0.78 mm) demonstrated a mean

Table 2 Supracrestal bone gains resulting from augmentation in Group B (treated with autogenous bone chips)

Implant no.	Patient	Implant Site (FDI)	Implant Length (mm)	Implant exposure Baseline (mm)	Implant exposure Reentry (mm)	Bone gain Height (mm)	Bone gain Percentage	Healing Time (mos)	Comments
1*	SP	35	10	−7.0	+1.0	8.0	114	7	Uneventful
2*	SP	36	10	−7.0	0.0	7.0	100	7	Uneventful
3*	SP	45	10	−7.0	0.0	7.0	100	7	Uneventful
4*	SP	46	10	−7.0	0.0	7.0	100	7	Uneventful
5*	BR	15	15	−3.0	0.0	3.0	100	7	Uneventful
6*	BR	16	13	−7.0	0.0	7.0	100	7	Uneventful
7*	BR	17	13	−4.0	+2.0	6.0	150	7	Uneventful
8*	BR	24	15	−5.0	0.0	5.0	100	7	Uneventful
9*	BR	25	13	−7.0	0.0	7.0	100	7	Uneventful
10*	BR	26	10	−6.0	+1.0	7.0	100	7	Uneventful
11*	PG	36	10	−4.0	0.0	4.0	100	7	Uneventful
12*	PG	37	10	−4.0	0.0	4.0	100	7	Uneventful
13*	VA	36	10	−3.0	0.0	3.0	100	7	Uneventful
14*	VA	37	10	−5.0	0.0	5.0	100	7	Uneventful
15*	VA	35	10	−5.0	−1.5	3.5	70	7	Uneventful
16	CF	11	13	−4.0	−3.0	1.0	25	7	Exposure
17	CF	12	13	−2.0	−1.0	1.0	50	7	Exposure
18	CF	21	13	−2.0	−1.0	1.0	50	7	Exposure
19	IA	32	15	−5.0	+1.0	6.0	120	7	Uneventful
20	IA	41	15	−5.5	+1.0	6.5	118	7	Uneventful
21	IA	42	15	−7.5	+1.0	8.5	113	7	Uneventful
22	OC	11	13	−4.0	−1.0	3.0	75	7	Abscess
23	OC	12	13	−3.0	−1.0	2.0	67	7	Abscess
24	OC	22	13	−3.5	−1.0	2.5	71	7	Abscess
25	RL	35	10	−3.0	0.0	3.0	100	7	Uneventful
26	RL	36	10	−3.0	0.0	3.0	100	7	Uneventful
27	SP	45	10	−2.0	0.0	2.0	100	7	Uneventful
28	SP	46	10	−3.0	0.0	3.0	100	7	Uneventful
29	PJ	35	10	−3.0	0.0	3.0	100	7	Uneventful
30	PJ	36	10	−3.0	0.0	3.0	100	7	Uneventful
Mean				−5.09	−0.02	5.02	95		
SD				1.75	1.05	2.33	23		

*Implant sites treated with a staged approach.

vertical bone gain of 3.14 mm (SD = 0.9 mm, range 1 to 5 mm), with a mean percentage of bone gain of 124% (SD = 46.6%). The measurements from Group B (initial mean defects = 5.09 mm, SD = 1.75 mm) showed a mean vertical bone gain of 5.02 mm (SD = 2.3 mm, range 1 to 8.5 mm) with a mean percentage of bone gain of 95% (SD= 26.8%).

The percentage of vertical bone gain in sites with complications during the healing period was visibly less (75%, SD = 18.8%, range 50% to 100% in Group A and 56.3%, SD = 18.7%, range 25% to 75% in Group B) than sites in which the membrane remained covered for the entire healing period (147.1%, SD = 36.6%, range 100% to 233% in Group A, and 103.5%,

Table 3 Histomorphometric evaluation of bone density in the biopsies of Group A (Percentage of mineralization)

Patient	Preexisting cortical bone	Regenerated bone	Preexisting spongious and regenerated bone
CG	85.2	61.8	
VG	80.4	58.1	
MN	61.2	39.6	
PM			46.3
RJ			19.8
MP			43.6
Mean	75.6	53.1	36.6
SD	12.7	11.8	14.6

In the spongious bone the pre-existing and the regenerated bone were evaluated together because it was impossible to make a clear distinction.

Table 4 Histomorphometric evaluation of bone density in the biopsies of Group B (Percentage of mineralization)

Patient	Pre-existing bone	Regenerated bone
PG	80.5	71.5
SP	80.5	58.5
SP	60.1	60.0
BR	80.2	35.2
VA	75.1	30.1
Mean	75.3	51.1
SD	8.7	17.6

SD = 13.5%, range 70% to 150% in Group B). All 58 implants were judged to be osseointegrated at the abutment connection (100% implant survival) and were used as anchorage for provisional and final prostheses.

Histologic results

The amount of vertical bone regeneration and the general features of the regenerated bone, as assessed by histologic analysis, varied widely between the treated cases. Nevertheless, it was possible to distinguish two different situations: *(1)* specimens demonstrating pre-existing cortical bone; and *(2)* specimens demonstrating pre-existing spongious bone. This distinction is important because the pattern of the regenerated tissue varied depending on the quality of the original bone (Tables 3 and 4).

Specimens demonstrating pre-existing cortical bone. In the specimens that demonstrated pre-existing cortical bone (mean density = 75.6%, SD = 12.7%), the vertically regenerated tissue demonstrated dense bone with a mean density of 53.1% (SD = 11.8%).This tissue was characterized by large trabeculae composed of a core of woven bone and graft particles surrounded by lamellar or parallel-fibered bone. Particles of the graft were randomly embedded in the regenerated trabeculae or in the marrow connective tissue.

In these specimens, the demarcation between the original crestal bone and the newly formed bone was easily distinguishable (Figs 2k and 5). Usually the original crest could be recognized because of the presence of longitudinally arranged lamellar bone. Above these lamellae, a cement line linked the pre-existing bone to the newly formed woven bone. The retrieved titanium screw from Group A showed a percentage of bone-titanium direct contact of 63.6% (SD = 23.4%) in the pre-existing bone and of 56.4% (SD = 12.3%) in the regenerated bone. In Group B the percentage of bone-titanium contact was 67.6% (SD = 14.9%) in the pre-existing bone and 63.2% (SD = 15.4%) in the regenerated bone.

Specimens demonstrating pre-existing spongious bone. In the case of vertical regeneration in original spongious bone, the regenerated tissue was composed of thin bony trabeculae hardly distinguishable from the pre-existing bone (Fig 6). In these cases the pre-existing cortex was not visible, but it was always possible to identify the new crestal cortex. The medium bone density was 36.6% (SD = 14.6%), and it was impossible to

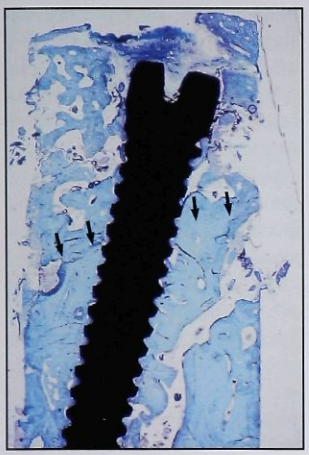


Fig 5 *Histologic section of a bone biopsy from a site treated with DFDBA allograft. The arrows indicate the location of the pre-existing cortical bone plate. The regenerated bone extends to the top of the pin and exhibits extensive areas of direct contact with the surface of the titanium pin. (Original magnification × 12; light green stain.)*

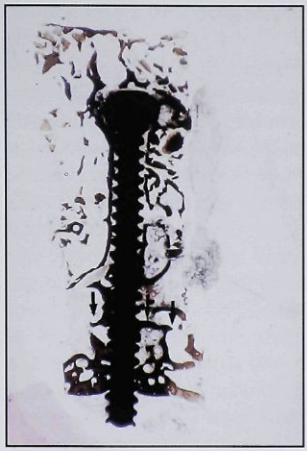


Fig 6 *Histologic section of a biopsy collected from a site with spongy bone. The site was treated with DFDBA. The limit between the regenerated bone and the native bone (arrows) is almost indistinguishable. The newly formed bone extends over the top of the pin, but direct contact with the surface of the titanium pin is limited. (Original magnification × 10; von Kossa stain.)*

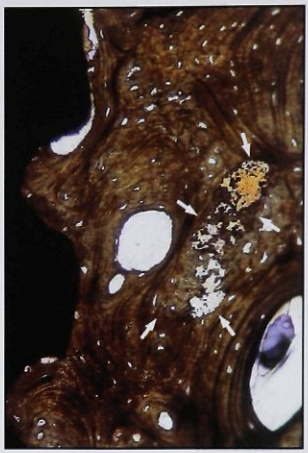


Fig 7 *Higher magnification of a specimen treated with DFDBA. Some remnants of demineralized bone particles (arrows) are still present in the newly formed bone. Round bodies of remineralization are visible in the particles (Original magnification × 100; von Kossa stain.)*

differentiate the regenerated from the pre-existing bone. The mean percentage of bone contact with the retrieved screws was 39.1% (SD = 13.6%).

The particles of demineralized freeze-dried bone were either completely embedded in the newly formed trabeculae or only partially embedded in medullary tissues, showing osteoconductive properties. Signs of ongoing remineralization were evident, as demonstrated by the presence of rounded bodies of remineralization (20 to 30 µm in diameter). These bodies, which were positive in the von Kossa staining, were visible inside the demineralized matrix and tended to coalesce (Fig 7). In some particles this process was almost complete, and the matrix assumed the aspect of the woven bone with some remnants of confluent mineralization bodies in the central area.

The particles of autogenous bone were easily distinguishable because of their different affinity to basic fuchsin. They appeared to be completely surrounded and integrated with the newly formed bone (see Fig 3). The largest bone particles appeared to have fractured in some areas, and new bone formation was evident in the rims of the fractures. The smallest particles showed signs of resorption with osteoclasts and Howships lacunae.

Discussion

In the last few years, a membrane technique has been proposed to obtain alveolar bone regeneration in a vertical direction.[15-17] In a previous study, Simion et al[15] have shown that when a mechanical barrier was used to protect the blood clot and to prevent the invasion of fibrous connective tissue, it was possible to achieve vertical bone regeneration up to 3 to 4 mm after a submerged healing period of 9 months. The same study, on the other hand, demonstrated an incomplete bone fill of the space available for bone regeneration and the regular presence of a fibrous connective tissue layer separating the membrane from the regenerating bone. Similar findings have been presented by Jovanovic et al[16] in an experimental study in dogs. Some of the possible explanations for the incomplete new bone formation proposed by the authors were: *(1)* the shrinkage of the blood clot under the membrane during the early stage of healing; *(2)* the entrapment of air under the membrane; *(3)* membrane micromovement; and *(4)* an insufficient healing period. The aim of the present study was to investigate the potential beneficial effect of DFDBA allograft or autogenous bone chips when used as grafting materials in vertical ridge augmentation procedures.

Both the clinical and histologic results from the present study indicate a beneficial effect from the use of DFDBA and autogenous bone as grafting materials for vertical ridge augmentation procedures. In fact, in all the sites analyzed, the thickness of the soft tissue layer observed between the regenerated bone and the membrane was limited to less than 1 mm, and almost all the space available under the membrane was occupied by new bone. The limit of vertical bone regeneration seemed to be determined more by the space available under the membrane than by the limits of the surgical technique itself. These findings demonstrated an improvement when compared to the above-mentioned human study,[15] which reported a mean connective tissue thickness of 2.1 mm (range 0.5 to 4.5 mm) and a maximum vertical bone gain of 4 mm.

Possible explanations for more effective vertical bone regeneration in the present study are:

1. The grafted particles, after implantation, became completely soaked by blood, allowing coagulum stabilization and preventing the formation of an empty space under the membrane.

2. The presence of filling materials could have reduced the micromovement of the membrane.
3. The osteoconductive activity of the DFDBA and autogenous bone chips clearly demonstrated by the histologic observations, could have enhanced the new bone formation.
4. The possible osteoinductive activity of the grafted materials used.

The results of the present study are in accordance with a recent study by Tinti et al[17] showing enhanced vertical bone regeneration using autogenous bone chips and powder as filling materials under e-PTFE membranes.

The histomorphometric analysis in the present study clearly demonstrated a direct correlation between the density of the pre-existing bone and the density of the regenerated bone. When the newly regenerated bone originated from cortical pre-existing bone (mean density = 75.60%, SD = 12.69%) it showed dense bone regeneration (mean density = 53.16%, SD = 11.89%). When the regenerated bone originated from spongious bone (mean density = 36.63%, SD = 14.63%), it demonstrated similar characteristics and was almost indistinguishable from the pre-existing bone.

Histologic observations from the retrieved miniscrews have confirmed the findings of previous studies[15,16] demonstrating the capability of the supracrestally regenerated bone to grow in direct contact with a titanium implant surface. The mean percentage of bone-titanium contact was from 56.4% (Group A) to 63.2% (Group B) in cortical bone specimens and 39.1% in spongious bone specimens. These data are within the normal range for implants inserted in native bone.

Both demineralized freeze-dried bone and autogenous bone particles were still visible in the histologic sections after a healing period of 7 to 11 months. They appeared completely surrounded and integrated with the newly formed bone.

The exposed sites (18%) showed a lower percentage of bone regeneration (56% to 75%) when compared to sites with uneventful healing (103% to 147%), and this is in accordance with the results of previous studies[20-23] that demonstrate that early exposure and premature removal of the membrane may reduce bone regeneration.

Conclusions

The results from the present study demonstrate the beneficial effect of the addition of DFDBA and autogenous bone to the membrane technique for vertical ridge augmentation. The histomorphometric analysis clearly demonstrated a direct correlation between the density of the pre-existing bone and the density of the regenerated bone. The regenerated bone appeared in general to be slightly less dense than the pre-existing bone.

The titanium miniscrews demonstrated a percentage of direct bone-implant contact with the newly formed bone within the normal range for implants inserted in native bone.

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