Clinical evaluation of particulate allogeneic with and without autogenous bone grafts and resorbable collagen membranes for bone augmentation of atrophic alveolar ridges

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Key words: alveolar bone augmentation, collagen, GBR, membrane

Abstract
Objectives: The evaluate the clinical outcome of bone augmentation with the use of particulate mineralized freeze-dried bone allograft (FDBA) with or without the addition of autogeneous bone chips, applied in a bi-layered (BL) technique, covered by a resorbable cross-linked collagen membrane.

Material and methods: Fifty patients presenting with a vertical and/or lateral ridge deficiency of at least 3 mm were included: Group FDBA, N = 27 patients, particulate FDBA was the only graft; and Group BL, N = 23 patients, a BL bone grafting procedure where autogenous bone chips were the inner layer and FDBA the outer. Bone graft was covered with a ribose cross-linked collagen barrier membrane. Ridge dimensions were clinically or radiographically (computerized tomography scan) measured at the time of the bone augmentation procedure and at implant placement or uncovering and the maximum linear vertical or horizontal calcified tissue gain was calculated. Statistical analysis consisted of linear regression analysis, with maximum bone gain being the dependent variable.

Results: In the FDBA group, mean vertical bone gain was 3.47 mm (SD 1.25) and the horizontal, 5 mm (SD 1.28), while in the BL values were 3.5 mm (SD 1.2) and 3.6 mm (SD 1.72), respectively. Addition of autogenous bone does not appear to statistically significantly enhance the results. Spontaneous membrane exposure occurred in 24% of the cases and was the only variant that significantly influenced results (P < 0.001).

Conclusions: Large vertical and/or horizontal ridge deficiencies may be treated with FDBA and ribose cross-linked collagen barrier membranes with good clinical outcome. No added effect of the application of a layer of autogenous bone in these bone augmentation procedures could be demonstrated.

Spontaneous membrane exposure was the only parameter to affect the degree of new calcified tissue formation.

Advanced alveolar bone atrophy may prevent appropriate implant placement. Various alveolar bone augmentation approaches have been suggested to enlarge the bone volume before or at the time of implant placement (Chiapasco et al. 2006; Esposito et al. 2006). Guided bone regeneration is a well-documented surgical procedure to increase limited alveolar bone volume and properly develop the implant site (Chiapasco et al. 2006). This treatment approach is based on the application of barrier membranes, excluding epithelial and connective tissues, to enable bone progenitor cell proliferation and differentiation into the isolated area (Nyman et al. 1987; Polimeni et al. 2004a). Cell occlusion and space provision are critical factors
for alveolar bone regeneration [Polimeni et al. 2004c]. Both resorbable and non-resorbable barrier membranes have proven clinical effectiveness (Moses et al. 2005). Non-absorbable membranes, mostly made of polytetrafluoroethylene, require a second surgical procedure for their retrieval. Therefore, bio-absorbable membranes [Simion et al. 1997; Moses et al. 2003] have become a suitable alternative in bone regeneration procedures.

Spontaneous membrane exposure leads to decreased new bone formation [Nowzari & Slots 1995; Moses et al. 2005]. This event appears to be frequent in bone augmentation procedures. A 41.2% exposure rate has been reported for implants placed together with non-resorbable membranes [Moses et al. 2005]; however, lower incidences have also been reported [Donos et al. 2008; Rocchi et al. 2008]. Early exposure of non-resorbable membranes to the oral environment and the subsequent contamination [Nowzari & Slots 1995] command their entire early removal [Moses et al. 2005]. Spontaneously exposed resorbable membranes disintegrate, losing their barrier function at the exposed site; however, part of the membrane remains functional within the tissues [Simion et al. 1997; Tal et al. 2008a, 2008b]. Successful regeneration is possible provided cell exclusion and space maintenance are continued throughout the required time. This can vary between 3 and 12 months; depending on the dimensions of the bony defect [Schlegel et al. 1997; Hämmerle et al. 1998].

Treatment of large vertical and horizontal ridge deficiencies with the use of non-resorbable barrier membranes and particulate bone grafts [Simion et al. 1998, 2007a, 2007b; Tinti & Parma Benfenati 2001; Donos et al. 2008; Rocchi et al. 2008] and titanium mesh [Artzi et al. 2003, Rocuzzo et al. 2007] has been reported; however, these kinds of procedures using resorbable collagen membranes have been sparsely tested [Llambés et al. 2007, Merli et al. 2007, Donos et al. 2008; Rocchi et al. 2008].

Autogenous bone has been considered the gold standard due to its osseointegrative and conductive properties; however, several inconveniences such as fast resorption rate, limited available amount and patient morbidity limit their application. The vitality of autografts is not evident, the majority of the osteocytes of monocular bone grafts do not survive grafting and non-vital bone is progressively remodeled into new vital bone 7 months after grafting [Zerbo et al. 2003]. Allografts, xenografts and alloplastic materials are currently applied with successful clinical outcomes. Bone substitutes may replace autogenous bone for sinus lift procedures even in extremely atrophic sinuses [Esposito et al. 2006, Aghaloo & Moy 2007]. Mineralized freeze-dried bone allograft (FDBA) both in particulate [Feuille et al. 2003, Fromm et al. 2006, Galski et al. 2006, Kolerman et al. 2008] and block shapes [Nissan et al. 2009] have been applied in bone augmentation procedures with a successful clinical and histological outcome. However, the clinical advantage of combining autogenous bone with FDBA has not been evaluated.

The purpose of the present study was to compare the clinical outcome of augmentation of atrophic alveolar ridges with the use of particulate FDBA with or without the addition of autogenous bone, applied in a bi-layered (BL) technique, in conjunction with a resorbable ribose cross-linked collagen membrane.

Material and methods

This study was performed at the Department of Periodontology at the Maurice and Gabriela Goldschleger School of Dental Medicine, Tel Aviv University, Tel Aviv, Israel and in the authors’ private practices. The study was approved by the Tel-Aviv University Helsinki convention committee. Fifty consecutive partially edentulous patients (39 females, 11 males, average age 51.9, SD = 12.43), with largely atrophic mandibular or maxillary residual alveolar ridges with vertical and/or lateral deficiencies presenting to the different clinics between January 2007 and August 2008 were included. Only one procedure per patient was included in this study. Pre-operative planning consisted of clinical and radiographic examinations. A comprehensive patients’ evaluation assessed systemic health and the status of all remaining teeth. Patients presented no contraindications for implant therapy. Exclusion criteria were heavy smokers (>10 cigarettes a day), uncontrolled periodontal disease, pregnancy and any medication or systemic diseases that could interfere with the treatment. In all cases, periapical radiographs and computerized tomography (CT) scans were performed to provide diagnostic information about residual alveolar ridge width and height. The clinical and radiographical findings were thoroughly discussed with the patients and the available treatment options were presented. Patients who accepted the treatment plan including placement of an allograft were included in the study and signed an informed consent.

All patients presented with vertical and/or horizontal ridge deficiencies [Tinti & Parma Benfenati 2003]. Only patients requiring a vertical and/or lateral alveolar ridge augmentation of at least 3 mm were included in this study. A staged approach where bone augmentation was performed before implant placement was applied in extreme cases where primary implant stability and predictable implants osseointegration could not be guaranteed. For these cases, bone augmentation procedure was performed in the first stage while implants were placed in a second procedure performed after 5–7 months. Where needed, another bone augmentation procedure was performed together with implant placement. In these patients, only results from the first procedure were taken in consideration.

Patients were assigned [by serial number, in each treatment center, odds to FDBA and pairs to BL] to one of the two treatments regarding the type of graft used. In Group FDBA (27 patients), particulate mineralized allogeneic bone graft was used as the only graft and in Group BL (23 patients), a BL bone grafting procedure consisting of autogenous bone chips as an inner layer and particulate mineralized allogeneic bone graft as the outer layer was applied. In each group, two subgroups were established according to whether mainly vertical or lateral bone augmentation was performed; therefore, altogether four groups were established. Group FDBA, with vertical ridge deficiency (Group 1), comprised 15 patients and with horizontal (Group 2) of 12. Group BL with vertical augmentation (Group 3) was composed of eight patients and with horizontal (Group 4) of 15. It should be noted that the assignment was not fully blinded because patients where the possibility of collecting...
autogenous bone from the treatment area, without opening a second surgical site, were limited, were always included in the FDBA group; therefore, groups are not identical, which could have an impact in the analysis. Twenty-six procedures [52%] were in the maxilla and the remaining 24 [48%] in the mandible. In 44 patients [88%], the augmentation procedure was performed before implant placement while in the remaining six [12%] implants were placed together with bone grafting.

Researchers’ standardization was performed in the first five cases, where measurements were performed by all three of them and then compared, and no significant differences were found. The procedure outcome was evaluated by the maximum vertical- or horizontal-calciﬁed tissue gain in the treatment area. In horizontal ridge deﬁciencies, the minimal ridge width was clinically measured during the bone augmentation procedure: this site was recorded and related to the nearest implant or tooth and measured again at the second surgery (implant placement or implant uncovering) using a UNC periodontal probe [Hu-Friedy Mfg. Co. Inc., Leimen, Germany].

In cases with a vertical ridge deﬁciency, where implants were placed together with the bone augmentation; the largest distance from the implant platform to the bone crest was recorded and clinically measured during implant surgery and the same site was measured at the second-stage implant surgery with a similar periodontal probe. When vertical bone augmentation was performed before implant placement, pre-operative minimal distance between the bone crest to a radiographical reference point in the treatment area such as the inferior alveolar canal, ﬂoor of the nose or of the sinus was measured in the CT scan. In these cases, shortly before the procedure for implant placement, another CT scan was performed and the vertical alveolar ridge gain in the site measured previously was calculated and recorded. It should, therefore, be noted that in these cases, measurements were performed only in the CT scans and not clinically. The same surgeon who performed the surgical procedure was also responsible for the measurements. Measurements were rounded to the closest full millimeter.

One hour before each surgical procedure, either 1000 mg of amoxycillin [Moxypen Forte, Novopharm Limited, Toronto, ON, Canada] or 875 mg amoxycillin plus 125 mg clavulanic acid [Augmentin 875, SmithKline Beecham Pharmaceuticals, Cambridge, UK] were administered as antibiotics and continued for 7 days (500 mg of amoxycillin every 8 h or 875 mg amoxycillin plus clavulanic acid twice a day). Pre-procedural rinse was performed with 10–15 ml of a 0.2% chlorhexidine digluconate [TardentC, Taro Pharmaceutical Industries Ltd, Haifa Bay, Israel] solution for 1 min. Patients continued to rinse with the same solution for the following 7 days.

Local anesthesia using 2% lidocaine with 1:80,000 epinephrine [LignospanC Special, Septodont, Saint-Maur-des-Fosses Cedex, France] was administered.

The allograft (Ora graftC, Lifenet, Virginia Beach, VA, USA) was rehydrated with a solution of sterile saline before use. Access was achieved through a mid-crestal incision with buccal vertical releasing incisions. Full-thickness muco-periosteal buccal and lingual ﬂaps were raised through a blunt dissection. On the lingual side of the mandible, the ﬂap was raised deeply into the floor of the mouth. Periosteal fenestration was performed only at the base of the buccal ﬂap.

In the FDBA group [Fig. 1a–i], numerous perforations in the cortical bone were performed to expose medullar spaces and enhance bleeding. In certain cases with a vertical ridge deﬁciency, where a two-stage procedure was performed, supporting stainless-steel screws [OsteoMed Corporation, Addison, TX, USA] were inserted in the treatment area, leaving part of their extension exposed, thus serving as supporting tenting posts. The rehydrated bone allograft was then applied to achieve the desired volume. In cases where the supporting screw/s became spontaneously exposed, they were retrieved shortly before implant placement, to allow for complete soft tissue healing before the next surgical procedure.

In the BL group [Fig. 2a–k], autogenous bone particles were harvested using a bone scraper [SafescraperC META, Reggio Emilia, Italy] from an area close to the surgical site, thus avoiding a second surgical site. In this group, slight decortication was performed with the scraper; therefore, drills were not used for intra-marrow perforations. Bone grafting was performed using a BL technique; autogenous graft was applied in contact with the pristine bone (inner layer). FDBA [Ora graftC] served as the outer layer, and ﬁlled the necessary volume (Fig. 2d and e).

In both groups, the bone graft was covered with a resorbable ribbon cross-linked collagen barrier membrane [OssixC-Plus, ColBar LifeScience Ltd, Herzliya, Israel]. The membrane was applied in a selective BL technique; the deeper layer completely covered the bone graft and underlying bone and the upper layer covered mainly the defect area; for vertical deﬁciencies, it was the occlusal and for lateral, it was the buccal (Figs 1d and 2f). Membranes were not necessarily ﬁxed to the underlying bone. Primary soft tissue closure was obtained with modiﬁed internal mattress sutures using a resorbable material [4-0 VicrylC coated, Johnson & Johnson Intl., Woluwe, Belgium] (Fig. 1c). Remaining sutures were removed after 14 days.

Altogether 106 implants were placed, between one and four per area, mean 2.12, SD = 0.82. Implants were SevenC, BiocomC, or MistralC [MIS Implant Technologies, Shlomi, Israel], Tapered Screw-VentC [Zimmer Dental Inc., Carlsbad, CA, USA] or Legacy [Implant DirectC, Calabasas Hills, CA, USA]. In areas where bone height was not at least 10 mm, implants were not fully inserted [Fig. 1c], leaving part of their surface exposed. The bone augmentation procedure was similar as described for each group; however, because the implants supported the bone graft and membrane, no tenting screws were inserted. Second-stage surgery for implant uncovering was performed 5–7 months after placement.

Control follow-up visits after bone augmentation and implant placement were performed 1 week, 2 weeks, 1 month, 3 months and 5–7 months after each procedure. Inconveniences or complications such as infection, exposure, or exfoliation of the membrane, supporting screws, bone graft and/or implants, or lower lip paresthesia were recorded.

**Statistical analysis**

This consisted of linear regression analysis where the dependent variable was maximum bone gain and the independents were...
groups, vertical or horizontal augmentation, age, minimal pre-operative ridge height or width, spontaneous membrane exposure, number of implants placed, whether augmentation was performed previously or together with implant placement and upper or lower jaw. Separate backward models of the linear regressions were also performed for membrane exposure and non-exposure situations.

Results

Pre-operative bone dimensions and gains for the different groups with and without membrane exposure are presented in Tables 1 and...
Mean bone gain was very similar in Groups 1 (3.47 mm, SD 1.25), 3 (3.5 mm, SD 1.2) and 4 (3.6 mm, SD 1.72), and slightly higher in Group 2 (5 mm, SD 1.28), however, differences between groups were not statistically significant.

Backward linear regression analysis had an $R^2$ value of 0.528. Logarithmic transformation (L) of pre-operative alveolar ridge
Ninety-five percent confidence interval for coefficient (membrane exposure, the unstandardized result, which was a linear dependence. For significant negative influence on the regression analysis was performed for cases where the assumptions of the linear normal distribution. Membrane exposure, only pre-operative height or width was obtained and the lower bound was -2.826 and the upper bound was -1.407. For pre-operative height or width, B = -0.785, SD 0.236. The assumptions of the linear regression were satisfied. Separate linear regression analysis was performed for cases either with or without membrane exposure [R² value 0.242]. For cases with no membrane exposure, only pre-operative height or width had a negative statistically significant influence (B = -0.884, SD 0.261). However, when only cases without spontaneous membrane exposure were analyzed, no independent variable showed statistically significant effects upon maximum bone gain.

Spontaneous membrane exposure was recorded in 12 (24%) cases out the 50 patients: five cases in the FDBA group with vertical augmentation (33.3% within group), one case in the FDBA group with horizontal augmentation (8.3% within group), two in the BL group with vertical augmentation (25% within group) and four in the BL group with horizontal augmentation (26.7% within group). Although Pearson’s χ² did not show any statistically significant association between membrane exposure and any of the other parameters, half of the events of early membrane exposure in the whole study were recorded in six of the 13 patients where vertical augmentation in the posterior mandible was performed (46%). In these 13 patients, the mean initial ridge height over the inferior alveolar canal was 5.92 mm (SD = 1.12) and the mean calcified tissue gain was 3 mm (SD = 1.15). However, bone gain was 3.86 mm (SD = 0.69) among the cases with no membrane exposure and only 2 mm (SD = 0.65) where exposure had occurred, and differences were statistically significant (P<0.001).

During the first days following the procedures, swelling and certain inconveniences such as pain and hematomes were noticed in several patients. Most of these disappeared toward the 4–5 post-operative days. In three out of the 24 patients (12.5%) with a mandibular procedure, a slight paresthesia of the lower lip was reported after the procedure; however, in all cases it disappeared shortly afterwards. All implants placed together with bone augmentation procedures were stable at the second-stage implant surgery. Augmented sites allowed for proper implant placement; however, in 15 patients (34%), another bone augmentation procedure was performed at this time. Of these patients, seven were from Group 1, 2, from Group 2, 3 from Group 3 and 3 from Group 4. All these implants also showed clinical integration at their uncovering. Remnants of the barrier membrane could usually be appreciated in the treatment area at the time of the second surgery, especially in cases where no spontaneous membrane exposure had occurred [Figs 1h and 2i].

Discussion

The present study evaluated the clinical outcome of augmentation procedures in largely atrophic alveolar ridges with the use of particulate FDBA with or without the addition of autogenous bone, applied in a BL technique, in conjunction with a resorbable ribose cross-linked collagen membrane. Results in groups FDBA and BL were similar, therefore, there was no added clinical effect of the use of autogenous bone. New bone formation within the defect appears to be completed by 7 months [Ersanli et al. 2004]. Bone grafts effectively enhance space provision, and this appears to be the principal mechanism by which biomaterials actually support bone regeneration [Polimeni et al. 2004b]. A BL (sandwich) bone augmentation procedure, for treatment of dehiscence defects based on the application of mineralized human cancellous allograft (inner layer), mineralized human cortical allograft (outer layer) and coverage with a barrier membrane [Wang et al. 2004; Park et al. 2008] has reported good clinical results. Histomorphometric
analyses of sites augmented with FDBA have revealed predictable acceptable results, showing over 40% new bone formation (Feuille et al. 2003; Cammack et al. 2005). The present study evaluated only the clinical outcome of vertical and lateral bone augmentation procedures, and no histologic evaluation was performed, and therefore the characteristics of the newly formed calcified tissue with the different bone grafts are not known. In vertical bone augmentation procedures, without simultaneous implant placement, with the use of non-self-supporting barriers and particulate bone grafts, tenting screws or osteosynthesis microplates may be used to prevent membrane and biomaterial collapse and preserve the space where bone can grow (Fugazzotto 1993; Hempton & Fugazzotto 1994; Dooblin et al. 1996; Merli et al. 2007).

Although, initially, a randomized-controlled study was planned, randomization regarding the whole population could not be really applied, because in large ridge deficiencies, mostly vertical, the availability of autogenous bone from the treatment area was sparse, and these patients were included in the FDBA group. Thus, it could be possible that patients in the FDBA groups presented with larger deficiencies, than that appearing according to the values presented in Table 1. Despite the lack of significant differences in the present clinical study, the possibility that the BL could perform better than FDBA in cases of [equally] large defects cannot be completely excluded. In other words, BL-treated sites in the present study might have been insufficiently big to disclose potential differences in effect. The importance of intramarrow perforations in bone augmentation procedures is not completely clear (Romp- en et al. 1999; Slotte & Lundgren 2002); however, it should be noted that in the BL, differently from the FDBA group, intramarrow perforations were not performed, because decortication was performed with the bone scraper.

Evidently, in small defects, the need for augmentation, and therefore the expected gain was slightly smaller than in larger defects. Although, pre-operative residual alveolar ridge height or width had a significant influence on bone gain for cases without membrane exposure, the most significant parameter to affect the degree of new calcified tissue formation between the two procedures for the whole sample was the spontaneous barrier membrane exposure. Similar findings have already been reported extensively (Nemcovsky & Artzi 2002; Moses et al. 2005; Park et al. 2008; Schwarz et al. 2008a). In the present study, this event was recorded in 24% of the cases. In a previous report, where the bone augmentation procedure was performed together with implant placement, with the use of a similar collagen barrier membrane, early exposure was appreciated in 39% of patients (Moses et al. 2005). In the present study, bone grafting was performed before implant placement in 88% of the patients; therefore, in these cases, small wound dehiscences could have healed without being noticed (Friedmann et al. 2001). Interestingly, 50% of the cases with membrane exposure occurred in vertical bone augmentation cases in the posterior mandible, accounting for 46% of cases where this kind of procedure was performed. Other studies have reported membrane exposures in only three out of 22 cases where vertical ridge augmentation was performed together with implant placement and covered by a non-resorbable barrier membrane (Tinti & Parma Benfenati 1998); however, a later report on vertical bone augmentation in 22 patients with dental implant placement reported complications in 40% of patients, mainly spontaneous membrane exposure, with major complications in 15% of them (Merli et al. 2006).

In the present study, a resorbable ribose cross-linked barrier membrane was applied in a selective BL technique; the deeper layer completely covered the bone graft and underlying bone and the upper layer covered mainly the defect area; for vertical deficiencies, it was the occlusal and for lateral, it was the buccal. The rational for this application is that in case of small wound dehiscence and subsequent membrane exposure, the superficial layer may resorb, while soft tissue may heal and cover the deep layer [Tal et al. 2008a]. Furthermore, the use of a double-layered membrane results in a barrier of increased collagen area and thickness, compared with the application of a single layer [Kozlovsky et al. 2009]. Collagen membranes can reduce bone graft resorption, and the BL technique has proven more effective than the single in experimental bone augmentation procedures [Kim et al. 2009]. Remnants of the membranes could usually be appreciated, in cases with undisturbed healing; however, they were not always present where spontaneous exposure had occurred [Tal et al. 2008a]. Membrane degradation starts shortly after implantation [von Arx et al. 2005]. Membranes should be appropriate to the clinical demands of each case, and barriers with high degradation rates might have a shorter than desired effect (Moses et al. 2008). The effect of collagen membrane coverage on bone graft volume maintenance is dependent on the membrane integrity and stability during healing (Adeyemo et al. 2008). Although, the advantage of slowly resorbable collagen barrier membranes in healing of bone defects is still not clear (Bornstein et al. 2009) and no definite landmarks are available, it has been suggested that the 1-month barrier function time for each millimeter of bone regeneration is needed; accordingly, 2–3-month barrier function time will be required for small dehiscence and fenestration defects; however, larger defects, as treated in the present study, may require longer barrier function times (Smiler & Soltan 2006). In small surgically created defects, bone-to-implant contact and bone fill values increased over time in membrane-covered defects; however, membrane exposure was associated with the loss of supporting alveolar bone even occurring 10–12 weeks postimplantation [Schwarz et al. 2008a]. Collagen membranes with a higher degree of cross linking remain intact for longer periods [Rothamel et al. 2005; Moses et al. 2008]. This could enable improved healing of larger defects (Brunel et al. 1996; Bunyaratavej & Wang 2001), and therefore may offer advantages for the treatment of large non-self-contained bone defects, where prolonged membrane barrier functions are desirable [Tal et al. 2008b]. Slow-resorption collagen membranes have the potential to promote vertical ridge augmentation when used with autogenous bone at the time of implant placement [Llambés et al. 2007]. However, cross-linked membranes present reduced tissue integration and vascularity [Rothamel et al. 2005; Schwarz et al. 2006], and in clinical trials, have shown a higher incidence of spontaneous exposure.
following their application in the oral cavity [Moses et al. 2005].

In 15 out of 44 cases (34%), where bone augmentation was performed before implant placement, another bone augmentation procedure was considered necessary at the time of implant placement. The mean amount of new calcified tissue formation was 3.5 mm among cases with vertical and slightly higher for cases with lateral ridge deficiencies. Therefore, a staged procedure appears to be indicated for cases with larger defects.

Conclusions

Large vertical and/or horizontal ridge deficiencies may be treated with mineralized bone allograft and ribose cross-linked collagen barrier membranes with good clinical outcome. No added clinical effect of the application of a layer of autogenous bone in these bone augmentation procedures could be demonstrated.

The main parameter to negatively affect the degree of new calcified tissue formation was spontaneous membrane exposure, which occurred in 24% of all the cases in this study, and a large percentage [46%] of vertical bone augmentation procedures in the posterior mandible were accompanied with spontaneous early membrane exposure.

References


Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1. Supporting information in accordance with the CONSORT Statement 2001 checklist used in reporting randomized trials.

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