The Wafer Technique: Histomorphometric Results

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Bone augmentation procedures are applied to patients with an inadequate quantity of bone necessary for implant placement for prosthetic rehabilitation.\textsuperscript{1} A recent umbrella review showed that in terms of vertical defects, short implants should be used when possible because of the reduced number of complications compared to longer implants with bone augmentation.\textsuperscript{2} If short implants cannot be used, no conclusions can be drawn regarding the comparison between different vertical bone augmentation techniques in the atrophic posterior mandible because quantitative meta-analyses were not performed.\textsuperscript{2}

The most widely applied procedures for the treatment of vertical bone defects are: guided bone regeneration (GBR), distraction osteogenesis, and autogenous inlay or onlay bone graft from intra- and extraoral donor sites.\textsuperscript{3,4} However, these techniques are often associated with high complication rates, costs, and patient discomfort.\textsuperscript{2} Complications and discomfort at the extraoral donor site have been previously reported.\textsuperscript{5,6} Intraoral donor sites could lead to fewer and less severe complications.\textsuperscript{7-9} The data obtained from a systematic review showed that GBR is a predictable technique that allows for the placement of implants in atrophic areas.\textsuperscript{10} In recent years, new approaches for

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GBR, such as the Fence Technique, localized Fence Technique, and the Wafer Technique, have been described.\textsuperscript{11-13} These two-stage techniques allow for the formation of large quantities of regenerated bone in horizontal and vertical dimensions, with limited discomfort to the patients.\textsuperscript{11-13} In particular, the Wafer Technique provides a retaining space for the compressed particulate grafting biomaterial using thin autogenous cortical bone plates, harvested intraorally, fixed to the residual bone by means of osteosynthesis screws.\textsuperscript{13} Collagen membranes are also used. The Wafer Technique can be applied for 3D reconstruction of osseous defects in partially or totally edentulous patients whose anatomy or prostheticic requirements do not permit the use of short or tilted implants.\textsuperscript{13}

Histomorphometric analysis is considered to be the gold standard method to estimate the amount of newly formed bone, residual graft particles, and soft tissue components.\textsuperscript{14} A recent systematic review evaluated the histomorphometric results of different grafting materials, based on the analysis, autogenous bone graft resulted in the highest amount of new bone formation and in the lowest amount of residual graft in comparison to other grafting materials.\textsuperscript{15} Bone substitute materials seemed to be good alternatives to autogenous bone and could be considered grafting materials to avoid the disadvantages related to autografts, such as morbidity rate, limited availability, and high volumetric change.\textsuperscript{15}

Histomorphometric data were recently presented for the Fence Technique in a randomized controlled trial.\textsuperscript{16} At implant placement, 6 months after grafting, no significant differences were evident in the histomorphometric comparisons between 100% autogenous bone vs 50% deproteinized bovine bone matrix with 50% autogenous bone, even if the percentage of residual graft was obviously greater in the 50:50 group.\textsuperscript{16}

Five clinical cases were treated in the article illustrating the Wafer Technique.\textsuperscript{13} All patients recovered well, and complications were not observed.\textsuperscript{13} Histomorphometric analysis was performed only for two of these cases, and it is unclear if this technique can result in a percentage of new bone formation and residual graft comparable to that of other techniques based on GBR.\textsuperscript{13}

The aim of this study is to report the histomorphometric results of the Wafer Technique in a series of patients with vertical defects requiring dental implants for prosthetic rehabilitation.

\section*{Materials and Methods}

This was a case series of consecutive patients. Eligible participants for this study were adult partially edentulous patients, 18 years old or older, in need of implant treatment, and with an extremely atrophic edentulous alveolar crest in the mandible or the maxilla. Patients were excluded if they had systemic or local contraindications to implant surgery, had undergone irradiation in the head and neck area, were treated with intravenous aminobisphosphonates, had poor oral hygiene (full-mouth plaque and bleeding scores $\geq 20\%$) and lack of motivation, had uncontrolled diabetes (reported levels of glycaemia over the normal threshold), were pregnant or lactating, had an allergy to collagen, were substance abusers, and had a smoking habit ($\geq 20$ cigarettes per day).

The study took place at a private center in Rimini, Italy. The dental office obtained the approval of the local authorities to conduct clinical studies (protocol no. 221722/P).

The investigators explained the nature of the trial, the aim, and the methods to the patients, anticipating benefits, potential risks, as well as any form of discomfort that participation might entail. The patients read and asked questions inherent to the study prior to signing the informed consent.

The study was conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki. The principal investigator (M. Merli) has 30 years of experience in dental implant surgery and dental implant prosthesis rehabilitation.

\section*{Surgical Procedure: The Wafer Technique}

All interventions were conducted with a two-stage procedure in combination with intravenous sedation and antibiotic preoperative administration.

The procedure was described in a previous article.\textsuperscript{13} In brief, preoperative CBCT limited to the atrophied arch was performed to evaluate...
the 3D morphology of the hard tissue. The data of the CT were used to build a stereolithographic model using thermosetting, light-sensitive epoxidic resins. This anatomical model served for presurgical planning of the bone-reconstruction procedure. The area of the region to be augmented is measured by a piece of aluminium foil that mimics the horizontal and vertical dimensions of the bone plate to be harvested. The model can also be used to simulate the entire clinical procedure.

At surgery, a horizontal midcrestal incision is designed and a mucoperiosteal flap is raised to obtain access to the hard tissue. The preferred site of bone harvesting is the corpus-ramus of the mandible, along the external oblique line where a sufficient quantity of cortical bone can usually be found. The adopted technique for bone harvesting is by means of a piezoelectric device (Piezosurgery, Mectron) with the use of a dedicated set of angled inserts with sharp, 0.25-mm-thick indented tips.

The advantage of this device is that the surgeon can sculpt multiple plates of autologous cortical bone that are ≤ 1 mm thin. The first plate harvested is used to build the external wall of the area to be regenerated. The cortical plate is smoothed—all sharp edges are removed in order to avoid trauma to the superficial soft tissue during the healing period—and fixed to the receiving site by means of 1-mm–diameter steel osteosynthesis screws (stoma micro-screw, Storz am Mark). The autogenous plate must be firmly fixed as follows: A groove is created with a round bur in the apical bone-receiving site where the plate is positioned vertically, supported partially by the residual basal bone, transforming the vertical component of the defect into a horizontal defect. The screws are then obliquely inserted from the vestibular side to the apical basal bone at both the proximal and distal extremities in order to leave space for application of the biomaterial.

To stimulate bone regeneration, intramarrow penetration is performed by drilling microperforations into the residual bone crest. The bone-marrow blood is extracted using a blunt-tipped syringe and mixed with demineralized bovine bone matrix (DBBM; Bio-Oss spongiosa granules, Geistlich Pharma) and the particulated autogenous bone, which was obtained by milling the second plate. In addition, autogenous bone dust was also harvested through a filter connected with the surgical suction unit during the previous procedures. The ratio of autologous bone and DBBM is approximately 50:50.

The space between the native bone and the inner surface of the cortical plate is entirely filled with the mixture of blood, particulate autogenous bone, and DBBM, and the filled defect is covered by a resorbable collagen membrane (Bio-Gide, Geistlich Pharma) fixed with titanium tacks first on the lingual side and then, after the defect is filled with the graft, on the buccal side. The size of the membrane used is strictly dependent on the mesiodistal dimension of the edentulous area.

The creation of a perfect seal along the primary horizontal incision lines is fundamental to avoid the risk of dehiscence and possible exposure of the membrane surface and infection of the grafted bone. A strategy followed in order to avoid this type of complication is the application of a flap extension technique (muscular dissection and/or perioplasty) fixed with a double-layer suturing technique.17–19

Amoxicillin (875 mg) and clavulanic acid (125 mg) twice a day for 6 days; ibuprofen (600 mg) twice a day for 2 days and then as needed; and betamethasone for 5 days (doses decreasing daily from 4 mg to 0.5 mg) is prescribed to the patients. Ice packs are given to the patients to be applied intermittently for the first 2 to 3 hours after surgical treatment. Patients are instructed to refrain from mechanical plaque removal in the treated area for 1 week, to use chlorhexidine mouth rinse (0.12%) twice a day from the third postoperative day, and to apply chlorhexidine gel to the wound area twice a day for 15 days. Patients are advised to avoid smoking 15 days before surgery and during the prescribed recovery period. Patient-centered outcomes were pain related to surgery (using a visual analog scale [VAS]; 0 equal to no pain and 10 equal to very intense pain) and analgesic intake (number of tablets) during 1 week postsurgery. Duration of surgery was also assessed.

The implants are inserted 6 months postsurgery and loaded 0 to 3 months after placement. The presence of complications is assessed during the entire procedure.
Fig 1  Patient 3. (a) Peri-implantitis seen in the radiograph. The three implants were explanted. (b) Presurgical CBCT. (c) A mucoperiosteal flap is raised to obtain access to the hard tissue. (d) The autogenous plate is firmly fixed as follows: A groove is created with a round bur in the apical bone-receiving site, where the plate is positioned vertically and is partially supported by the residual basal bone, transforming the vertical component of the defect in a horizontal defect; the screws are then inserted, leaving space for application of the biomaterial. (e) A resorbable collagen membrane, fixed with titanium tacks on both the lingual and buccal sides, covers the filled defect. (f) Postsurgical CBCT. (g) After 6 months, the area has been regenerated. A biopsy sample was harvested from the regenerated bone area. (h) Patient smile. (i) Provisional prosthesis. (j) Radiographic view 18 months after implant placement.
The surgical procedure of one representative case is shown in Fig 1.

**Histologic Evaluation**

During the second-phase surgery for implant placement, bone core biopsy samples, corresponding to the regenerated bone areas comprehensive of the zone of the autologous plate, were harvested using a trephine bur under copious irrigation. Biopsy samples were immediately fixed in 10% buffered formalin solution (Sigma-Aldrich) at 4°C for 24 hours and processed for histologic and histomorphometric analyses. The specimens were dehydrated in an ascending series of alcohols and embedded in London White resin (LR White, London Resin). After resin polymerization, specimens were sectioned with a high-precision diamond disk at 150 μm and ground to approximately 40 μm with a specially designed grinding machine (Micromet, Remet). The nondecalcified ground sections were stained with acid fuchsine and toluidine-blue staining. The specimens were observed under normal transmitted light with an optical microscope (Eclipse, Nikon). Histomorphometric analysis was carried out using the light microscope connected to a high-resolution video camera, and the images were elaborated using ImageJ software (National Institutes of Health). The tested variables in the histomorphometric analysis were: native bone (mineralized tissue), newly formed bone, residual graft particles, empty marrow space, and soft tissue. These values were expressed as a percentage.

**Outcome Measures and Sample Size**

Percentages of new bone, mineralized native tissue, residual graft, empty marrow space, and soft tissue were calculated. An a priori sample size was not performed. All patients who underwent at least a biopsy were included in this study.

**Statistical Analysis**

Descriptive statistical analysis was performed at the patient level for age, gender, smoking, and VAS scores. Mean and standard deviations were calculated for quantitative variables, and frequency and percentage for qualitative data.

Percentages of new bone, mineralized native tissue, residual graft, empty marrow space, and soft tissue were calculated. For histomorphometric variables, 95% CI was calculated for each variable.

A single statistician (M.N.) performed all statistical analyses. The statistical software was JMP v. 13 (SAS Institute).

**Results**

This histologic study was performed on 12 patients consecutively treated with the Wafer Technique at a private center in Rimini, Italy. All 12 patients were analyzed, and there were no dropouts. Baseline information is presented in Table 1.

Mean chair time was 93.3 ± 6.5 minutes, and mean VAS pain was 2.3 ± 0.5. The mean number of anti-inflammatory tablets consumed was 10.7 ± 1.8. The patients recovered well, and only one complication was observed: One patient developed a hypoesthesia of the lower lip after surgery. The patient was treated with lipoic acid and vitamin B complex (Tiobec 800, Laborest), and the hypoesthesia disappeared in 4 weeks.

During the second surgical phase for implant placement, a total of 19 biopsy samples were taken from the 12 patients.

Histologic appearance of the regenerated area revealed new bone formation in contact with mature bone and with grafting particles. Several areas of concentrated grafting particles were also noted, as well as marrow spaces and osteocytes (Figs 2 and 3).

The histologic data were analyzed using the percentage of new bone, mineralized native bone, residual graft, marrow spaces, and soft tissue. The means, standard deviations, and 95% CI of the means are reported in Table 2. The percentage of new bone was 16.4% (95% CI: 9.5% to 23.2%), and the percentage of the mineralized native bone was 42.6% (95% CI: 28.2% to 57.0%).

During the second surgical phase for implant placement, a moderate adjunct of autogenous graft was performed in two patients. Bio-Oss...
Table 1 Baseline Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Demographics</th>
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<tbody>
<tr>
<td><strong>Age, y</strong></td>
<td></td>
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<tr>
<td>Mean ± SD</td>
<td>56.3 ± 8.3</td>
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<tr>
<td>Minimum</td>
<td>37</td>
</tr>
<tr>
<td>Maximum</td>
<td>66</td>
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<tr>
<td><strong>Female patients, n (%)</strong></td>
<td>7 (58%)</td>
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<tr>
<td><strong>Smokers, n (%)</strong></td>
<td>4 (33%)</td>
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<tr>
<td><strong>Arch, n (%)</strong></td>
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<tr>
<td>Mandible</td>
<td>9 (75%)</td>
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<tr>
<td>Maxilla</td>
<td>3 (25%)</td>
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<tr>
<td><strong>Opposing arch, n (%)</strong></td>
<td></td>
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<tr>
<td>Teeth</td>
<td>3 (25%)</td>
</tr>
<tr>
<td>Implants</td>
<td>1 (8%)</td>
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<tr>
<td>Mixed</td>
<td>8 (67%)</td>
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</table>

A total of 12 patients underwent the Wafer Technique.

Fig 2 Patient 3. Histologic specimens are stained with toluidine-blue and acid fuchsine. (a) Histologic appearance of the regenerated area showing new bone formation (NB) in contact with mature bone, with minor marrow spaces (MS) containing osteocytes (-Oc). Original magnification ×4,000. (b) Higher magnification (×10,000) shows intense NB formations in contact with mature bone, with minor MS containing -Oc. (c) Higher magnification (×20,000) of mature bone surrounded by NB areas. MS containing -Oc are evident.
was also added in one of the two patients.

A total of 25 implants were positioned in the second surgical phase. The length of the implants was $9.1 \pm 1.9$ mm and the diameter was $4.0 \pm 0.4$ mm. The insertion torque was $\geq 35$ Ncm for all implants.

## Discussion

The aim of this study was to report the histomorphometric results of the Wafer Technique in a series of Table 2

<table>
<thead>
<tr>
<th>Table 2 Histologic Data</th>
<th>Mean</th>
<th>SD</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>New bone, %</td>
<td>16.4</td>
<td>10.8</td>
<td>9.5, 23.2</td>
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<tr>
<td>Mineralized native bone, %</td>
<td>42.6</td>
<td>22.7</td>
<td>28.2, 57.0</td>
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<tr>
<td>Residual biomaterial graft, %</td>
<td>15.0</td>
<td>18.9</td>
<td>3.0, 27.0</td>
</tr>
<tr>
<td>Empty marrow space, %</td>
<td>5.5</td>
<td>2.8</td>
<td>3.7, 7.3</td>
</tr>
<tr>
<td>Soft tissues, %</td>
<td>20.6</td>
<td>14.0</td>
<td>11.7, 29.5</td>
</tr>
</tbody>
</table>

The histologic data of the 12 patients who underwent the Wafer Technique were analyzed.

Fig 3 Patient 12. Histologic specimens are stained with toluidine-blue and acid fuchsine. (a) Histologic appearance of the regenerated area showing new bone (NB) formation in contact with mature bone grafting particles. An area of concentrated grafting particles is also evident, as well as marrow spaces (MS) and osteocytes (-Oc). Original magnification $\times 4,000$. (b) Higher magnification ($\times 10,000$) shows NB in contact with mature bone, with large MS and -Oc. (c) Even higher magnification ($\times 20,000$) shows mature bone areas and grafting particles, with MS containing -Oc.
cases of patients with vertical defects requiring dental implants for prosthetic rehabilitation. Twelve patients were consecutively treated with the Wafer Technique, and the histomorphometric results showed a considerable percentage of new bone (16.4%) and a considerable percentage of mineralized native bone (42.6%).

The Wafer Technique utilizes osteosynthesis screws to fix a thin cortical layer of bone vertical to the residual bone bed, delimitating the area of regeneration. The Wafer Technique can be distinguished from the Fence Technique in that the osseous plate containing the particulated grafting material is itself resorbable as well as providing growth factors and mineralized tissue for the regeneration, while a titanium plaque is used in the Fence Technique. Additional growth factors could also stem from the intramarrow penetration, as well as the resulting blood profusion collected and mixed with the particulated autogenous bone and the DBBM. The 12 cases reported showed positive outcomes resulting in a substantial reconstruction of the alveolar crest deficiencies, allowing for a second surgical phase of implant placement with high primary stability.

In the present study, the regenerated bone was well organized and after 6 months the histologic analyses revealed the presence of a homogenous and compact bone substrate, mainly characterized by newly formed bone and mineralized native bone, where spotted regions of residual grafted bone particles were detectable.

The percentage of residual biomaterial graft, new bone formation, and mineralized native tissue of this study are very similar to those obtained in a recent study on the Fence Technique using a 50:50 mixture of autogenous bone and biomaterial. In that study, the DBBM residual graft constituted 13.9% (15.0% in the present study), new bone formation was 19.4% (16.4% in the present study), and the mineralized native tissue was 36.3% (42.6% in the present study). The greater percentage of native bone compared to the Fence Technique could be due to the Wafer Technique’s presence of the cortical bone layer.

Only one transient complication was observed. For this reason, the Wafer Technique seems to be a favorable option in cases of atrophied edentulous regions when compared to alternative and more invasive solutions, such as the use of onlay bone grafts harvested from extraoral donor sites, or the use of zygomatic fixtures.

The clinical cases presented were carried out by a surgeon experienced in GBR (M. Merli), and it is unclear whether these results can be obtained by less experienced operators.

Conclusions

The results of this case series are very promising. The Wafer Technique has proven to be safe and reliable, although randomized clinical trials are needed to validate the effectiveness of this procedure.

Acknowledgments

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References


