Microstructural and densiometric analysis of extra oral bone block grafts for maxillary horizontal bone augmentation: a comparison between calvarial bone and iliac crest

Maxillary bone resorption following tooth extraction or due to pneumatization of maxillary sinus often results in inadequate residual ridges for ideal implant placement (Pietrokowski & Massler 1967). To provide enough ridge height and width for proper 3-dimensional (3D) implant placement, bone augmentation procedures have been proposed. While procedures such as ridge splitting and guided bone regeneration (Hammerle et al. 2002) have been applied for horizontal ridge augmentation, autogenous block grafting is still considered the main method for extensive reconstruction of the maxilla (Tessier et al. 2005).

Autogenous block grafts can be harvested from different sources and depending on the embryonic origin of the donor site, the graft will possess different properties. They can be harvested from intraoral or extra oral sites and the nature of the block grafts can be of endochondral or intramembranous origin. For instance, intramembranous block grafts from intraoral sites and the calvarium have less amount of bone resorption and the process of bone remodeling or "creeping substitution" is longer (Burchardt 1983), comparing to endochondral bones (e.g. tibia, iliac crest) (Zins & Whitaker 1983). Intraoral block grafts

Key words: alveolar ridge augmentation, bone, dental implant, grafting, maxillary ridge augmentation

Abstract

Purpose: The main purpose of the present clinical study was to compare architectural metric parameters using micro-computed tomography (μ-CT) between sites grafted with blocks harvested from the intramembranous origin calvarium and endochondral origin iliac crest for horizontal bone augmentation in the maxilla. The second aim was to compare primary stability of implants placed in both types of block grafts.

Material and Methods: Nine consecutive healthy partially edentulous patients requiring extensive horizontal bone reconstruction in the maxilla were included in this study from July 2011 to March 2012. A total of 14 block grafts (seven each from the calvarium and iliac crest) were studied. After 6–7 months of the bone regeneration surgery, 43 implants were placed. Twenty-four implants (55.2%) were placed on calvaria (group 1) and 19 (44.8%) on iliac crest (group 2). All implants were clinically stable. A resonance frequency analysis (RFA) and μ-CT analysis were performed. Furthermore, two randomly biopsies were selected for histomorphometric analysis.

Results: Micro-CT analyses evidenced completely different parametric values between intramembranous and endochondral extra oral bone block grafts, being the group 1 higher in density and in % of bone volume. However, these parametric values cannot be considered statistically different due to the sample size, excepting the trabecular thickness, which is statistically higher for group 1 (P = 0.06).

Conclusion: Calvarial bone blocks for horizontal maxillary augmentation provided a higher degree of bone volume and density than the iliac crest bone grafts. Nonetheless, both grafts provide implant with the same primary stability, as assessed by RFA.

Maxillary bone resorption following tooth extraction or due to pneumatization of maxillary sinus often results in inadequate residual ridges for ideal implant placement (Pietrokowski & Massler 1967). To provide enough ridge height and width for proper 3-dimensional (3D) implant placement, bone augmentation procedures have been proposed. While procedures such as ridge splitting and guided bone regeneration (Hammerle et al. 2002) have been applied for horizontal ridge augmentation, autogenous block grafting is still considered the main method for extensive reconstruction of the maxilla (Tessier et al. 2005).
Materials and methods

Nine consecutive healthy partially edentulous patients requiring extensive horizontal bone reconstruction in the maxilla were recruited for this study from July 2011 to March 2012. A total of 14 onlay block grafts were placed, which were harvested either from the calvarial (group 1) or the iliac crest (group 2) (Table 1), depending of patient's preference. The present study was independently reviewed and approved by the local ethical committee of the University Hospital Infanta Cristina (Badajoz, Spain). Written consent of each subject was signed prior to the treatment.

**Harvesting procedures of the iliac crest block graft**

Under general anesthesia with local anesthesia, an incision is performed in the anterior iliac crest. A rectangular-shaped bone block was marked with a fissure bur and harvested using a saw blade and then harvested using a chisel very gently. The amount of bone harvested was according to patient's needs.

**Harvesting procedures of the calvarial block graft**

Under general anesthesia with local anesthesia, an incision is performed in the parietal area, parallel to the cranial major axis. A rectangular-shape bone block was marked with a fissure bur and harvested using a chisel very gently. The amount of bone harvested was according to patient's needs.

**Recipient site preparation and delayed implant placement**

At the recipient site, a mid-crestal incision was performed with intra-sulcular and vertical releasing incisions, after which a full-thickness flap was reflected. The block graft, either from the calvarium or from iliac crest, was adapted to the recipient sites and anchored to the residual ridge by one or two 1.5-mm-diameter titanium fixation screws (Level One 1.5 Neuro, KLS Martin LP, Jacksonville, FL, USA). After achieving stability of the graft, sharp edges were smoothed using a fissure bur. A bone substitute of bovine origin (Bio-Oss, GeistlichPharma AG, Wolhusen, Switzerland) was packed around the graft to fill any voids. Then, a collagen absorbable membrane (Bio-Gide, GeistlichPharma AG) was placed over the graft. Finally, the facial flap was scored to ensure a tension-free closure, and the flaps were sutured with both resorbable and non-resorbable sutures (Cytoplast™Suture, OsteogenesisBiomedical Inc, Lubbock, TX, USA). After 6–7 months of healing (mean 6.16 ± 1.2 months) (Fig. 1), implants were placed according to the initial treatment plan. The implant insertion torque ranged from 35 to 45 N/cm. In addition, all implants had more than 50% ridge width that was supported by the grafted bone block.

**Resonance frequency analyses**

A RFA device (Ostell™ Mentor, Integration Diagnostics AB, Göteborg, Sweden) was used following the manufacturer's recommendations for the measurement of primary implant stability. Basically, a designated metal rod (Smartpeg, Integration Diagnostics AB) was screwed into the implant screw.
vent. Then, a probe was placed close to the rod at the mid-facial side and the buccal side of the implant. The ISQ was generated and recorded for both sides. The two measurements were averaged to represent the primary stability of each implant.

**Micro-CT analysis**
Cylindrical bone core biopsies were obtained by a 2-mm-inner-diameter trephine bur from where the implants were planned to be placed. Then, bone biopsies were preserved at 20°C. They were scanned with the high-resolution μ-CT SkyScan 1172 in 100 voltage and 100 micro-amperage (Fig. 2). The exposure time was 450 ms. Images were reconstructed by the software (Nrecon; SkyScan NV, Aartselaar, Belgium) that used the modified algorithm described by Feldkamp et al. (1989) (Feldkamp et al. 1989) to obtain the axial sections of the specimen. The analyzed morphometric variables includes the following: [1] percentage of bone volume (BV/TV), [2] relation surface/bone volume (BS/BV), [3] Density of bone surface (BS/TV), [4] trabecular thickness (Tb.Th), [5] trabecular separation (Tb.Sp), [6] trabecular number (Tb.N), [7] trabecular pattern factor or inverse connectivity (Tb.Pf), which is an inverse connectivity index: the higher it is the trabecules are less connected, [8] structure model index (SMI), which gives information about preponderance of trabecular morphology (0 is an ideal plate, whereas 3 is an ideal cylinder), and [9] degree of anisotropy (DA), which is the presence or absence of aligned trabecules in a particular direction (1 is considered isotropic, >1 is considered anisotropic).

**Table 2. Biopsy distribution of the onlay block grafts included in the study**

<table>
<thead>
<tr>
<th>Biopsy no.</th>
<th>Donor site</th>
<th>Age</th>
<th>Sex</th>
<th>Initial ridge thickness at the level of the crest (mm)</th>
<th>Recipient site</th>
<th>Implants (N)</th>
<th>Mean ISQ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Calvarium</td>
<td>22</td>
<td>M</td>
<td>2.3</td>
<td>Anterior</td>
<td>2</td>
<td>73.5</td>
</tr>
<tr>
<td>2</td>
<td>Calvarium</td>
<td>22</td>
<td>M</td>
<td>2</td>
<td>Anterior</td>
<td>2</td>
<td>76</td>
</tr>
<tr>
<td>3</td>
<td>Calvarium</td>
<td>49</td>
<td>F</td>
<td>1.8</td>
<td>Anterior/posterior</td>
<td>4</td>
<td>70.2</td>
</tr>
<tr>
<td>4</td>
<td>Calvarium</td>
<td>49</td>
<td>F</td>
<td>2</td>
<td>Anterior/posterior</td>
<td>4</td>
<td>65</td>
</tr>
<tr>
<td>5</td>
<td>Calvarium</td>
<td>56</td>
<td>F</td>
<td>2.4</td>
<td>Anterior/posterior</td>
<td>4</td>
<td>70.5</td>
</tr>
<tr>
<td>6</td>
<td>Calvarium</td>
<td>56</td>
<td>F</td>
<td>1.9</td>
<td>Anterior/posterior</td>
<td>4</td>
<td>70</td>
</tr>
<tr>
<td>7</td>
<td>Calvarium</td>
<td>38</td>
<td>F</td>
<td>2</td>
<td>Anterior/posterior</td>
<td>4</td>
<td>73</td>
</tr>
<tr>
<td>8</td>
<td>Iliac crest</td>
<td>56</td>
<td>F</td>
<td>2.2</td>
<td>Posterior</td>
<td>4</td>
<td>65.5</td>
</tr>
<tr>
<td>9</td>
<td>Iliac crest</td>
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<td>F</td>
<td>1.7</td>
<td>Posterior</td>
<td>4</td>
<td>76</td>
</tr>
<tr>
<td>10</td>
<td>Iliac crest</td>
<td>44</td>
<td>F</td>
<td>2.2</td>
<td>Posterior</td>
<td>2</td>
<td>67</td>
</tr>
<tr>
<td>11</td>
<td>Iliac crest</td>
<td>60</td>
<td>F</td>
<td>1.6</td>
<td>Anterior/posterior</td>
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<td>73.3</td>
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<td>12</td>
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<td>55</td>
<td>M</td>
<td>2.3</td>
<td>Anterior</td>
<td>2</td>
<td>62</td>
</tr>
<tr>
<td>13</td>
<td>Iliac crest</td>
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<td>M</td>
<td>1.5</td>
<td>Posterior</td>
<td>2</td>
<td>74.5</td>
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<td>14</td>
<td>Iliac crest</td>
<td>47</td>
<td>M</td>
<td>2.1</td>
<td>Anterior/posterior</td>
<td>2</td>
<td>78.5</td>
</tr>
</tbody>
</table>

ISQ, implant stability quotient.

**Fig. 3.** Plot for implant stability quotient (ISQ) values for the calvaria and iliac crest group.

**Table 3. Micro-CT analysis and statistical differences of the biopsies obtained.** Units are patients and for each one, mean values are considered.

<table>
<thead>
<tr>
<th>Type of graft</th>
<th>Mean BV/TV</th>
<th>Mean BS_BV</th>
<th>Mean BS_TV</th>
<th>Mean Tb.Th</th>
<th>Mean Tb.Sp</th>
<th>Mean Tb.N</th>
<th>Mean Tb.Pf</th>
<th>Mean SMI</th>
<th>Mean DA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calvaria</td>
<td>50.44</td>
<td>18.00</td>
<td>11.91</td>
<td>0.25</td>
<td>0.26</td>
<td>2.14</td>
<td>0.18</td>
<td>0.18</td>
<td>3.05</td>
</tr>
<tr>
<td>Iliac crest</td>
<td>35.75</td>
<td>20.68</td>
<td>7.39</td>
<td>0.16</td>
<td>0.30</td>
<td>2.20</td>
<td>1.19</td>
<td>1.19</td>
<td>3.03</td>
</tr>
</tbody>
</table>

ISQ, implant stability quotient.

* Mann–Whitney test for patients (mean values).
† Two-way ANOVA for original values.

**Histomorphometric analysis**
Two biopsies, one for each group, were randomly selected. The mineralized biopsies were progressively dehydrated in ethanol 60% for 2 h, ethanol 80% for 4 h, ethanol 90% for 12 h, and ethanol 100% for 24 h. After this, bone biopsies were introduced in a solution of acetone 50% and ethanol 98% for 6 h, and later in xylene 100% for 2 h.
Finally, the specimens were drained and dried in an incubator at 60°C for 25 h. Afterwards, specimens were sectioned along their long axis to 50 μm. The slides including the middle portion of the biopsies were used for the analyses. In each group, one slide was prepared for low-vacuum surface scanning electron microscopy (SEM) (JEOL JSM-5500LV, Akishima City, Tokyo, Japan) and the other for optic microscopic scans (OM). The slides were stained with toluidine blue before the optic microscopic analyses with 20× and 25× magnifications.

Slide preparations and scans of SEM followed standard protocols. Microphotographs of the entire specimens were obtained with a digital microphotograph system (Nikon Kodak Ltd, Rochester, NY, USA) and analyzed with a software package [Image-Pro AMS 5.1®, Media Cybernetics®, Inc., Bethesda, MD, USA and Adobe 7 portable®, Adobe Systems Incorporated; San José, CA, USA]. The software was used to define the areas of interest and to calculate the percentage of bone tissues [% bone] and Bio-Oss [% Bio-Oss].

Statistics
Statistical package [SPSS 15.0; SPSS Inc., Chicago, IL, USA, STATISTICA version 7.1; StatSoft, Inc, Tulsa, OK, USA and R 2.14.0] were used to analyze the data. Descriptive statistical analysis for continuous and categorical variables was performed. In the case of variable ISQ, a two-way ANOVA for a mixed nested model (being type of graft the main effect and patient the random secondary one) was performed. For the rest of variables, we obtained for each subject the mean of its values and then applied the Mann–Whitney test to compare both groups of patients. P value < 0.05 was considered statistically significant.

Results
The mean age of the patients was 47.5 years old, with a 1:1 male/female distribution. A total of 14 block grafts (seven each from the calvarium and iliac crest) were placed on extremely reabsorbed ridges (<2.5 mm at the level of the crest). After 6–7 months of the bone regeneration surgery, 43 implants were placed. Twenty-four implants (55.8%) were placed on calvaria and 19 (44.2%) on iliac crest (Table 2). All implants were clinically stable.

RFA between both groups
The mean ISQ value was 70.20 ± 4.67 and 71.61 ± 6.10 for groups 1 and 2, respectively (Fig. 3). The minimum ISQ value was 68.5 and 62, while the maximum value was 79 and 78.5 for groups 1 and group 2, respectively. Hence, no statistical significant difference was observed between both groups according to two-way ANOVA (P = 0.714). In this study, patient effect turned out to be the only source of variability (P = 0.003).
Micro-CT results

Table 3 lists the mean, standard deviation and median values for the average values of all the subjects in the study for every measured parameter, distinguishing between types of graft, as well as the mean of original data for ISQ. It was evidenced different parametric values between intramembranous and endochondral extra oral bone block grafts, being the group 1 higher in density and in % of bone volume (Fig. 4). It was also observed a greater variability between the biopsies included in group 1. However, these parametric values cannot be considered statistically different due to the small sample size, excepting the trabecular thickness, which is slightly statistically higher for group 1 (P = 0.06).

Histomorphometric evaluation

Under SEM, sample #7 (Group 1) showed 33.6% bone tissues and 6.2% Bio-Oss, whereas sample #8 (Group 2) had 24.4% bone and 4.9% Bio-Oss (Fig. 5). Under optical microscopy, sample #7 (Group 1) revealed 32.6% of bone and 3.9% of Bio-Oss and sample #8 (Group 2), 20.5% of bone and 5.7% of Bio-Oss (Fig. 6).

Discussion

This study showed completely different parametric values between intramembranous and endochondral extra oral bone block grafts, as evidenced by μ-CT. It is also observed a greater variability between the biopsies included in group 1. However, due to the sample size, eight of nine parametric values analyzed were not found to be significant between both groups. On the contrary, trabecular thickness is clearly statistically favored toward calvaria group 1 (P = 0.06). Moreover, while cancellous block grafts (i.e., iliac crest) undergo “creeping substitution,” which new bone is deposited first, followed by resorption of necrotic bone, cortical block grafts (i.e., calvarial) undergo “creeping substitution,” whereas bone resorption precedes bone apposition (Burchardt 1983). Therefore, it is understandable that, even not being statistically difference, the parametric values analyzed indicate a denser microstructure and higher% of bone favored for group 1 due to its intramembranous origin. Nevertheless, precautions must be exercised when interpreting our results due to the small sample size and the lack of randomization. Otherwise, it might be appreciate statistically significant differences between both groups.

Primary stability is represented by the lack of mobility immediately after implant placement (Albrektsson & Zarb 1993). To assess it, many methods were proposed. However, most of them are no longer available due to their invasiveness and proved inaccurate (Huang et al. 2002). On the other hand, RFA is so far remained as a valid method to assess osseointegration (Huang et al. 2002). Primary stability is related to the percentage of bone-to-implant contact and the bone density around the implant (Meredith 1998). Interestingly, the present study showed no difference between the ISQ values of both groups. This result suggests that even group 1 had higher bone microstructure and density but ISQ value resulted to be similar to the group 2. This implies that having higher bone density does not play role for ISQ values once the block graft is integrated into the maxilla. Additionally, all implants in both groups achieved primary stability, suggesting that even partially supported by native bone, both grafts provide high mechanical strength for achieving implant stability.

Since clinical performances of both studied block grafts are similar, the choice between the two is dependent on other factors. Contrary to the use of intraoral block grafts, iliac crest and calvarial bone blocks provide a much large amount of bone for horizontal augmentation. Even though for extremely large defect, iliac crest is greater in thickness and thus it might be harvested a greater amount of bone. However, early bone resorption rates are considerably different (Mertens et al. 2012). Findings of previous studies showed a higher 3D stability of the calvaria (Turrriaga & Ruiz 2004). Chiapasco et al. found a minimal resorption of the calvaria ranging from 0 to 15% while for the iliac crest was reported a major resorption ranging from 12 to 60% (Chiapasco et al. 2006). This may be explained due to the greater thickness of the cortical bone layer of the calvarial. Hence, from the restorative standpoint, it will be more predictable over the time the use of calvarial bone blocks. On the other hand, iliac crest harvesting can be set-up by two-team approach which will reduce the surgery time (Mertens et al. 2012). The present study showed that both grafts provide enough amount of bone for proper implant placement. In addition, there was minimal to no complications noted if the procedures were properly executed.

As regard to the accuracy μ-CT, it was reported that the mean difference in density between μ-CT and histomorphometric analysis is 2.5% (Muller et al. 1998). Furthermore, the feasibility between the SEM and blue toluidine stains in detecting bone and surrounding tissue is comparable. However, SEM seems to be more precise for determining areas with high calcium concentration (Gonzalez-Garcia & Monje 2012). These histomorphometric
analyses were randomly selected and added to the study to confirm and evaluate the bone morphology. However, these results cannot be conclusive due to the small sample size analyzed. This study is not in concordance with the study developed by Ozaki and Buchman in which it was demonstrated by μ-CT and histomorphometric less density for endochondral origin bone (Ozaki & Buchman 1998). Nonetheless, in agreement with theirs, our study showed no statistically difference between both groups for bone volume fraction, higher trabecular thickness and a less organized internal structure. Nevertheless, it is noteworthy that there is a trend towards calvaria bone blocks regarding the % of bone volume.

Conclusion

Based upon the μCT evaluation, the calvarial bone blocks, for horizontal maxillary augmentation, provided a higher degree of total bone volume and density than the iliac crest bone grafts. However, due to the small sample size, the results cannot be considered conclusive. Nonetheless, both grafts and correspondent native bone provide implant with the same primary stability, as assessed by RFA.

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References


