Histomorphometric comparison of maxillary pristine bone and composite bone graft biopsies obtained after sinus augmentation

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Abstract

Introduction: Sinus grafting is a technique oriented to facilitate implant placement in posterior atrophic maxillae. Several modifications of the original technique and a wide variety of materials have been proposed; most of them associated with implant survival rates. However, the quality of the bone obtained after the application of certain grafting materials has not been fully elucidated yet. The aims of this multicenter study were to analyse histomorphometrical samples obtained 6 months after sinus grafting using a composite graft consisting of anorganic bovine bone (ABB) + autologous bone (AB), and to compare these samples with maxillary pristine bone biopsies.

Material and methods: Ninety maxillary sinus augmentations were performed for delayed implant placement (N = 90) in 45 consecutive patients (test group). Bone cores were harvested 6 months after grafting for histomorphometric and ultrastructural study. Control pristine bone biopsies were taken from the posterior maxilla of 10 patients (control). Bone radiographic changes were assessed up to 24 months after implant loading.

Results: The total mean values after analysis of test cores revealed a proportion of 46.08 ± 16.6% of vital bone, 42.27 ± 15.1% of non-mineralized connective tissue, and 37.02 ± 25.1% of the remaining ABB particles. Significant bone remodeling activities were noticed in sinus grafting samples when compared with pristine bone. A statistically significant difference was observed in the number of osteoid lines between two groups, with higher values in the test one (15.1 ± 11.48% vs. 2.5 ± 2.2%, P = 0.0005).

Ultrastructural study showed that vital trabecular bone was in intimal contact with ABB particles. Radiographic analysis revealed that the higher the proportion of remaining ABB, the lower the total vertical resorption of the graft.

Conclusion: Sinus grafting constitutes an excellent model for the study of de novo bone formation patterns and graft consolidation, when a combination of different bone substitutes is applied. The combination of ABB + AB yields highly satisfactory outcomes from both a clinical and a histologic perspective.

Bone availability is the key for successful implant placement (Block & Kent 1997). Implant primary stability is often challenging in the posterior segments of the maxilla. This is because of the poor osseous density typically found in this region, insufficient bone availability mainly due to sinus pneumatization, and alveolar bone atrophy as a consequence of tooth loss (Yildirim et al. 2000). Although the use of short or tilted implants has been shown to be a suitable therapeutic option to overcome these obstacles (Aparicio et al. 2001; Morand & Irinakis 2007), in cases of...
anatomical limitations and consideration of prosthesis long-term biomechanical stability, sinus grafting has emerged as a good option for implant site development to facilitate implant placement.

Since the early introduction of sinus grafting in implant dentistry (Boyne & James 1980), several modifications of this technique have been proposed in the literature throughout the last two decades (Tatum 1986; Summers 1998). Bone grafting material selection is one of the differential factors that may play a significant role in sinus grafting outcomes. The search for an ideal bone grafting material that would enable clinicians to obtain the best mature bone formation in the shortest period of time, with minimal complications, is one of the most investigated topics in this field.

Anorganic bovine bone (ABB) is a biocompatible material that presents similar structure and physical properties as human cancellous bone. When ABB combines with autogenous bone, it may represent an excellent grafting material for maxillary sinus augmentation (Wallace & Froum 2003). Autologous bone (AB) provides cellularity and growth factors while ABB facilitates space maintenance and slows down the process of rapid bone remodeling of AB. This may be explained by ABB’s slow resorption rate and its osteoconductive properties (Schlegel et al. 2003).

A modified sinus grafting technique using a bone scraper to simultaneously access the Schneiderian’s membrane and collect a part of the bone that lines the lateral wall of the sinus cavity, to be later used as a grafting material in combination with ABB, has been previously proposed by our group (Galinus-Moreno et al. 2007).

Several authors have reported excellent clinical outcomes after using a combination of ABB and AB for sinus grafting (Yildirim et al. 2001; Hallman et al. 2002; Galindo-Moreno et al. 2008). In spite of this, little information is available regarding the biologic response after performing sinus grafting using a combination of xenogenic material (ABB) and autogenous bone (AB). Therefore, the main aim of the present study was to analyze, morphologically and histomorphometrically, samples obtained 6 months after sinus grafting of grafted sinuses of human subjects using a composite graft consisting of ABB + AB, and to find out the differences with maxillary pristine bone.

### Material and methods

#### Study design

In this multicenter, prospective, controlled clinical study, 45 patients (28 males and 17 females) in need of a bilateral sinus grafting procedure to allow replacement of missing posterior maxillary teeth with implant-supported restorations were recruited for the study after informed consent. Information regarding medical and dental history was recorded following a questionnaire. To be included in the study, patients had to be adults between 18 and 85 years of age, present a physical status according to the American Society of Anesthesiologists (ASA) of I or II, have neither an uncontrolled systemic disease nor a condition known to alter bone metabolism (i.e. osteoporosis, diabetes mellitus etc., adequate oral hygiene (O’Leary plaque score ≤ 20%), and have <5 mm bone height remaining after radiographic measurement over panoramic films (Zinner & Small 2004). Subjects who had taken antibiotics in the previous 3 months, or reported long-term use (>6 months) of medications known to modify bone metabolism (such as bisphosphonates, corticosteroids etc.), women who were pregnant or attempting to get pregnant at the time of the screening, and patients who presented existing sinus conditions, sepsis, a history of cancer and/or radiation to the oral cavity, or complications derived from any of these conditions affecting the sinus area were excluded from the study.

A total of 90 grafting procedures were conducted. Patients underwent a delayed implant placement approach. Additionally, 10 subjects (five males and five females) consented in order to harvest control biopsies. Ten pristine bone core biopsies were harvested from the posterior maxilla at the time of implant placement from sites where bone grafting was not necessary.

This study was conducted according to the principles of WHO Declaration of Helsinki (Schuklenk & Ashcroft 2000).

#### Surgical and restorative procedure

All patients took 875/125 mg of amoxicillin/clavulanic acid, every 8 h starting 1 day before the surgery. Clindamycin, 300 mg every 8 h, was prescribed to penicillin-allergic patients. This medication was maintained for 7 additional days. All surgical procedures were performed under local anesthesia (Ultracain®, Aventis Inc., Frankfurt, Germany). A modification of the conventional lateral wall approach was used to perform the sinus grafting in all patients. A bone scraper (Safe scraper™, Meta corp., Remigia, Italy) was used to harvest autologous cortical bone and to expose the Schneiderian membrane. After membrane elevation, all sinus cavities were grafted with AB in combination ABB (Bio-Oss® particle size 250–1000 μm – Geistlich Pharma AG, Wohlen, Switzerland), mixed in a 1:1 volume ratio. After bone grafting, an absorbable collagen membrane (Bio-Gide® – Geistlich Pharma AG) was placed over the window to minimize soft tissue invasion. Primary closure was achieved in all cases, suturing with 3–0 silk suture (Laboratorio Aragó, Barcelona, Spain).

After a 6-month healing period, a 3-mm internal diameter, 4-mm external diameter trephine was used to collect bone core biopsies from the sites where implants had to be placed, according to the restorative plan. Hence, the initial osteotomy was performed using a trephine, and not with the conventional drills of each implant system. According to the study protocol, just one bone core per sinus was analyzed for conventional histological and histomorphometrical study. The implant placed in the ideal position of the maxillary first molar was the preferred site for bone sample collection. Other cores were taken from different implant positions for ultrastructural analysis. A total of 90 implants represented the group that was followed up: 52 Microdent® (Microdent Implant System, Barcelona, Spain) and 38 Astra Tech (Osseospeed™, Astra Tech, Mömlad, Sweden) with a range 13–16 mm of length and 4.2–5 mm of width. A staged approach was performed in all cases; therefore, 6 months after implant placement, a second-stage surgery was performed. After soft tissue healing, impressions were taken. The final screw-retained implant-supported PFM crowns were delivered – approximately 2 weeks later – and an occlusal adjustment was performed. After implant loading, all patients were placed in a 3-month maintenance program.

#### Radiographic variables

Standardized digital panoramic films of each patient were obtained before and immediately after sinus grafting surgery,
before implant insertion, at the time of
prosthesis delivery, and 24 months after
functional loading [Kodak ACR-2000,
Eastman Kodak Company, Rochester,
NY, USA]. Total bone height, at the point
of shortest remaining alveolar bone pre-
operatively, as well as the maximum ver-
tical augmentation after grafting and on
the day of implant placement was measured
by the same examiner using a specific soft-
ware [Digident Dent-A-View Version 1.0,
DigiDent, DIT, Nesher, Israel].

Histological study
Bone core biopsies were fixed in 10%buf-
ered formalin for 24 h. They were
subsequently decalcified in a decalcifier contain-
ing formaldehyde (10% w/v), formic acid
(8% w/v), and methanol (1% w/v) [Surgipath1
Europe Ltd., Peterborough, UK] for at least 8 days, and then embedded in paraffin.
Sections were dewaxed, hydrated, and 4 μm
sections following the central axis of the
biopsies were obtained and stained with
Hematoxylin–Eosin, periodic acid Schiff’s,
Masson’s trichrome, and Goldner’s tri-
chrome techniques. A millimeter scale in
the eyepiece of a microscope BH2 (Olympus
Optical Company, Ltd., Tokyo, Japan) with
a × 40 objective was used to count the
osteoblasts, osteoclasts, and osteocytes per
mm². The results were expressed as the
number of positive cells per mm².

Bone histomorphometry was performed
semi-automatically on Masson trichrom-
stained sections, assessing 10 randomized
images with ×10 objective, using a micro-
scope equipped with a digital camera DP70
(Olympus Optical Company, Ltd., Tokyo, Japan) with
a × 40 objective was used to count the
osteoblasts, osteoclasts, and osteocytes per
mm². The results were expressed as the
number of positive cells per mm².

Electron microscopy study
Several 1 mm³ fragments, obtained at three
different levels of each sample [coronal,
medial, and apical], including trabecular
and cortical bone, were fixed in 2.5% glu-
taraldehyde solution for 24 h and subse-
quently decalcified for 8 days [Decalcifier
1°, Surgipath1 Europe Ltd., Peterborough,
UK]. Samples were then postfixed in 1% OsO₄
at 4°C for 2 h, washed in distilled
water, dehydrated in increasing concentra-
tions of acetone, and embedded in Epon.
Semithin sections were stained with tolu-
dine blue solution. Ten blocks of LC cluster
areas were sampled. Ultrathin (about 70 nm
thick) sections were obtained using an ultra-
microtome [Reichert-Jung ULTRACUT E,
Leica Microsystems, Barcelona, Spain] and
stained with lead citrate and uranyl acetate.

Statistical analysis
A multilevel model technique with the
Box & Cox transformation was carried
out and a Skewness/Kurtosis test was used
to determine the normality of the variables.
Because of the high values of intraclass
correlation coefficient and non-normal dis-
tribution of variables, we decided to use
each patient separately as the unit analysis.
This was chosen in order to eliminate
intrapatient variable high resemblance; hence,
we calculated the average value of each variable taken of each patient (con-
sidering both sinuses). This required to
redifine a new statistical sample where the ‘n’ value was 45, which finally ac-
counted for non-related samples from 45
different patients to determine the statisti-
significance of the findings. As a con-
sequence, Welch’s t-test for values with
different variance and Pearson’s correlation
coefficient and non-normal dist-
tribution of variables, we decided to use
each patient separately as the unit analysis.

Morphologic and histomorphometric
analysis
Analysis of maxillary pristine bone biop-
sies [Fig. 1a] revealed the presence of
mineralized tissue, including mainly trabec-
ular bone, and bone marrow. The mean
values after morphometric analysis showed
a predominance of bone marrow with adi-
ocytes (51.2 ± 8.1%), while mineralized
tissue was found in a lower (45.7 ± 7.9%),
but similar proportion (Table 1). A scant
number of osteoid lines were observed in
association with mineralized structures.
The number of osteoblasts, osteoclasts,
and osteocytes per squared millimeter is
also shown in Table 1.

Woven and lamellar type of trabecular
bone, in the presence of ABB particles, was
observed in samples obtained from patients
who underwent sinus augmentation, after 6
months of healing (Fig. 1b). At this time,
biores from sinus floor elevation showed
trabecular bone in a wide range of propor-
tion (from 29.5% to 75%), with normally ap-
pearing bone marrow and ABB particles in
and between the trabecular bone, with an
appearance of a ‘terrazzo floor’, especially in
the apical portion of the sample (Fig. 2).

The total mean values after image analy-
sis revealed a proportion of 46.1 ± 16.6%
of vital bone, 42.3 ± 15.1% of non-miner-
alized tissue, and 37 ± 25.1% of remaining
ABB particles. Significant bone remodeling
activities were noticed in sinus grafting
samples as compared with pristine bone.
For example, a statistically significant dif-
derence in the number of osteoid lines was
observed between the two groups, with
higher values in the test group (15.1 ±
11.48% vs. 2.5 ± 2.2%, P = 0.0005) (Table
1). No acute or chronic inflammatory cell

Results

Patients
Patients’ age ranged from 35 to 72 years
(mean 50.4 years) in the test group. On the
other hand, control patients’ ages ranged
from 38 to 61 years (mean 48.3 years).
Only one perforation of the Schneiderian
membrane after elevation and no other
complications related to the grafting tech-
nique was observed over the study period.
At the time of harvesting the bone core
biopsy, all cores presented similar dimen-
sions (approximately 14 mm). All patients
completed the study.

Radiographic parameters
The most significant finding regarding
radiographic changes was that more bone
resorption was observed in patients whose
biopsies showed a lower proportion of ABB particles (<30% ABB: 0.42 ± 0.13 mm vs.
≥ 30% ABB: 0.31 ± 0.1 mm, P = 0.0005) (Welch’s test), and a higher proportion of
non-mineralized tissue (nMT) (<40%
MT: 0.22 ± 0.11 mm vs. ≥ 40% nMT:
0.39 ± 0.14 mm, P = 0.01 Welch’s test).
infiltrate or foreign body reactions were observed in any sample, other than a tendency from the osteoclasts to show activity around ABB particles (Fig. 3). Ultrastructural study showed that vital trabecular bone was in intimal contact with ABB particles. Also, cement lines between vital bone and ABB were observed (Fig. 4a), a finding that was previously observed under light microscopy as well (Fig. 4b).

Non-mineralized tissue appeared to be directly correlated with osteoblast number per mm² (\( p = 0.329, P = 0.043 \)). In fact, stratified data analysis of samples depending on the proportion of non-mineralized tissue showed that there was a significant difference in osteoblasts/mm² when the proportion of interposed soft tissue was higher than 40% \([ \geq 40\% \text{ mean } 344.9 \pm 284 \text{ vs. } <40\% \text{ mean } 88.7 \pm 59, P = 0.0008\) Welch’s test), but the number of osteoid lines was not significant \([21.25 \pm 8.64 \text{ vs. } 12.42 \pm 11.68, P = 0.99\) Welch’s test]. However, the remaining ABB particle proportion seemed to be important for osteoid formation \([r = 0.509, P = 0.001]\), as a significant increase of osteoid lines was observed when ABB proportion was higher than 30% \([19.32 \pm 9.01 \text{ vs. } 12.69 \pm 12.22, P = 0.012, \text{ Welch’s test}]\). Table 2 shows the Pearson’s correlation coefficients between morphometric variables.

**Discussion**

Primary stability is one of the key factors for successful osseointegration. It is widely

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**Table 1. Morphometric comparison between pristine maxillary bone and grafted bone core biopsies after sinus augmentation**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pristine bone ((N = 10))</th>
<th>Grafted bone ((N = 45))</th>
<th>( P )-values (Welch’s test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteoblasts (no./mm²)</td>
<td>247.31 ( \pm ) 251.6*</td>
<td>267.26 ( \pm ) 266.3</td>
<td>0.92</td>
</tr>
<tr>
<td>Osteoclasts (no./mm²)</td>
<td>48.38 ( \pm ) 72.13</td>
<td>151.1 ( \pm ) 192.74</td>
<td>0.51</td>
</tr>
<tr>
<td>Osteocytes (no./mm²)</td>
<td>1575.28 ( \pm ) 196.94</td>
<td>1042.66 ( \pm ) 807.96</td>
<td>0.0006</td>
</tr>
<tr>
<td>Osteoid lines (no.)</td>
<td>2.5 ( \pm ) 2.17</td>
<td>15.1 ( \pm ) 11.48</td>
<td>0.0005</td>
</tr>
<tr>
<td>Vital bone (%)</td>
<td>45.73 ( \pm ) 7.98</td>
<td>46.08 ( \pm ) 16.63</td>
<td>0.81</td>
</tr>
<tr>
<td>Anorganic bovine bone (%)</td>
<td>–</td>
<td>37.02 ( \pm ) 25.09</td>
<td>–</td>
</tr>
<tr>
<td>Non-mineralized connective tissue (%)</td>
<td>51.23 ( \pm ) 8.1</td>
<td>42.27 ( \pm ) 15.1</td>
<td>0.12</td>
</tr>
</tbody>
</table>

\*Values are expressed as mean \( \pm \) standard deviation.
Acknowledged that primary stability is highly associated with the proportion of mineralized tissue present in a particular location (Turkyilmaz et al. 2007; Turkyilmaz 2008). Therefore, a lack of bone height in the posterior maxilla often requires bone augmentation before implant placement. Sinus grafting is aimed at increasing the amount of available bone in the posterior segments of the maxilla, thus providing an adequate condition for osseointegration after implant placement. The aims of the present study were to evaluate the bone maturation status and histomorphometric features of samples obtained 6 months after sinus grafting, and to compare these parameters with a pristine bone control. Mineralized vital bone proportion in maxillary pristine bone varies depending on the age and gender, ranging from 23% to 28% as shown by previous studies (Trisi & Rao 1999; Ulm et al. 1999). In the present study, the mean proportion of mineralized vital bone was found to be $45.73 \pm 7.98\%$, which is significantly higher than the values reported by other authors. This difference may be explained by the low number of subjects analysed and the particular characteristics of our population. A high percentage of vital bone was noted in pristine bone sections, but it had less signs of bone remodeling when compared with sinus grafting samples.

Sinus grafting success may be evaluated by performing a histologic analysis to determine the features and the amount of newly formed bone that could potentially be in intimate contact with an implant fixture placed in the grafted area (Yildirim et al. 2000). Thus, it has been proposed that maximum osteogenesis of qualitatively high-grade living bone can be used as a way to assess the success outcome of sinus augmentation (Wallace et al. 1996). In a previous pilot study, our group found that the mean value for mineralized vital bone was approximately 31% (Galindo-Moreno et al. 2008). This percentage is in accordance with the results previously reported, which found a similar % of vital bone (ranged from 22.2% to 34.2%) in their samples (Yildirim et al. 2000; Fromm et al. 2008; Simunek et al. 2008). In contrast, the mean percentage of vital bone obtained in the present series is one of the highest reported so far for this particular combination of biomaterials (46.08%), closely followed by the results reported by Cordaro and colleagues (45.2%) and by Artzi and colleagues (41.6% after 9 months) (Artzi et al. 2008).

![Fig. 4. A narrow relationship between trabecular bone and ABB particles in the absence of gaps or fibrous interface: (a) ultrastructural image of a cement line between ABB particles and decalcified vital bone (TEM × 4600), (b) light microscopy (Masson trichrome × 100).](image-url)

<p>| Table 2. Statistical correlations between morphometric variables in grafted bone core biopsies after sinus augmentation |
|--------------------------------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>Osteoblasts (mm²)</th>
<th>Osteoclasts (mm²)</th>
<th>Osteocytes (mm²)</th>
<th>Vital bone (%)</th>
<th>Osteoid lines (no.)</th>
<th>ABB particles (%)</th>
<th>Connective Tissue (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteoblasts (mm²)</td>
<td>1*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteoclasts (mm²)</td>
<td>0.345 (0.033)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteocytes (mm²)</td>
<td>0.676 (0)</td>
<td>0.193 (0.245)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vital bone (%)</td>
<td>0.454 (0.004)</td>
<td>0.325 (0.046)</td>
<td>0.734 (0)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteoid lines (no.)</td>
<td>0.35 (0.03)</td>
<td>0.562 (0)</td>
<td>0.544 (0)</td>
<td>0.628 (0)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABB particles (%)</td>
<td>−0.247 (0.133)</td>
<td>0.296 (0.071)</td>
<td>−0.243 (0.141)</td>
<td>−0.192 (0.247)</td>
<td>0.509 (0.001)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Non-mineralized tissue (%)</td>
<td>0.329 (0.043)</td>
<td>−0.349 (0.031)</td>
<td>0.023 (0.889)</td>
<td>−0.076 (0.649)</td>
<td>−0.414 (0.009)</td>
<td>−0.613 (0)</td>
<td>1</td>
</tr>
</tbody>
</table>

*Pearson’s coefficient (r) (bilateral signification). ABB, anorganic bovine bone.
Cordaro et al. 2008). This may explain the previously reported higher success rates of implants inserted into grafted sinuses [Olson et al. 2000; Scarano et al. 2006].

Our group has advocated for a surgical approach in which cortical AB is obtained from the lateral wall of the sinus cavity using a bone scraper, and then combined with a xenogenic bone substitute [e.g., ABB] [Galindo-Moreno et al. 2007, Martin Diaz et al. 2007]. This approach not only allows to harvest AB to be used as a part of the grafting material, but also eliminates the need for a second surgical site to harvest autogenous bone. Despite AB being regarded as the gold standard in bone grafting material, it undergoes a rapid resorption when compared with other grafting materials. This resorption can be as much as 60% when it is used as a single biomaterial [Jensen & Sindet-Pedersen 1991]. In this sense, Schlegel and collaborators observed that AB may be resorbed up to 40% after 6 months of healing when used as a sinus grafting material. They also found a lower bone-to-implant contact for implants placed in areas where AB was used vs. ABB: 42% vs. 68%, respectively [Schlegel et al. 2003]. Hence, the combination of xenogeneic and autologous bone may provide some benefits when compared with the sole use of AB. These include unlimited ABB availability, allowing recolonization and apposition of osteogenic cells, and a slow resorption rate [Piattelli et al. 1999, Taylor et al. 2002]. Although it has not been fully elucidated what is the average amount of resorption in a determined time span, it has been shown that ABB particles may be present even 9 years after grafting [Traini et al. 2007]. Our results showed a mean percentage of remaining ABB particle of 37.01% after 6 months of healing. This supports the notion of a slow resorption rate. ABB is an adequate scaffold for dimensional graft maintenance, a concept that is reinforced by the findings of this study, because the higher the proportion of ABB, the lower the total mean resorption of the graft. Stratified data analysis of ABB percentage present in our samples showed that, above 30% of the remaining xenogeneic particle, graft resorption was significantly lower (0.21 ± 0.1 mm vs. 0.42 ± 0.13 mm, P = 0.0006, Welch’s test). We assume that a slow degradation of the material occurs and not a complete replacement by AB in < 5 months after grafting, as reported by Tadjoedin et al. (2003). In addition, the composite grafting material proposed here promotes an early osteogenesis/osteoinduction elicited by the cells and growth factors of the AB, which may in turn allow for a more efficient implant osseointegration [Tadjoedin et al. 2003].

Newly formed bone showed slightly more osteoblasts (mean #/mm² = 267.3), more osteoid lines [P < 0.46 Welch’s test], significantly more osteoclasts (mean #/mm² = 151.1), and a higher proportion of osteocytes (four times higher than osteoblasts), but less than mature pristine bone [P < 0.0006, Welch’s test]. A direct, statistically significant correlation found between the number of osteoblasts and the quantity of non-mineralized tissue supports the observations of Zerbo et al. (2005), who showed that early differentiation towards preosteoblastic undifferentiated cells may be mediated by the expression of Run × 2, sialoprotein, and osteopontin.

On the other hand, the remaining ABB particle proportion seemed to be important for osteoid formation [r = 0.509, P = 0.001], as a significant increase of osteoid lines was observed when the ABB proportion was >30% (19.32 ± 9.01 vs. 12.69 ± 12.22, P = 0.012, Welch’s test). This finding may be explained by the osteoconductive nature of ABB [Schlegel et al. 2003]. Ultrastructural analysis revealed that the remaining ABB particles were surrounded by newly formed mature bone, throughout all the samples. Cement lines could be clearly identified, in the absence of gaps or fibrous interfaces. In the light of the findings of this study, we can state that ABB shows a different behavior from other bone substituts, such as tricalcium phosphate graft materials: at 6 and 9 months in humans. Clinical Oral Implants Research 19: 886–892.

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