BIPHASIC HYDROXYAPATITE AND ß-TRICALCIUM PHOSPHATE BIOMATERIAL BEHAVIOR IN A CASE-SERIES OF MAXILLARY SINUS AUGMENTATION IN HUMANS

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Running title: Osteon™ II suitability as bone graft biomaterial in humans.

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Author Contribution

A.O. and G.M.A. conceived the ideas, conducted the surgeries and collected the samples; F.O. and N.M.M. evaluated the histological samples; M.P.M. and P.G.M. wrote the manuscript.

ABSTRACT

Objectives: The aim of this study was to evaluate and compare the morphometric components and the histological properties of pristine bone and bone grafted with a biphasic ß-tricalcium phosphate in humans using the maxillary sinus model. Reparative mesenchymal stem cells in the pristine bone and graft were also evaluated.

Material/methods: For this prospective case-series, sinus augmentation was performed using a biphasic ß-tricalcium phosphate. After 6 months of healing, a core of remnant native alveolar bone and grafted bone was collected with a trephine. Histological, histomorphometrical, and immunohistochemical techniques were performed. Radiological analysis through cone beam computerized tomography was also conducted.

Results: A total of 10 patients were enrolled in this study. Radiologically, patients showed an average increase of crestal bone of 8.03±1.72 mm. Morphologically, the grafted area was composed by 34.93±14.68 % of new mineralized tissue, 9.82±11.42 % of remnant biomaterial particles and 55.23±11.03 % non-mineralized tissue. Histologically, we found no differences in the number of osteocytes per mm^2 (p=0.674), osteoblasts (p=0.893) and blood vessels (p=0.894) in the grafted area compared to the pristine bone. Differences were found on the number of osteoclasts (15.57±27.50 vs. 5.37±16.12, p=0.027). The number of Musashi-1 positive mesenchymal cells (239.61 ± 177.4 vs. 42.11 ± 52.82, p=0.027) was also significantly higher in the grafted area than in the pristine bone.

Conclusion: Biphasic ß-tricalcium phosphate is a suitable biomaterial to be used in the formation of new bone in sinus floor elevation procedures in humans, not only from the histomorphometrical point of view, but also regarding the cellular and vascular quality of the regenerated bone.

INTRODUCTION

Bone regeneration is a daily procedure conducted in Dentistry. Many different families of biomaterials have been proposed to achieve the best results. These biomaterials claim to have a series of biological properties in order to induce an effective reparation of the treated bone defects. The final goal is to get *ad integrum* reparation of the tissues. Among all these biomaterials, autogenous bone has been preconized as the gold standard because this biomaterial exhibits all the regarded as ideal biological properties. However, the unique biological property shared by all biomaterials is osteoconduction (Albrektsson & Johansson 2001). In fact, osteoconduction is absolutely needed for bone apposition, and its role is key in the bone formation in adults. By knowing this, alloplastic biomaterials are being design accordingly and will play an important role in a close future. This family of biomaterials will become an ideal substitute of those other materials that come from living beings.

There are many types of alloplastic biomaterials. Traditionally, they have shown poorer conditions than other biomaterials, in terms of quicker resorption and poorer induction of biological responses, such as absence of osteogenesis and osteoinductivity and limited ostoconductivity. Among these synthetic biomaterials, biphasic calcium phosphates have been widely investigated, with promising results (Ebrahimi et al. 2017). These biomaterials consist of a combination of synthetic hydroxyapatite and ß-tricalcium phosphate (ß-TCP) at different ratios. The rationality for this combination is based in the rapid dissolution of the ß-TCP, to allow its substitution by newly formed bone, combined with the slow resorption of the hydroxyapatite, to maintain the volume of the grafted area.

Osteon™ II (Dentium, Seoul, South Korea) is an alloplastic biomaterial composed of a hydroxyapatite scaffold coated with ß-TCP. Regarding this biomaterial, some different concentrations of these two components are available in the market, showing some

biological differences. Osteon™ II contains a concentration of 70 % HA and 30 % β-TCP. It has a porous structure, which can accelerate new bone ingrowth and maturation (Cha et al. 2011). Among its properties, the particle size ranges between 0.5 and 1.0 mm, with pore size between 300 and 500 μm; its porosity is estimated about 70 % with macropore size of 250 μm. Its crystallinity is about 97 %, and a crystal size of approximately 0.043 μm (Kim et al. 2013). In some previous studies, Osteon has demonstrated to be a suitable material for sinus augmentation, based not only on histologic analysis (Kim et al. 2008), but also clinically (Bae et al. 2010). However, potentially interesting data, such as the analysis of the expression of particular markers, is not available.

Biological response to biomaterials is governed by several key markers. Musashi-1 (gene *MSI1*) is a RNA-binding protein that has been reported as a marker of mesenchymal stromal cells. It un-inhibits *Notch* signaling favoring cell proliferation and differentiation (Messerli et al. 2013). Our group has previously detected increased immunohistochemical expression of Musashi-1 in mesenchymal repair in bone healing (O'Valle et al. 2015) and maxillary sinus floor elevation (Galindo-Moreno et al. 2018; O'Valle et al. 2018).

Thus, the aims of this study were to analyze the clinical and radiographical behavior of this biphasic ß-TCP in humans, using the maxillary sinus model (Avila-Ortiz & Galindo-Moreno 2014), to evaluate the histological and morphometrical components, and to analyze the expression of markers of reparative mesenchymal stem cells. The histological data was then compared with the data obtained from pristine crestal bone within the same biopsy.

MATERIAL AND METHODS

Study population

This clinical study was approved by the Ethical Committee for Research in Humans of the Universidad Científica del Sur (Lima, Perú) (Code: 00015). All procedures were performed according to the principles of the Declaration of Helsinki and the results are presented according to the PROCESS guidelines (Preferred reporting of case series in surgery). A written informed consent was obtained from every patient before any study procedure was initiated.

Patients were selected from those referred to the Implant Department of UCSUR (Universidad Científica del Sur, Lima, Perú) to be subjected to a maxillary sinus floor elevation. All patients were partially or totally edentulous and needed a sinus floor elevation procedure for a future implant-supported prosthesis. The edentulous space distal to the canine had to have a residual bone height of less than 4 mm. To be included, patients had to be between 18 and 80 years old, non-smokers and mentally and physically healthy. In those patients where a bilateral sinus augmentation was required, only one was included in the current study.

The exclusion criteria were: subjects with medical problems (uncontrolled diabetes, heart diseases, osteoporosis, etc.), smokers, alcohol-consumer patients, pregnancy, patients with chronic or acute sinusitis, any kind of sinus pathology detectable in CBCT, uncontrolled periodontal disease and inadequate habit of dental hygiene, or the presence of any disease previously known to alter bone metabolism.

Surgical protocol

A lateral window technique to perform the maxillary sinus augmentation was performed by 2 experienced periodontists (AO and GMA). Prior to surgery, local infiltration of anesthesia was performed with mepivacaine hydrochloride (Scandonest 3 %, Septodont, Saint-Maur-des-Fossés, France). A supracrestal incision was done, with releasing incisions in the mesial and distal aspect of the proposed area to access the sinus and a

mucoperiostal flap was elevated. Then, a round drill of 8 mm in diameter was used to conduct the lateral access to the sinus cavity performing an oval shaped osteotomy, following the recommendations proposed by DASK technique (Dentium Advanced Sinus Kit, Dentium, South Korea) (Monje et al. 2016). The sinus membrane was elevated carefully to minimize its perforation; after elevation of the Schneridian´s membrane, a graft composed totally by Osteon II biomaterial ([HA 30 % + ßTCP 70 %], Dentium) was used to fill the created space. A collagen absorbable membrane (Dentium) was placed in the buccal aspect of the maxilla bone and stabilized with absorbable surgical coated vycryl 4/0 (Ethicon, Norderstedt, Germany). Postoperative medical instructions were given to all patients, and medication was prescribed. Patients were prescribed with antibiotic therapy (amoxicillin 875mg + clavulanic acid 125mg, 1 tablet every 8 hours for 7 days). Patients were also provided with anti-inflammatory medication (ibuprofen 600 mg, tablets as needed). Sutures were removed 14 days after the surgery.

Patients were followed-up for six months, in 6 to 8 weeks intervals. After six months of healing, implant placement surgery was performed. A full-thickness flap was elevated and, in the implant location according to the prosthetic treatment plan, a 2 mm internal / 3 mm external diameter trephine was used to harvest a bone core. This bone biopsy was used for the histological examination of the newly formed bone (FO and NMM).

Clinical data recorded

Gender, age, type of edentulism and history of periodontal disease were recorded for each patient. History of periodontal disease was determined by the assessment of clinical attachment loss (CAL) using a Michigan-O probe (Hu-Friedy, Chicago, IL, USA). Patients with no teeth were included as patients with previous history of periodontitis when the records or the patients themselves reported it. Partially edentulous patients who presented at least 3 sites in non-adjacent teeth exhibiting clinical attachment loss (CAL)

≥ 3mm (excluding third molars) were classified as part of the periodontitis group (Tonetti & Claffey 2005). Edentulism was considered in two categories, according to patients who presented at least one missing maxillary posterior tooth, excluding third molars, considered as partially edentulous, or if they have no teeth at all, then considered as complete edentulous.

All these data were collected in a standardized form by the same examiner (AO), during the screening appointment.

Radiographic evaluation

Cone beam computerized tomography (CBCT) scans (Planmeca Promax® 3D MID) were performed before the sinus floor elevation and 6 months later before implant placement. Remnant alveolar crest (RAC) and vertical bone height (VBH) before and after sinus floor elevation were measured by an experienced surgeon (MPM) with the Romexis Viewer (Planmeca Romexis, Finland). Measures were made by drawing a horizontal line at the crest and then moving it up to the lowest point of the sinus floor and to the maximum height achieved 6 months after grafting. Sinus floor elevation (SFE=VBH-RAC) was subsequently calculated for each patient.

Histopathological analysis

For conventional morphology, bone cores were immediately fixed in 10% buffered formalin during 48 h at room temperature. They were then decalcified during 24 h at 37ºC (Decalcifier I®, Surgipath Europe Ltd., Peterborough, United Kingdom), dehydrated, paraffin-embedded in an automatic tissue processor (Thermo Scientific Excelsior AS, Thermo Fisher Scientific, Waltham, Massachusetts, USA) and sectioned. Then, sections were deparaffinized in xylol (3 passes of 5 minutes) and re-hydrated in ethanol of decreasing gradation (absolute, 96%, and 70%, 2 passes of 3 min, respectively). Tissue

sections were stained with hematoxylin and eosin, and Masson's trichrome. The morphological study was done in blinded fashion on 4-micrometer sections with BX42 light microscopy (Olympus Optical Company, Ltd., Tokyo, Japan), by using the 40x objective in a microscope with an attached scale (BH2, Olympus Optical Company), the number of osteoblasts, osteoclasts, osteocytes and vessels were quantified per mm^2. Histomorphometrical quantification was performed semiautomatically using the Masson's trichrome stain. 10 random images were captured from each sample with a 10x objective in a microscope with a digital camera attached (DP70, Olympus Optical Company). Images were then analyzed with the software ImageJ (NIH, http://imagej.nih.gov/ij/) to quantify the percentage of new mineralized tissue, remnant biomaterial particles and non-mineralized tissue.

Immunohistochemical analysis

Rehydrated 4-micrometer sections were also termically treated in a pre-treatment module (Thermo Fisher Scientific Inc., Waltham, MA, USA) containing a 1mM EDTA buffer (pH8) at 95ºC for 20 minutes. Primary polyclonal antibody against Musashi-1 (MSI1) was then applied and incubated at 1:100 dilution for 1 h at room temperature. A non-immunospecific IgG was used as negative control. All antibodies were obtained from Master Diagnóstica (Granada, Spain). The immunostaining was developed in an automatic immunostainer (Autostainer480S, Thermo Fisher Scientific Inc.) using a peroxidase-conjugated micropolymer and diaminobenzidine (Master Diagnóstica).

Immunopositivity was then evaluated quantitatively in bone and non-mineralized tissue (osteocytes, osteoblasts, osteoclasts, mesenchymal cells, and fibroblasts) per mm^2.

Statistical analysis

IBM SPSS-Windows 20.0 (SPSS Inc., Chicago, IL) was used for the analyses. Results were recorded independently for the area of regenerated bone and for the pre-existing crestal native bone. Data are presented as mean±standard deviation for continuous variables and as percentage (frequency) for categorical data. Primary outcome variables were those related to the histomorphometrical analysis. Secondary outcome variables included the cellularity of the grafted and non-grafted areas, the expression of musashi-1 and the radiographical and clinical data. Non-parametric Wilcoxon test was used to compare morphological and histomorphometrical related variables. Results were considered statistically significance when p values were below 0.05.

RESULTS

Ten healthy patients (5 male, 5 female) with a mean age of 63.5 (min. 49 to max. 74). 50 % of the patients were partially edentulous, and 50 % were totally edentulous. All surgeries were conducted and evolved uneventfully.

The initial remnant alveolar crest (RAC) measured by CBCT showed an overall mean residual bone height of 3.02 ± 0.85 mm. The final mean vertical bone height (VBH) after 6 months of healing was 11.05 ± 1.35 mm. Thus, a mean SFE of 8.03 ± 1.72 mm was achieved.

Morphological study of native bone showed normal cortical and trabecular bone (area=46.01 ± 7.35%) with low remodeling activity. Medullary stroma (area=53.99 ± 7.35%) was mainly composed by adipocytes with few fibroblasts, sparse hematopoietic and lymphoid components (**Figure 1**). Grafted areas presented new mineralized tissue (area=34.93 ± 14.68%). The differences with the results obtained in pristine bone were not statistically significant. The presence of remnant biomaterial particles (area=9.82 ± 11.42%) was observed as latent image due to the tisular decalcification process. Non-

mineralized tissue (area=55.23 ± 11.03 %) was mainly constituted by an important number of fibroblastic-like cells, contained in a highly dense and highly vascularized non-mineralized tissue. Mature adipocytes in the medullary stroma were found in some areas (**Table 1**).

Histologically, compared to the pristine bone, although not statistically significant, the number of osteocytes per mm^2 was higher in the pristine bone (235.30 ± 99.77 vs. 134.67 ± 111.07, pristine vs. grafted, respectively; p=0.674, Wilcoxon test). However, the number of osteoblasts per mm^2 (9.83 ± 15.33 vs. 30.86 ± 31.04, pristine vs. grafted, respectively; p=0.893, Wilcoxon test) was higher but not statistically significant in the grafted bone than in the remnant pristine alveolar crest. The number of vessels per mm^2 was also higher in the grafted areas (25.62 ± 19.72 vs. 30.07 ± 28.21, pristine vs. grafted, respectively; p=0.894 Wilcoxon test). Interestingly, the number of osteoclasts per mm^2 (5.37 ± 16.12 vs. 15.57 ± 27.50, pristine vs. grafted, respectively; p=0.027, Wilcoxon test) was significantly higher in the grafted bone than in the remnant pristine alveolar crest, which reflects the remodelation process undergoing in response to the graft (**Table 2**).

Interestingly, the mesenchymal cells of the non-mineralized compartment of native bone showed weak nuclear expression of MSI1. In contrast, high nuclear intensity was detected in fibroblastic-like cells in biopsies from grafted sites (**Figure 2**). In fact, the number of MSI1 positive fibroblastic-like cells was significantly higher in the grafted area than in the pristine bone (239.61 ± 177.4 vs. 42.11 ± 52.82 cells/mm^2, grafted vs. pristine, respectively; p=0.027, Wilcoxon test). Moreover, the detection of MSI1 in osteocytes, osteoblasts and osteoclasts was also higher in biopsies obtained from grafted areas, although not statistically significant (**Table 3**).

DISCUSION

The aim of this study was to analyze the behavior of a biphasic tri-calcium phosphate in humans, using the maxillary sinus as a clinical model to study tissue regeneration. We have presented results from morphometrical analyses, as previous studies had also done (Kim et al. 2008; Bae et al. 2010; Cha et al. 2011). Additionally, histological data, regarding vascularity and cellularity, and immunohistological detection of the expression of important osteogenic markers, are also presented for the first time. These data have not been described previously in relation to this biomaterial nor in comparison with the pristine bone located in the same area of the maxilla.

In this study, after 6 months of graft consolidation, we observed 34.93 ± 14.68 % of mineralized tissue, 55.23 ± 11.03 % of non-mineralized tissue and 9.82 ± 11.42 % of remnant biomaterial in the grafted area. To our knowledge, this is the first histomorphometrical description of this alloplastic biomaterial in humans. Although there are some other studies with Osteon™ II in sinus augmentation in humans (Kim et al. 2008; Bae et al. 2010; Cha et al. 2011), those authors did not analyze the results from the histopathological point of view. Only Nevins and coworkers have reported a mean percentage of vital bone of 46.6 ± 5.5 % (that our group prefers to refer to as mineralized tissue (Galindo-Moreno et al. 2018)) and a mean percentage of remaining graft particles of 10.4 ± 2.0 %, without describing the percentage of non-mineralized tissue in their study. In addition, this study was conducted in dogs, with only 3 months of healing and conducted in defects created in inferior alveolar ridges (Nevins et al. 2013).

Although our findings are in accordance with the expectable effect of this biomaterial in this location, they are not in total concordance with other studies on biomaterials of the same family. Maxillary sinus augmentations with a biphasic bone substitute (Maxresorb®, Botiss Biomaterials, Berlin, Germany) showed an average of 36.16 ± 9.37

% of new bone matrix, 30.26 ± 11.7 % of residual biomaterial and 34.29 ± 18.32 % of non-mineralized tissue (Jelusic et al. 2017). Ohayon found, after grafting 10 sinuses with a biphasic calcium phosphate (Bone Ceramic, Straumann, Basel, Switzerland), that the mean composition of the samples harvested from the grafted areas was 26.1 ± 6.3 % of newly formed bone, 29.3 ± 9.1 % of remaining biomaterial particles, and 44.7 ± 7.7 % of non-mineralized tissue (Ohayon 2014). The same author described, in a posterior study, a histomorphometrical evaluation of 7 grafted sinuses with the same biomaterial showing similar mean values: 27.4 ± 4.6 % of new mineralized tissue, 26.9 ± 5.4 % of remaining biomaterial particles, and 45.7 ± 6.0 % of non-mineralized tissue (Ohayon et al. 2016). The differences observed between different biomaterials, even within the same family, can be explained by different reasons: 1. Manufactured with different procedures (Cao et al. 2008; Nevins et al. 2013; Gao et al. 2014; Monje et al. 2017); 2. Clinical characteristics of the patient habits and demographic variables, diseases such as periodontitis, can influence the final histological outcome of our studies. Galindo-Moreno and coworkers demonstrated that age, gender, alcohol and tobacco consumption or absence of teeth due to previous history of periodontitis can be associated to final quantity of mineralized tissue in the grafted area (Galindo-Moreno et al. 2012). 3. The surgical technique used to create the lateral window to access the maxillary sinus as well as the specific anatomic characteristics of the sinus (Avila et al. 2010; Monje et al. 2014; Velasco-Torres et al. 2016, 2017). In our study, all the lateral windows were created with a standardized drill, so all the lateral windows were similar in terms of size. Thus, the influence of that variable was minimized.

Although our histomorphometrical results differ slightly from those obtained using other biphasic alloplastic materials, they can be considered as normal in the biological context of maxillary sinus floor elevation. In the present study, we obtained 34.93 ± 14.68 % of

mineralized tissue and 55.23 ± 11.03 % of non-mineralized tissue. Among other factors, bone formation is function-dependent and genetic-dependent, keeping in mind that is the patient who forms bone and not the biomaterial. Accordingly, it is important to understand that the same biomaterial will promote the formation of different proportions of tissues in the same patient, according to the location where it is placed. Using histomorphometric analysis, the maxillary pristine bone, obtained by trephine, has shown, in other studies, mean values of bone marrow about 51.2 ± 8.1 % while mineralized tissue was found in a lower but similar proportion (45.7 ± 7.9 %) (Galindo-Moreno et al. 2010a). Lindhe and coworkers demonstrated similar proportion of lamellar bone in the posterior maxilla area, 47.4 ± 1.8 % (Lindhe et al. 2013). However, the use of biomaterials decreases partially this potential ratio of mineralized tissue, because of the persistence of the remnant biomaterial. To understand this idea, it is important to know the dynamics of the bone formation after grafting (Busenlechner et al. 2009). Other researchers using different biomaterials in the same location as the current study have reported similar proportion of new mineralized tissue. For instance, anorganic bovine bone (Bio-Oss®) has revealed similar percentage of mineralized tissue in several studies 34.88 ± 15.2 % (Galindo-Moreno et al. 2010b), or 34.50 ± 3.18 % (Galindo-Moreno et al. 2018). These results are not dependent on the ratio of the composite components and ratio of biomaterials. Mean proportion of vital bone was 36 ± 9.44 % for a ratio of 20 % of autologous bone and 80 % of xenograft while the mean proportion of vital bone for a ratio of 50 % of autologous bone and 50 % of xenograft was 37.38 ± 17.46 % (Galindo-Moreno et al. 2011). These data are similar to those reported by Hallman and coworkers, using the same biomaterial, with a quite similar percentage of new mineralized tissue, (39.9 ± 8 %) (ratio 20 % autologous: 80 % xenograft) (Hallman et al. 2002). Other biomaterials, such as allografts, show the same behavior. A recent study has shown a mean value of

39.54 ± 0.05 % of mineralized tissue for solvent dehydrated human allograft (Puros®) and 31.96 ± 0.08 % of mineralized tissue for freeze-dried human allograft (Minero-Oss®) (Monje et al. 2017). These data allow us to affirm that Osteon™ II can be considered as an adequate biomaterial to contribute to bone formation in humans.

Bone formation is correlated to the number and distribution of vessels in the non-mineralized tissue (Galindo-Moreno et al. 2010b). The presence of the proposed biphasic biomaterial promoted a microvascular density formation of around 30 vessels per mm^2, which is highly similar to the 25.6 vessels per mm^2 in the native bone. This vascular presence in the grafted area is similar to the reported for calcium phosphate (31.8 ± 1.9 vessel/mm^2) or hydroxyapatite (29.2 ± 3.0 vessel/mm^2) in other studies (Boëck-Neto et al. 2009).

The higher remodeling activity in the grafted area is also explained by the marked resorption of this biomaterial. The number of osteoclasts was significantly (p=0.027) higher in the grafted area (15.57 ± 27.50 cell/mm^2) compared to the native bone (5.37 ± 16.12 cell7mm^2). In perspective, Osteon™ II disappears quicker than other biphasic materials. Our mean remnant biomaterial is 9.82 ± 11.42 %, similar to the data presented by Nevins and coworkers in their study in dogs (10.4 ± 2.0 %) (Nevins et al. 2013). However, Jelusic and coworkers reported the presence of 32.66 ± 12.57 % of biphasic biomaterial in their samples after the same maturation time (Jelusic et al. 2017). Similarly, 26.9 ± 5.4 % and 29.3 ± 9.1 % of remaining biphasic calcium phosphate particles have been observed in similar studies (Ohayon 2014; Ohayon et al. 2016). More recently, Flichy-Fernández found the persistence of 22.0 ± 17.26 % of a similar biphasic calcium phosphate biomaterial (Flichy-Fernández et al. 2019).

In the described processes, the reparation and consolidation of the grafted area, mesenchymal stromal cells will play a fundamental role. Thus, in our study, we analyzed

the expression of some important markers of this aspect. We found a statistically significant higher number of MSI1 positive mesenchymal cells per mm^2 in the non-mineralized tissue of the grafted area than in the pristine area bone (239.61 ± 177.4 vs. 42.11 ± 52.82 cells/mm^2; p=0.027). MSI1 has been reported as a marker of mesenchymal stromal cells with neural differentiation potential (Messerli et al. 2013). O'Valle and coworkers had evidenced the role of MSI1 in bone cells (O'Valle et al. 2015). In fact, we had proposed the use of this marker to study bone regeneration (Padial-Molina et al. 2015). The presence of even more MSI1 positive cells in grafted bone in humans has also been previously reported for other biomaterials. Galindo-Moreno and coworkers found a higher number of positive MSI1 cells per mm^2 in xenograft and allograft composites grafts (291.70 ± 64.18 and 169.11 ± 142.62, respectively) (Galindo-Moreno et al. 2018). In contrast to the current study, those grafts contained 50 % of autogenous bone, which could explain these differences. As a consequence, our results indicate that the grafted area, after 6 months of healing, is still under remodeling. Thus, more mineralized tissue could be expectable in the area where implants are placed because the tissue has a high potential to do so.

Our study presents some limitations. The sample size is limited, but it is in accordance with many of the manuscripts published on the topic. We conducted a post-hoc power calculation for the number of MSI-1 positive MSCs and obtained a 92.62% of power achieved, which may justify the overcome of this limitation. In addition, a more detailed analysis of the significance of different proteins or these analyses through other technologies could be performed. In fact, some efforts are being done in order to improve our understanding of the biological events occurring in grafts placed in our patients.

CONCLUSION

Osteon™ II is a suitable biomaterial to be used in the formation of new bone in sinus floor elevation procedures in humans, not only from the histomorphometrical point of view, but also regarding the cellular and vascular quality of the regenerated bone.

COMPLIANCE WITH ETHICAL STANDARDS

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Conflict of Interest

The authors declare that although the biomaterial was donated by Dentium company, no resources and economical support was provided by the company. The authors declare no conflict of interest, either directly or indirectly, in any of the products listed in the manuscript.

Ethical aspects

All procedures performed in studies involving data from human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

This cross-sectional study was reviewed and approved by the Ethics Committee for Human Research of the Universidad Científica del Sur (Lima, Perú) (Code 00015).

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TABLES

Table 1: Porcentual area of the different tissue compartments analyzed in the morphological study. Data expressed as Mean ± SD.

	Pristine bone	**Grafted bone**
Mineralized tissue (%)	46.01 ± 7.35	34.93 ± 14.68*
Non-mineralized tissue (%)	53.99 ± 7.35	55.23 ± 11.03**
Remnant biomaterial (%)	NA	9.82 ± 11.42

*p=0.312, **p=0.953 (Wilcoxon test).

Table 2: Number of cells and vessels per mm^2. Data expressed as Mean ± SD.

	Pristine bone	**Grafted bone**	**P values (Wilcoxon test)**
Osteocytes	235.30 ± 99.77	134.67 ± 111.07	0.674
Osteoblasts	9.83 ± 15.33	30.86 ± 31.04	0.893
Osteoclasts	5.37 ± 16.12	15.57 ± 27.50	0.027
Vessels	25.62 ± 19.72	30.07 ± 28.21	0.894

Table 3: Number of Musashi-1 positive cells per mm^2. Data expressed as Mean ± SD.

	Pristine bone	**Grafted bone**	**P values (Wilcoxon test)**
Mesenchymal cells	42.11 ± 52.82	239.61 ± 177.4	0.027
Osteocytes	47.47 ± 58.60	96.06 ± 33.30	0.068
Osteoblasts	14.32 ± 15.06	40.70 ± 22.77	0.066
Osteoclasts	0.0 ± 0.0	2.29 ± 4.21	0.317

FIGURES

Figure 1: Panoramic microphotography of trephine biopsy from maxillary sinus lift with Osteon. A) Note the morphology of the pristine bone in comparison with the grafted area. B) The same microphotography with fluorescent light we can observe in yellow the bone trabecules in both areas of the biopsy. (Hematoxylin-eosin original magnification x0.2).

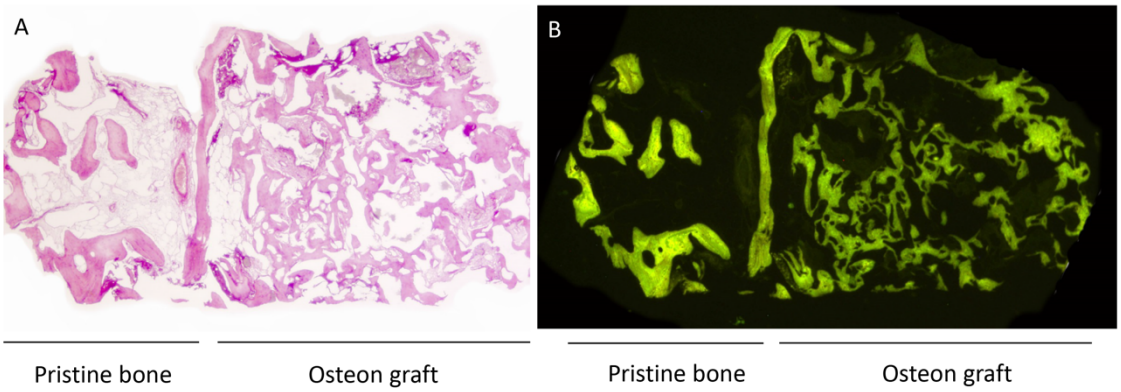

Figure 2: Detection of MSI1 in pristine bone (A) in comparison with grafted bone (B). Note the higher detection in mesenchymal cells in medullar stroma and osteoblasts in the grafted area (Micropolymer-peroxidase-based method, original magnification x20) (bar: 100 micrometers).