Sinus floor elevation using particulate PLGA-coated biphasic calcium phosphate bone graft substitutes: A prospective histological and radiological study

Running head: PLGA-coated biphasic calcium phosphate in sinus

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ABSTRACT

Background: PLGA is widely used for the development of delivery systems for drugs and therapeutic biomolecules in tissue engineering applications. Particles of biphasic calcium phosphate can be covered by PLGA to change their manipulating characteritics.

Purpose: Aim of this study was to investigate the radiological and histomorphometric results of the use of PLGA-coated biphasic calcium phosphate granules in sinus floor elevation and to analyze the underlying molecular processes by immunohistochemical staining.

Materials and Methods: A randomized clinical study was designed to include patients in need of sinus floor elevation. Patients were assigned to receive either PLGA-coated biphasic calcium phosphate particles (group I) or the equivalent but non-coated particles (group II). CBCT scans were performed before and 6 months after the procedure to assess the bone height gain. At the time of implant placement, bone core biopsies were obtained at the site of implant placement. Histological sections were subjected to histomorphometric and immunohistochemical evaluation of differentiation markers (Musashi-1).

Results: No statistically significant differences were observed between groups for the radiologic parameters. No differences were observed histologically or histomorphometrically. However, PLGA-coated particles (group I) were more colonized by Musashi-1-positive osteoblast precursors ($p=0.0001$, Chi-squared test) and were penetrated by more CD34-positive vascular structures ($p=0.001$, Chi-squared test) than non-coated particles (group II).

Conclusions: PLGA-coated particles are associated with more Musashi 1-positive cells and more extensive microvascularization than non-coated particles.

Keywords: sinus floor elevation; bone substitutes; biomaterials

INTRODUCTION

Tooth loss may induce dramatic consequences in the alveolar bone. Bone resorption has been explained through different mechanisms as an inevitable effect of bundle bone resorption due to a lack of mechanical stimulus by the periodontal ligament on the alveolar bone (1). Consequently, bone regeneration is required in many cases to permit dental implant placement. Bone regeneration requires the participation of cells, bioactive molecules, blood supply, and scaffolding materials (2). Different types of biomaterials have been proposed as scaffolds. Understanding the behavior of such biomaterials is essential for rational clinical treatment.

Different animal models have been used to investigate the *in vivo* performance of biomaterials. However, data from human studies are essential. In this sense, maxillary sinus floor elevation procedures require a substantial amount of grafting material; after a period of time, surgical re-entry for implant placement is available if it is less than 5 mm of the remaining crestal bone (3). Thus, these techniques allow the ethical procurement of human samples to study biomaterials or combinations and have been useful for the study of the behavior of bone grafts in humans (4). Moreover, the original lateral technique (5) and its modifications (6,7) have been associated with high success rates.

Calcium phosphates and other synthetic biomaterials have been proposed as effective alternatives to bone auto-, allo-, or xeno-grafts (8,9). Other biocompatible synthetic polymers have also been proposed. The most studied synthetic polymer is the group of poly (lactic-co-glycolic acid)-based (PLGA) biomaterials (10). The PLGA copolymer is completely biodegradable (11,12). It can be used for different purposes, including as an additive molecule, a carrier, or a degradable membrane, to avoid cellular competition. Currently, PLGA is widely used for the development of delivery systems for drugs and therapeutic biomolecules in tissue engineering applications (10).

In order to assess the induction of bone formation by biomaterials on a molecular level, different biomarkers, such as Musashi-1 (MSI1), CD34, CD56 and TRAP, have been evaluated. MSI1 is a RNA-binding protein reported to be marker of mesenchymal stromal cells (MSCs). Its expression in maxillary sinus floor elevation has been previously described by our group (13). The identification of adult MSCs is highly relevant to better understand the molecular pathways of key biological responses. CD34, CD56 and TRAP can help to specifically recognize vascular endothelial cells, osteoblast an osteoclast cells, respectively.

Thus, the aims of the current study were to investigate the use of PLGA-coated biphasic calcium phosphate granules to improve bone augmentation as assessed by radiological and histomorphometric techniques and to analyze the underlying molecular processes by immunohistochemical staining.

MATERIALS AND METHODS

A prospective, randomized, non-controlled, clinical study was conducted in the Oral Surgery Department (Faculty of Medicine and Dentistry, University of Valencia, Spain) from February 2012 to March 2014 and included patients who received lateral sinus lift surgery. The CONSORT statement (14) was followed (Figure 1).

The patients were informed about the study and procedures, and informed consent was obtained from all patients prior to inclusion. The study design was approved by the Ethical Committee of the University of Valencia (H1452514461215), Spain. The study was included in ClinicalTrials.gov with the identifier: NCT03474627. Criteria for inclusion were as follows: 1) partially edentulous patients, Kennedy class I or II in the upper jaw, from first premolar to second molar; 2) less than 5 mm of residual crestal bone; and 3) the need for the replacement of teeth with dental implants. Criteria for exclusion were: 1) sinus pathology (sinusitis, mucocele, cysts); and 2) smokers. The patients were randomly allocated to two treatment groups with SPSS-Windows 20.0 statistical software (IBM SPSS Inc., Chicago, IL, USA).

Group I (16 patients) received *in situ* hardening PLGA-coated biphasic calcium phosphate particles composed of hydroxyapatite and beta-tricalcium phosphate (HA/TCP=60/40) (GUIDOR *easy-graft* CRYSTAL, Sunstar Suisse SA, Switzerland), and the same biphasic particles without PLGA coating were placed in patients in group II (20 patients) (GUIDOR *calc-i-oss* CRYSTAL, Sunstar Suisse SA, Switzerland) (Figure 1).

Surgical technique

A sinus floor elevation by lateral approach was performed on each patient. All surgeries were performed by the same surgeon (AF). The procedure was performed under infiltrative local-regional anesthesia with 4% articaine and 1:100,000 epinephrine (Inibsa®; Barcelona, Spain). After a crestal incision with mesial and distal vertical releases, a full mucoperiosteal flap was elevated. A bone osteotomy was performed with the aid of a surgical ultrasound device (Variosurg®; NSK Europe, Eschborn, Germany) at the lateral aspect of the maxillary sinus to separate and elevate the Schneiderian membrane with the corresponding insert. After

verifying a proper elevation with manual curettes, the space created was filled with one of the two alloplastic materials according to the randomization. Once the elevated cavity was filled, a resorbable membrane was placed over the lateral wall of the maxilla (COLLAGENE AT®; Padova, Italy), and a tensionless soft tissue flap closure was performed with 4/0 sutures (Lorca Marín SA; Murcia, Spain). The sutures were removed after 1 week.

Prescribed medication included 2 g amoxicillin-clavulanic acid 1 h preoperatively, 750 mg amoxicillin 3 times a day for 7 days, 600 mg ibuprofen on demand, and 0.12% chlorhexidine rinses 2 times a day for 7 days.

At the time of implant placement after 6 months of healing, a bone core trephine sample was collected from each of the augmented sinus sites in all 36 patients for histomorphometric and immunohistochemical evaluation. In total, 57 TiCare-MozoGrau InHex implants (MozoGrau SL, Valladolid, Spain) were placed in the 36 augmented sinuses. Prostheses were delivered 3 months later.

Assessment of sinus floor height changes using CBCT

CBCT scans (Planmeca Romexis® 3D module) were performed before the sinus floor elevation and 6 months later before implant placement. Maxillary sinus width, buccal cortical bone, remnant alveolar crest (RAC), and vertical bone height (VBH) after sinus floor elevation were measured by an experienced surgeon with the Invivo 5 (Anatomage Medical; San Jose, CA, USA) (Figure 2).

Assessment of new bone formation in the augmented site by histomorphometry

A biopsy was obtained with 4 mm diameter trephines 6 months after sinus floor elevation. Bone biopsies were immediately fixed in 10% formalin for 48 h at room temperature, transferred to 70% ethanol, and transported to the Center for Biomedical Research at the University of Granada (CIBM-UGR) for evaluation. They were then decalcified for 24 h at 37°C (Decalcifier I®, Surgipath Europe Ltd., Peterborough, United Kingdom) and embedded in paraffin. Sections (4 μm) were obtained along the apico-coronal axis, deparaffinized, rehydrated, and stained with hematoxylin-eosin and Masson's trichrome. With the 40x objective in a microscope with an attached scale (BH2, Olympus Optical Company, Ltd., Tokyo, Japan), the numbers of osteoblasts, osteoclasts, osteocytes, and vessels were quantified per mm^2. Histomorphometric quantification was performed semi-automatically with Masson's trichrome-stained sections. Ten random images were captured from each sample with a 10x objective using a microscope equipped with a digital camera (DP70, Olympus Optical Company), and ImageJ software (NIH, http://imagej.nih.gov/ij/) was used to quantify the percentage of new mineralized

tissue, remnant particles, and non-mineralized tissue. All histological evaluations were performed by an experienced pathologist who was blinded to the type of biomaterial used.

Assessment of biomarkers of bone regeneration by immunohistochemical staining

Rehydrated samples were thermally treated at 95°C for 20 min in a pre-treatment module (Thermo Fisher Scientific Inc., Waltham, MA, USA) containing 1 mM EDTA buffer (pH 8) as an antigen unmasking solution. A primary polyclonal antibody against Musashi-1 (MSI1) was then applied and incubated at a 1:100 dilution for 16 h at 4°C. Staining with prediluted CD34 (clone QBEnd/10), CD56 (Clone MRQ-42), and TRAP (clone 9C5) for 10 min at room temperature was also performed. Vimentin (clone V9) (1:100 dilution) was used as positive control. A non-immunospecific IgG was used as a negative isotype 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 Polymer, Master Diagnóstica).

Immunopositivity was evaluated semi-quantitatively by a blinded examiner (0=no staining; 1=staining). Immunopositivity was evaluated in osteocytes, osteoblasts, osteoclasts, mesenchymal stromal cells, and endothelial cells.

Statistical analysis

SPSS-Windows 20.0 (IBM SPSS Inc.) was used for the analyses. The results are presented as the means±standard deviation for continuous variables and as percentage (frequency) for categorical data. Chi-squared tests were used to compare categorical variables, and Student's t test and the Mann-Whitney U-test were used for continuous variables. The statistical power for comparisons between both groups was estimated as 85%. The results were considered statistically significant when p-values were below 0.05.

RESULTS

The mean age of the enrolled patients was 43.11±9.32, and 38.8% of them were women (p=0.324)). One bone core biopsy was taken from every patient, leading to a total of 36 biopsies from the 36 augmented sinuses. Overall, 57 implants were placed in the area. One of the 57 dental implants failed (1.75%) within the first 3 months. No other implants failed during a total observation time of 24 months after implant placement.

The initial remnant alveolar crest (RAC) measured by CBCT showed an overall mean residual bone height of 3.19±1.15 mm (2.50±1.58 mm in group I vs. 3.46±0.87 mm in group II; p=0.143). The final mean vertical bone height (VBH) after 6 months of healing was 9.50±3.03 mm (10.60±4.27 mm in group I vs. 9.07±2.49 mm in group II, p=0.775). Thus, a mean sinus floor elevation (SFE=VBH-RAC) of 8.10±4.81 mm in group I vs. 5.61±3.00 in group II was achieved (marginal significance level, p=0.116) (Table 1).

Remnant biomaterial particles were visible in all biopsies after 6 months and exhibited a spherical morphology (Figure 3). The particles were surrounded by fusiform cells (adult stromal mesenchymal cells) and multinucleated osteoclastic cells. New mineralized tissue was more evident in group I, where more cells could also be detected in the interior of the biomaterial particles. However, no significant statistical differences were found between biomaterials in terms of new mineralized tissue (31.25±13.82 vs 34.09±14.11), non-mineralized tissue (46.0±16.63 vs. 43.9±17.75), or remnant biomaterial (23.38±24.52 vs. 22.0±17.26) between group I and group II, respectively. Compared to the pristine bone, the number of osteocytes per mm^2 was higher in the pristine bone (145.16±46.40 vs. 92.39±33.75, pristine vs. grafted, respectively) (p=0.0001, Student's t test). However, the numbers of osteoblasts (19.59±20.79 vs. 56.49±33.49, pristine vs. grafted, respectively) (p=0.001, Student's t test) and osteoclasts (0.00±0.00 vs. 14.81±12.12, pristine vs. grafted, respectively) (p=0.0001, Student's t test) per mm^2 were significantly higher in the grafted bone than in the remnant pristine alveolar crest (table 2).

Immunohistochemical analysis of the histological sections evidenced biomaterial particles surrounded by CD56-positive cells (osteoblast precursors) and giant multinucleated TRAP-positive cells (osteoclasts). In the interior of the PLGA-coated biphasic calcium phosphate particles (group I), CD34-positive vascular structures (p=0.001, Chi-squared test) and abundant MSI1-positive cells (p=0.0001, Chi-squared test) were detected, whilst none of these morphologic events were present in the biopsies from group II (Figure 4).

DISCUSSION

The purpose of this study was to quantitatively and qualitatively assess new bone formation after sinus floor augmentation with particulate or PLGA-coated hardening alloplastic biphasic calcium phosphate grafting materials *in situ* via CBCT technology, histomorphometrical, and immunohistochemical analyses of bone biopsies and to explore the associated underlying mechanisms.

It is important to emphasize the biological meaning of each tissue compartment obtained after the bone healing processes following regeneration: new mineralized tissue, non-mineralized tissue, and remnant particles. It is reasonable to think that an increase in mineralized components would be associated with improved quality of the regenerated bone. However, the non-mineralized portion plays a crucial role in bone homeostasis. This non-mineralized tissue is described in the literature as "*soft connective tissue*," which is inadequate in our opinion. The bone marrow or non-mineralized tissue compartment is essential for bone repair and maturation. For instance, cellular and vascular repair are mediated by the components found in this portion of the bone (15).

Radiographic results from CBCT showed that after the sinus floor elevation surgery, alloplastic biphasic calcium phosphate particles that contained hydroxyapatite (HA) and beta tricalcium phosphate (β-TCP), with or without the PLGA coating, were able to generate tissue that was radiographically compatible with bone. This radiographic image of new bone was sufficient in quantity and quality to allow the rehabilitation of the posterior edentulous maxilla with dental implants. Radiographically, our findings are in accordance with those reported by Baena (16). They found a mean vertical radiographic increase of 7.8 mm after using a PLGA/HA graft; for a deproteinized bovine bone (DBB) graft, the vertical increase was 9 mm. In our study, the final mean vertical bone height was 9.50±3.03 mm after 6 months of healing, slightly higher than for the same biomaterial and quite similar to the DBB biomaterial in the previously mentioned study. These differences can be explained by several plausible factors: the quantity of biomaterial placed during the surgery, the sinus size, and the random distribution of the biomaterial in the sinus cavity.

The final radiographic bone height obtained depends on the biomaterial properties, specifically on resorption rate and osteoconductivity. A higher biomaterial resorption rate is detrimental because it promotes graft collapse. On the other hand, higher osteoconductivity promotes quicker bone formation from all bony walls of the cavity, including the Schneiderian´s membrane, and therefore promotes space maintenance (17). In this sense, in terms of histological results, although clinical studies using ß-tricalcium phosphate are common, studies that use PLGA-containing materials are limited and restricted to indications, such as the treatment of periodontal intrabony defects (18) or ridge preservation (18,19). In this sense, 34% to 60% of vital bone formation has been reported with the use of PLGA in ridge preservation (20,21). Still, the application of these materials in different indications might lead to different results due to anatomical or physiological differences.

In the current study on sinus floor elevation, the mean percentage of new mineralized bone tissue formed, classically called “new vital bone,” was quite similar to the percentage reported for other types of biomaterials, such as anorganic bovine bone (32.75±14.0% to 35.44±16%) (21,22) or bio-glasses (38%) (24). The biphasic biomaterial used in this study showed less resorption than a classic calcium phosphate composed only of the tricalcium phosphate phase. In fact, in our patients, the quantity of remnant biomaterial was slightly higher than in other studies that used only ß-TCP (25, 26). This would induce an implosive maturation model with bone growing into the graft from all sides of the cavity, similar to DBB (27). This is in contrast to the vertical regeneration behavior of tricalcium phosphate proposed by other authors (8), where bone grows only from the side of the remaining alveolar crest.

Remarkable differences could be found when the grafted area was compared to the remnant alveolar bone. The number of osteocytes per mm^2 was higher in the pristine bone than in the grafted areas (145.16±46.40 vs. 92.39±33.75, respectively); however, the number of osteoblasts and osteoclasts per mm^2 was significantly lower in the remnant pristine alveolar crest area than in the grafted tissue (19.59±20.79 vs. 56.49±33.49 and 0.0±0.0 vs. 14.81±12.12, respectively). These differences were statistically significant in all cases ($p<0.001$). These findings are in accordance with previous reports. Mature bone contains more differentiated cells, such as osteocytes, surrounded by a more stable trabecular structure. In comparison, areas that are healing and undergoing remodeling require more cells to promote the resorption of the damaged components (i.e., osteoclasts) and to generate a mineralizable matrix formation (i.e., osteoblasts). Furthermore, these data are corroborated by previous studies that demonstrated a similar bone healing pattern after the placement of a xenograft in the sinus, where considerably more osteoclasts and osteoid lines were found in the grafted area than in the pristine maxillary bone (28). During healing after implant insertion, the osteocyte lacunar density is almost twice as high in woven bone as in mature cortical bone (29). This is exactly the same as in bone development, during which the density of osteocyte lacunae is higher in woven bone than in lamellar bone (30).

The PLGA coating of the biphasic calcium phosphate granules was developed to permit adherence of the particles during preparation in order to improve the handling, moldability, and *in situ* stability of the graft in the defect. Wildburger et al. (31) studied in eight sheep the effect of adherence of the particles with PLGA in HA/β-TCP bone graft respect the same biomaterial without PLGA; and observed after 21 weeks 19.82 % (±

6.29) of bone formation in PLGA samples respect 14.38 % (± 4.51) in the samples without PLGA, with better results in PLGA biomaterial. However, an interesting finding reported in the present study is the different biological behavior of the particles coated with PLGA. It is quite well-known that histomorphometric measures give us a temporal "*picture*" of the tissue composition. Histology also offers a glimpse into the future fate of those tissues. Cellularity and vascularity are essential for bone tissue development, maturation, and remodeling (15). Thus, it is necessary to pay attention to these important elements when studying the behavior of biomaterials in humans. In this sense, *in situ* hardened PLGA-coated particles consistently showed cellular and vascular penetration, which was not observed in the non-coated particles. This event might show a potential effect of the PLGA *per se* in bone healing. In the interior of the PLGA-coated biphasic calcium phosphate particles (group I), CD34-positive vascular structures (p=0.001, Chi-squared test) and abundant Musashi-1-positive cells (p=0.0001, Chi-squared test) were detected. None of these morphologic events were present in any of the biopsies of sites augmented with non-coated particles (group II).

MSCs are of high interest in regeneration procedures in the maxillofacial area (32). MSCs are characterized by their self-renewal potential and ability to differentiate into multiple cell types, including osteogenic, adipogenic, chondrogenic, or myogenic lineages. MSCs are, therefore, key elements in bone graft maturation and osseointegration processes used in implant dentistry (2). The expression of MSI1 has been reported as a marker of mesenchymal stromal cells (33, 34). Interestingly, we have also detected MSI1 in fusiform cells in biopsies from xeno-grafted maxillary sinuses (manuscript under revision). In our current samples, MSI1 was detected in osteoprogenitor cells in 97.4% of biopsies from grafted sites with PLGA-coated particles vs. 66.6% of biopsies from native bone. Although MSI1 was detected in osteoblasts, osteocytes, and osteoclasts, its function in these cell populations remains unknown. In any case, the presence of MSI1-positive cells inside the PLGA-coated calcium phosphate particles could contribute to the remodeling of bone tissue and increase osteoconductivity. Araujo-Pires et al. (35) have highlighted that PLGA is osteoconductive and provides a structural framework that permits the adhesion of pre-osteoblasts and osteoblasts that can then secrete *de novo* bone matrix on the scaffold surface. Therefore, the finding that PLGA can support the formation of an interconnected structure that allows the migration of new cells and new blood vessels was corroborated by our observations, as described above. However, the specific effect of PLGA on bone healing remains unclear. Further studies are needed to investigate whether PLGA *per se* might have a positive effect on bone formation

and maturation.

This study presents some limitations. As the majority of studies conducted in maxillary sinus, is important to highlight that anatomic variables of the maxillary sinus are numerous and associated with other clinical parameters (36, 37), and previously, anatomical variations were related to the response to the graft at the histomorphometrical level (38). In addition, a split-mouth design could improve the outcomes of the comparison considering that several patient-related factors such as type of edentulism, some deleterious habits as smoking and alcohol intake, or previous history of periodontitis, influence the response to the graft in maxillary sinus floor elevation (27). These factors could be truly important in order to balance different patient's characteristics but also from the site-specific configuration. However, these variable-effects have not been analyzed in the current study.

CONCLUSIONS

PLGA-coated particles increase the number of MSI1-positive cells and the degree of microvascularization in the regenerated bony area in comparison to non-coated particles. Further studies are needed to corroborate these initial findings.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ETHICAL ASPECTS

All procedures performed in studies on human participants were in accordance with the ethical standards of the institutional or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All patients were informed about the study and procedures.

Prior to participating, written informed consent was obtained. This prospective study was reviewed and approved by the Ethics Committee for Human Research of the University of Valencia (approval number: H1452514461215). Patient interventions were performed in the Department of Oral Surgery (Faculty of Medicine and Dentistry, University of Valencia, Spain).

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FIGURE LEGENDS

Figure 1: Consort Flow Diagram.

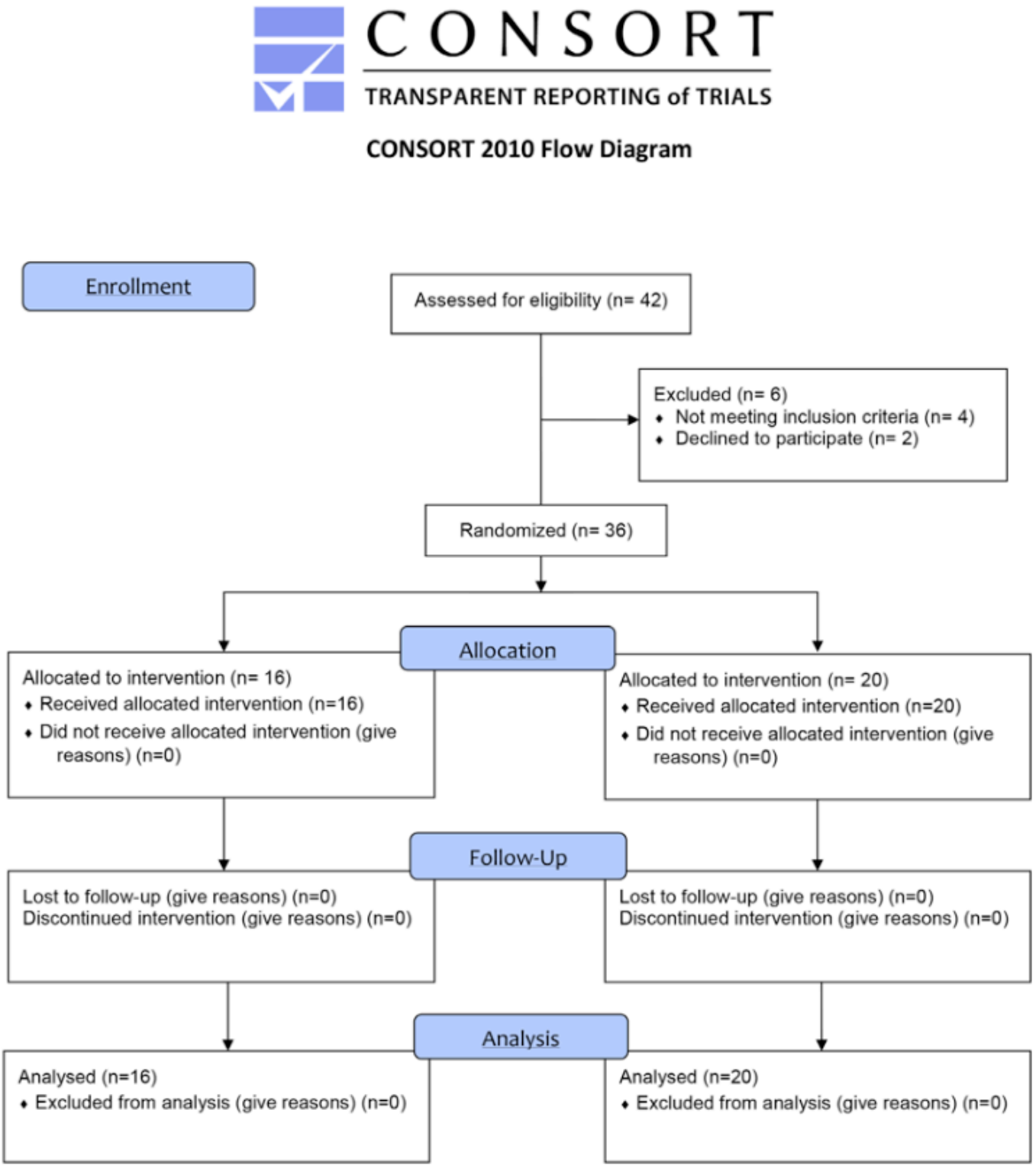


Figure 2: CBCT control: a) pre-surgery; b) 6 months after the sinus lift.

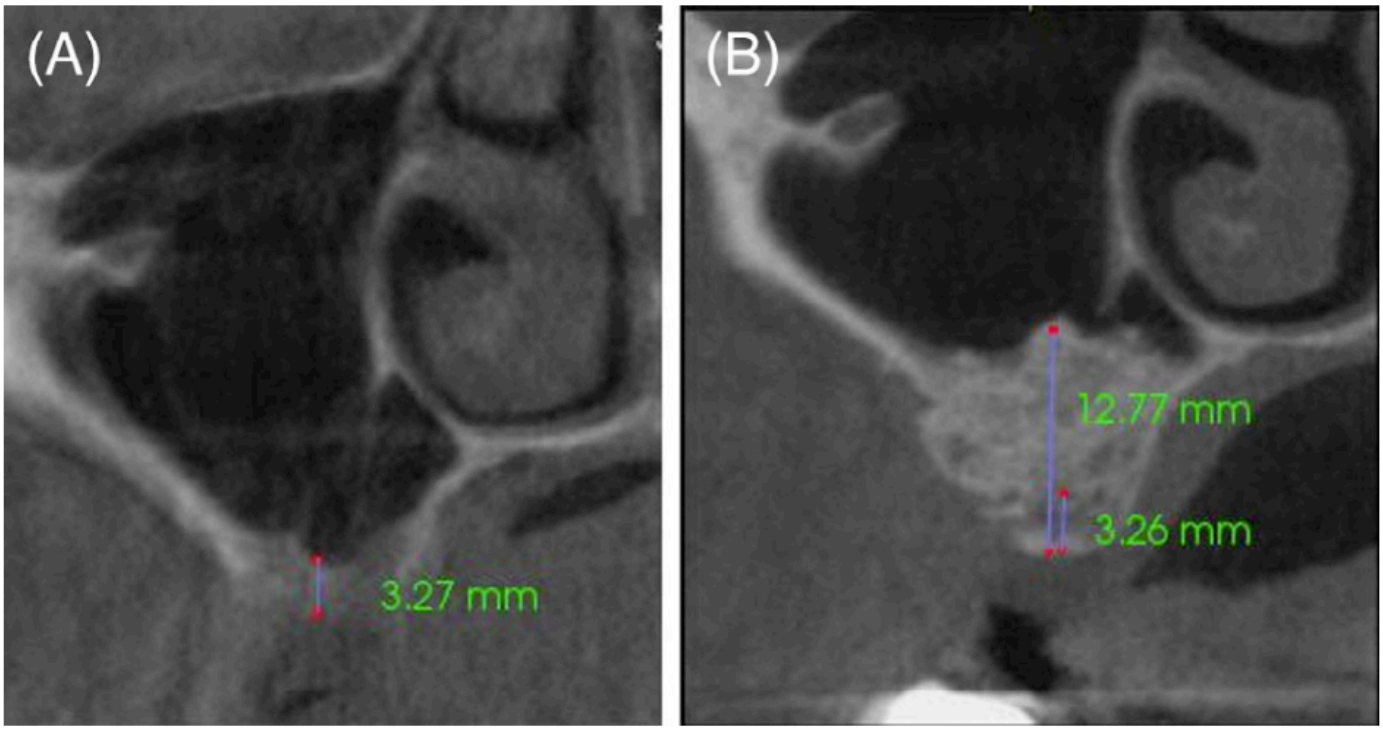


Figure 3: Microphotographs of biopsies from the maxillary sinus lift. A and B) Group I: PLGA-coated biphasic calcium phosphate; C and D) Group II: non-coated biphasic calcium phosphate. Note the presence of new mineralized tissue (nMT) surrounding the allograft particles (AP) and higher cellularity in non-mineralized tissue in group I (Masson trichrome stain). Scale bars = 500 µm (A and C) and 100 µm (B and D).

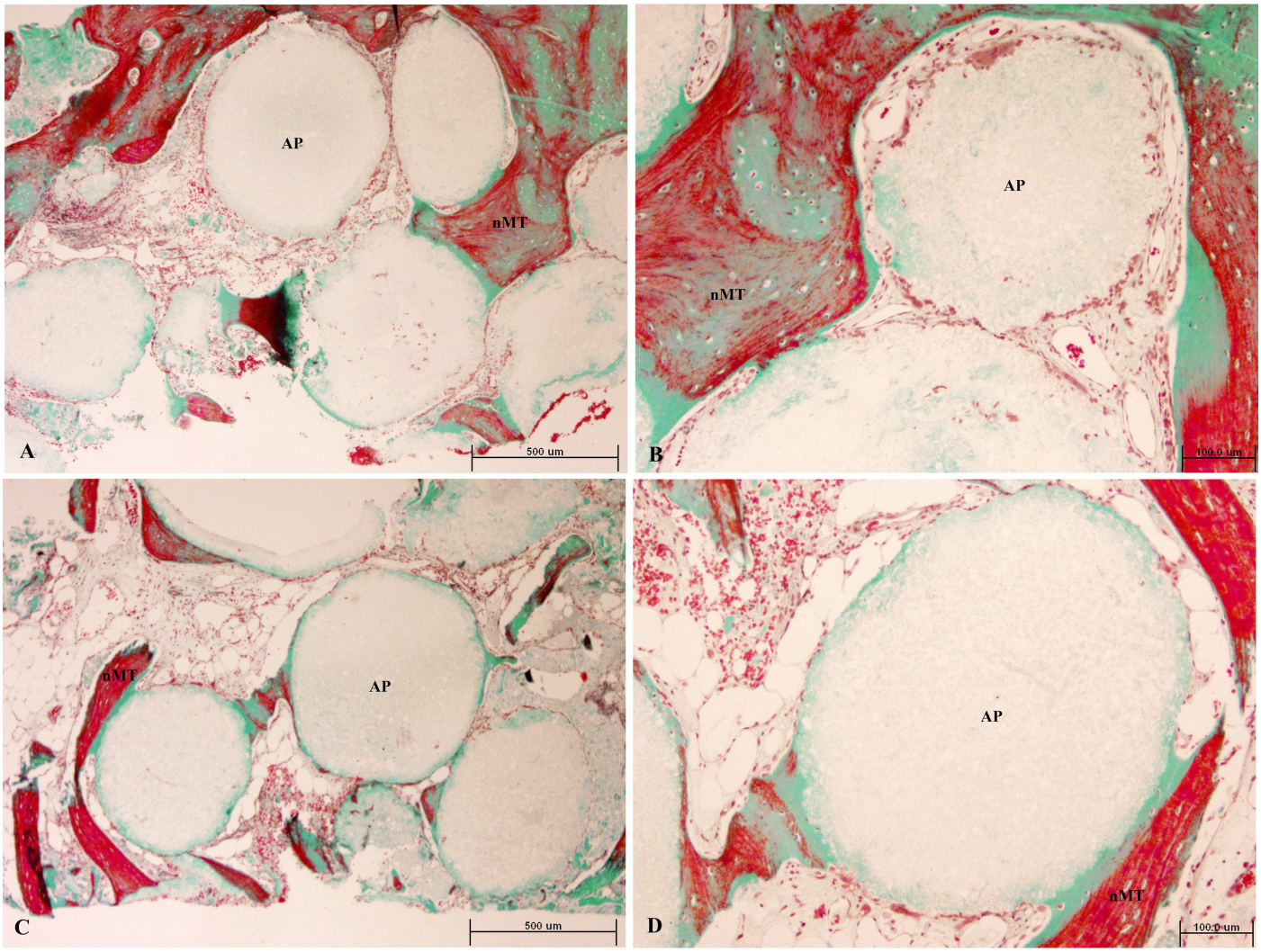
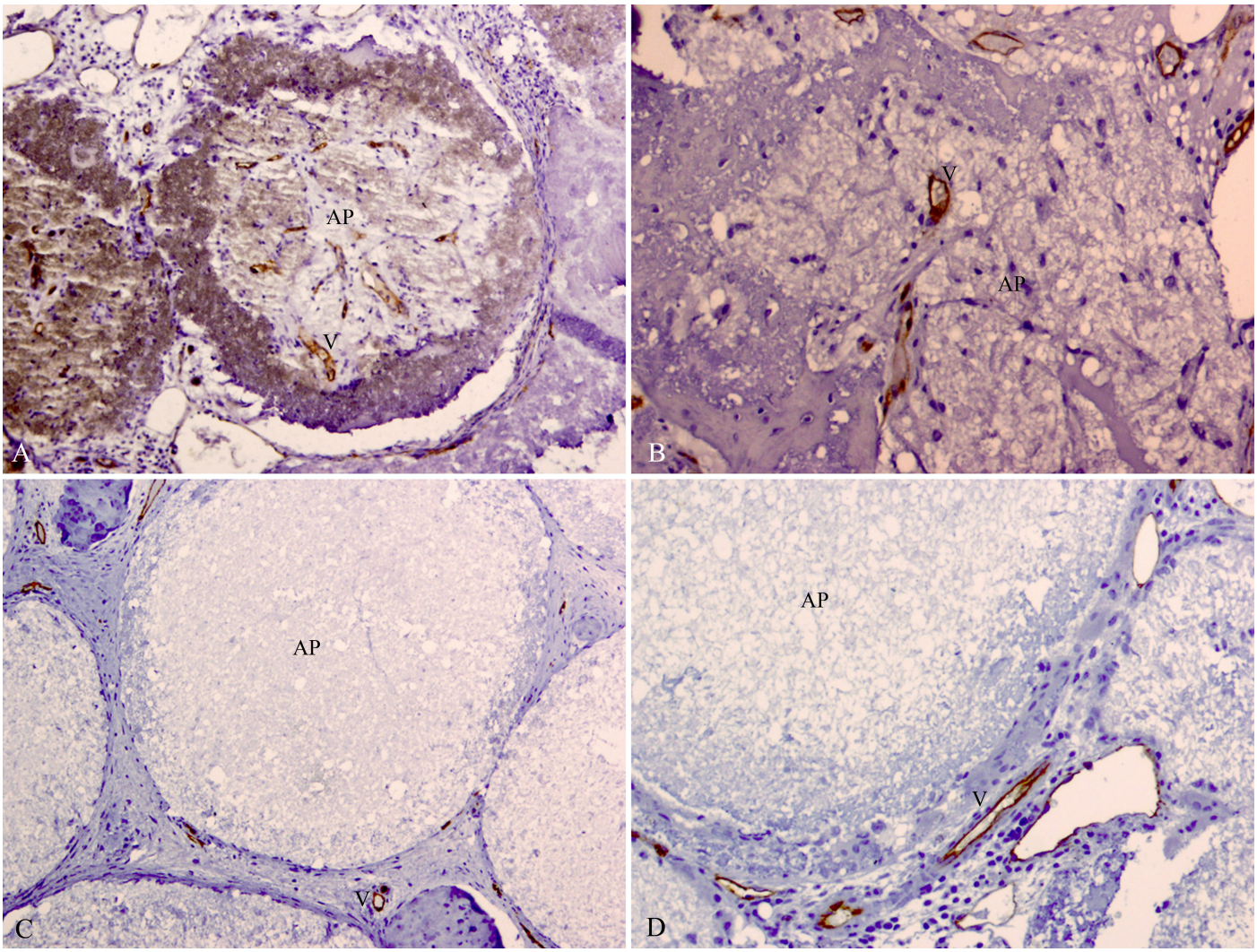


Figure 4: 1) CD34 expression in biopsies from maxillary sinus lifts. A and B) Group I: PLGA-coated biphasic calcium phosphate; C and D) Group II: non-coated biphasic calcium phosphate. Note the exclusive presence of CD34-positive vessels inside particles (AP) in group I (V) (peroxidase-conjugated micropolymer, original magnification 20x (A and C) 40x (B and D)). 2) Musashi-1 expression in biopsies from maxillary sinus lifts. A and B) Group I: PLGA-coated biphasic calcium phosphate; C and D) Group II: non-coated biphasic calcium phosphate. Note the exclusive presence of Musashi-1 expression (>) inside particles (AP) in group I (peroxidase-conjugated micropolymer, original magnification 20x (A and C) 40x (B and D)).

1)



2)

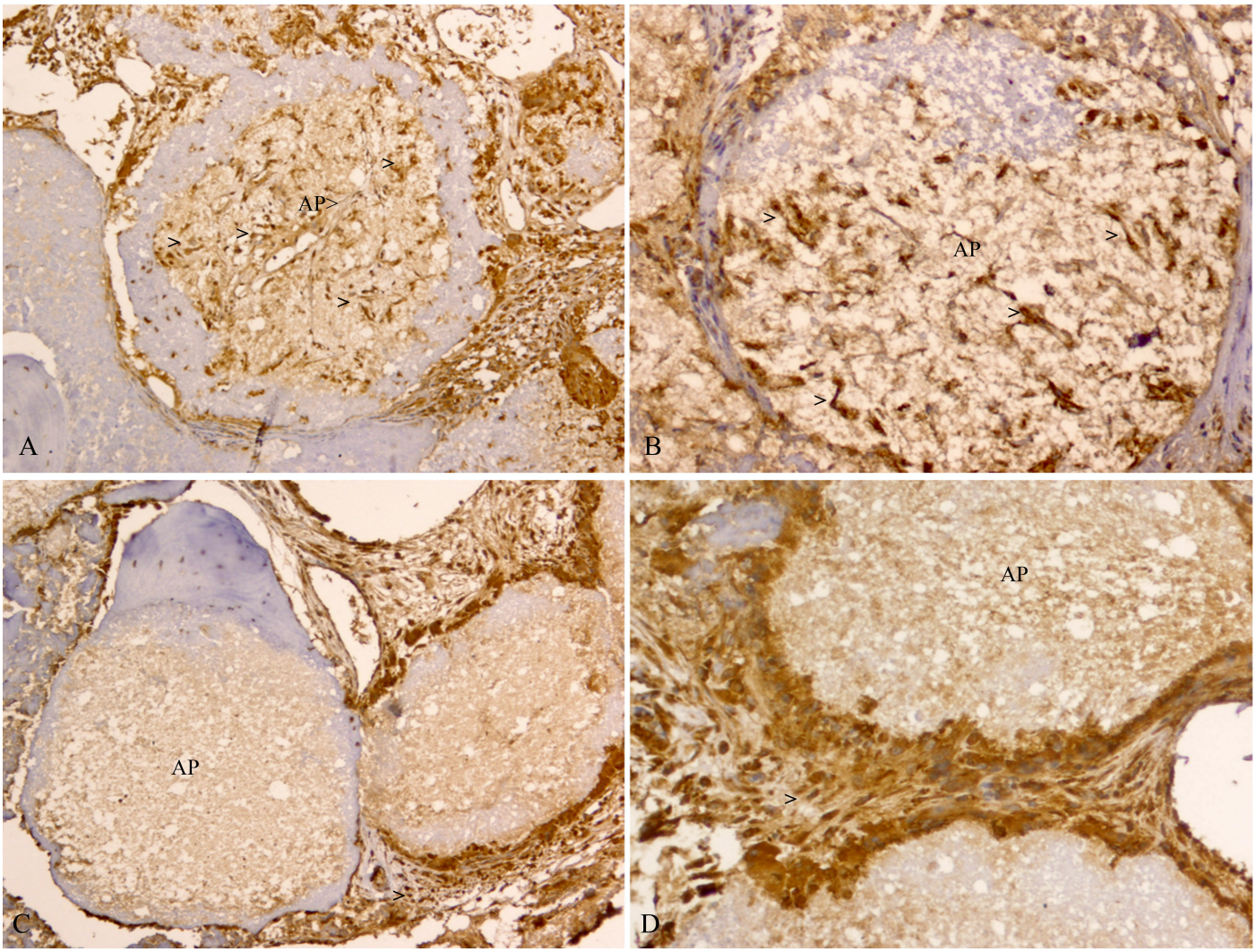


TABLE LEGENDS

Table 1. Demographic and radiological data of alloplastic bone grafts placed in the sinus cavity in both groups.

	Group I (n=16)	Group II (n=20)	P-values[†]
Age (years)	39.60±4.77	44.46±10.41	0.566
Maxillary Sinus Width (mm)	6.80±1.48	7.38±1.32	0.503
Buccal Cortical Bone (mm)	0.90±0.22	1.30±0.38	0.075
Remnant Alveolar Crest (RAC) (mm)	2.50±1.58	3.46±0.87	0.143
Vertical Bone Height (VBH) (mm)	10.60±4.27	9.07±2.49	0.775
Sinus Floor Elevation (SFE=VBH-RAC) (mm)	8.10±4.81	5.61±3.00	0.116

Values are expressed as the means±standard deviation. [†]Mann-Whitney U-test.

Group I: PLGA-coated biphasic calcium phosphate; Group II: non-coated biphasic calcium phosphate.

Table 2. Comparative study of cellularity depending on the area of the biopsy studied.

	Osteocytes (mm^2)	*P-values	Osteoblasts (mm^2)	*P-values	Osteoclasts (mm^2)	*P-values
Graft	92.39±33.75	0.0001	56.49±33.49	0.001	14.81±12.12	0.0001
Pristine	145.16±46.4		19.59±20.79		0.0±0.0	
Group I	107.97±51.39	0.781	34.29±30.28	0.066	7.46±12.20	0.206
Group II	112.10±39.52		54.53±36.19		12.41±11.8	

Values are expressed as the means±standard deviation. *Student's t test. Group I: PLGA-coated biphasic calcium phosphate; Group II: non-coated biphasic calcium phosphate.