Histomorphometric Comparison of New Bone Formed After Maxillary Sinus Lift With Lateral and Crestal Approaches Using Periostal Mesenchymal Stem Cells and Beta-Tricalcium Phosphate: A Controlled Clinical Trial

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Abstract: The present study investigated clinical and histomorphometric data after sinus lift procedures performed with and without mesenchymal stem cells (MSCs) added to a graft. Twenty-four patients underwent maxillary sinus lift for implant placement. Twelve patients each were assigned to control (Group 1) and test (Group 2) groups. An MSC suspension was added to the graft used in patients of Group 2. Five of 12 patients in both groups underwent crestal-approach sinus lift with immediate implant placement, while seven patients received a lateral-approach sinus lift. The MSC suspension was obtained using the Rigenera protocol. Samples from the grafted site were evaluated, processed, and stained using three staining techniques 90 days after surgery. Histomorphometric analysis was performed using an imaging software (ImageJ). Two types of tissues were defined: Type 1 'mature bone' and Type 2 'osteoid tissue'. The mean Type 1 tissue percentage was 27.24% in Group 1 and 44.45% in Group 2 ($P < 0.05$). The mean Type 2 tissue percentage was 10.86% and 7.04% in Groups 1 and 2, respectively. The mean Type 1 tissue percentages for the crestal approach were 24.52% for Group 1 and 50.78% for Group 2, while the mean Type 1 tissue percentages for the lateral approach were 29.18% for Group 1 and 39.92% for Group 2. Patients treated with grafts containing MSCs showed 63.18% increased bone formation compared to those treated with grafts not containing MSCs ($P < 0.05$). Although our data showed a positive trend in patients treated with MSCs, differences between subgroups were not significant ($P > 0.05$).

Key Words: Beta-tricalcium phosphate, bone graft, maxillary sinus lift, mesenchymal stems cell, Rigenera protocol

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Bone loss in the posterior maxilla often requires surgery to regenerate bone for proper implant positioning. Sinus floor elevation is a predictable technique that – when used with graft materials to improve bone regeneration – can ensure accurate implant positioning in the posterior maxilla, even in cases with a limited height of available bone.[1–4] Misch has described two main techniques for sinus floor elevation based on the height of available bone: the lateral window technique (<8 mm of residual bone height) and the transalveolar technique (the osteotome technique or Summers' technique; approximately 8–12 mm of residual bone height).

Autologous bone is considered to be the "gold standard" owing to its excellent osteoconductive, osteoinductive, and osteogenetic properties.[5] However, autologous grafting procedures increase patient morbidity. Autologous bone seems to resorb faster and - compared to synthetic bone substitutes – is harder to obtain and manipulate.[4-6] In contrast, the lack of osteoinductivity and osteo- genetic cells in synthetic bone substitutes lengthens the healing time by 6 months.[7,8] The use of autologous mesenchymal stem cells (MSCs, autologous pluripotent cells with differentiative properties) with a scaffold material can improve osteoinduction with no possibility of rejection. The most commonly used bone MSCs are obtained from the bone marrow,[9] although they can also be obtained from periosteal or adipose tissues.[10]

However, the most predictable and efficient procedure for using stem cells as bone-grafting enhancers remains controversial. Several clinical trials have demonstrated the ability of MSCs to form new bone, but further research on this topic is required.[9] The procedure for harvesting MSCs is often invasive and requires general anesthesia or sedation. Researchers, therefore, obtained MSCs from alternative sources of MSCs: adult dental tissues, such as dental pulp, periosteum, and periodontal ligament.[11] The periosteum is a thin membrane that covers the bone and is rich in pluripotent cells and molecular

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The data that support the findings of this study are available from the corresponding author upon reasonable request.

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factors.[12] Periosteal progenitor cells have featured widespread application in cartilaginous regeneration, bone healing, and tissue engineering in oral and maxillofacial tissues.[13] Cultured periosteal MSCs have been widely used in bone augmentation and sinus lift procedures. This approach has successfully achieved faster bone remodeling and lamellar bone formation than in conventional bone grafting, with consequent reliable implant placement,[14] and has thus helped to shorten recovery time after implant surgery.[15]

Bone formation usually occurs three months after the surgery. A mean bone formation of 31%[16] is associated with synthetic bone grafting materials, while 41.34%[17] is associated with periosteal MSCs with a mixture of tricalcium phosphate and hydroxyapatite as a scaffold. The combined use of MSCs and collagen reportedly accelerates new bone formation in post-extraction sites and reduces bone resorption after surgery. A previous study reported horizontal and vertical resorption in the test group to be less than 38.3% and 36.5%, respectively, relative to the control group (collagen only).[18]

The use of granulated autologous bone is considered to be the gold standard for sinus lift procedures. However, granulated autologous bone is often not easily available, and the procedure requires additional surgical operations. In contrast, MSCs are easy to obtain because the periosteum is readily accessible during sinus lift procedures. We hypothesized that the addition of periosteal MSCs to a synthetic graft material would enhance healing and bone regeneration, resulting in higher percentages of bone formation in the short term. This study thus aimed to compare the effects of using periosteal MSCs in combination with a synthetic scaffold material on bone regeneration after sinus lift procedures with those of using a scaffold alone.

MATERIALS AND METHODS

Study Design

All patients were recruited from the Department of Head and Neck, Division of Oral Surgery and Implantology, Institute of Clinical Dentistry, Catholic University of the "Sacred Heart," Gemelli Foundation for the University Policlinic, Rome, Italy.

Patients were included in this trial only after they provided informed consent. The principles of the Helsinki Declaration of 1975, as revised in 2000 for biomedical research involving human subjects; the ICH GCP; CIOMS ethical guidelines; and all national legal and regulatory protocols were followed. The study was approved by the Institutional Ethics Committee of the Faculty of Technical Medical Sciences of Elbasan "ALEKSANDËR XHU-VANI," Albania (Protocol identification: INTL_ALITSHCOOP/DentPath/2020_SLK).

All patients who required maxillary sinus lift procedures, were $\geq$ 18 years old, and were able to understand the study procedures and provide informed consent were eligible for this study. Considering the small sample of the study, the assignment of patients to various groups was performed according to the dynamic criterion of minimization for the factor of age. Data were collected from the 24 patients who underwent 24 sinus lift procedures, including clinical and histological data, between September 2020 and December 2020. Patients were categorized into one of two groups depending on the material used and surgical procedure performed: A control group of 12 patients treated using a synthetic bone graft, a mixture of beta-tricalcium phosphate (80%) and hydroxyapatite (20%), which is very similar to autologous bone in terms of biocompatibility and resorption rate[19–22]; and a test group of 12 patients treated using the same synthetic bone graft in combination with MSCs. Each group was further subdivided based on the surgical procedure performed: five patients underwent crestal sinus lift procedure and seven patients received a lateral sinus lift.

Clinical information included data pertaining to anamnesis, plaque, and the bleeding index, as well as digital pictures taken during the healing period. Radiological data included a preoperative radiograph (RX), preoperative Dentascan, and a control RX obtained 30 days after the surgery.

Histomorphometric analysis of samples obtained from regenerated bone 3 months after the surgery (bone samples are routinely obtained to evaluate osseointegration and bone healing before implant loading) was performed to investigate the extent of bone regeneration in the short term.

All patients exhibited full-mouth plaque scores and full-mouth bleeding scores of lower than 25%. Exclusion criteria included high-risk patients (American Society of Anesthesiologists' Physical Status Class 4); patients with severe systemic disease (severe heart disease, unstable angina pectoris, respiratory disease, endocrine disease, and spleen and kidney disease), untreated chronic periodontitis, alcohol and drug abuse, smoking habits (more than five cigarettes per day), uncontrolled diabetes, intravenous bisphosphonate therapy, and severe immunosuppression; patients who had undergone radiotherapy; and patients who were taking medications or had diseases that could alter bone metabolism.

Bone samples were processed, embedded in paraffin, cut, and stained. Newly formed bone, mature bone, and fibrous tissues were recognized and quantified using the dedicated imaging software ImageJ (public domain software developed at NIH-National Institutes of Health, Bethesda, Maryland, USA and LOCI-Laboratory for Optical and Computational Instrumentation, University Of Wisconsin, Wisconsin, Madison, USA; NIH v. 1.61, Http://rsb.in-fo.nih.gov/nih-image).

All patients were treated and evaluated by a single operator.

Surgical Procedure

Dentascan (computed tomography [CT] system, GE Medical Systems, Global Center, Milwaukee, Wisconsin, USA) was used to determine the implant positioning sites.

All patients were orally administered nimesulide 100 mg 2 h before surgery and amoxicillin 875 mg + clavulanic acid 125 mg, 2 g 1 h before surgery (antibiotic prophylaxis).

The procedures performed differed according to the amount of ridge resorption in each patient: a crestal approach sinus lift procedure was performed according to Summers' technique when the bone height was 8 to 10 mm, as determined by the Dentascan analysis; while sinus lift was performed according to the trapdoor technique when bone height was between 4 and 8 mm. Implant placement was delayed when the bone height was less than 4 mm. All implants were loaded 3 months after the surgery.

Sinus Lift Using the Trapdoor Technique

Patients who underwent sinus lift performed according to the trapdoor technique were treated using the Boyne & James' and Tatum's surgical protocols and the subsequent modifications of the protocols.[8]

Each patient was administered a chlorhexidine (0.20%)-based mouth rinse to be used for 60 s before surgery. Local anesthesia was achieved with 4% articaine infiltration.

A sub-crestal palatal incision was made to avoid an overlap between the incision line and the implant, and a full-thickness flap was raised and extended according to the sinus floor width and residual bone volume. Two vertical secondary incisions

were made to expose the anteroinferior bony wall of the sinus. Incisions were wide enough to ensure the comfortable use of manual and rotatory instruments and allow for good flap passivation.

Antrostomy was performed using piezoelectric equipment (ENAC OE-W10 All-in-One Piezoelectric Ultrasonic Oscillating System, Osada Inc., Los Angeles) to reduce the possibility of the accidental perforation of the sinus membrane. The bone incision was oval, without any sharp edges that could damage the membrane during the grafting procedure. The extension of the antrostomy was determined by the number of implants to be placed, with mean dimensions of 10 to 20 mm in width and 6 to 15 mm in height. The inferior margin of the antrostomy was placed 3 to 4 mm higher than the sinus floor.

When adequate mobility of the trapdoor was obtained, the sinus membrane was elevated manually using dedicated piezoelectric tips (Osada Inc., Los Angeles, CA) while avoiding any damage to the membrane. The instruments were always maintained in contact with the bone of the sinus floor; a continuous and constant force was applied to dislocate the sinus membrane safely. The sinus membrane was passivated to avoid perforation during the grafting procedures.

The grafting material MBCP$^+$ (micro/macroporous bone graft synthetic resorbable biphasic calcium Phosphate, Biomatlante, Vig- neux de Bretagne, France) was inserted through the trapdoor with a proprietary syringe-like dispenser. The material comprises granulated beta-tricalcium phosphate (80%) and hydroxyapatite (20%) and is easy to manipulate. In the test group, MSCs were added to the synthetic graft material prior to graft placement. Tetracycline (Ambramicina 250 mg, Scharper, Via Manzoni, Milano, Italy) and collagen particles were added to the granulated material, and a resorbable collagen membrane was used to protect the grafted site.[23]

When 3 to 4 mm of bone height was evident, implant placement was delayed due to the impossibility of achieving the primary stability of the implants.[24]

If immediate implant placement was performed, two-thirds of the graft was deposited before implant placement, and the remaining graft was inserted after implant placement.

The use of a resorbable membrane in sinus lift procedures has been shown to achieve higher vital bone regeneration percentages and better implant healing than has the use of the periosteum as the sole barrier (i.e., no membrane).[3]

The flap was sutured using non-resorbable 4–0 sutures (Polisoft 4–0, Sweden & Martina, Veneto 6, Due Carrare) after ensuring passivation. The sutures were removed 7 to 10 days after surgery.

Sinus Lift Using Crestal Approach

Each patient treated using crestal sinus lift protocol was given a chlorhexidine (0.20%)-based mouth rinse to be used for 60 s before surgery. Local anesthesia was achieved using 4% articaine infiltration.

A partial-thickness flap was raised to expose the crestal bone. Drills of increasing diameters were used for osteotomy until 0.5 to 1.5 mm of bone was left between the drill tip and the sinus floor. Osteotomes with smooth heads and increasing diameters were used to increase the volume of the preparation until a diameter slightly smaller than that of the implant to be used was obtained. The sinus floor was then pushed and fractured by the osteotome. The possibility of perforations of the membrane was investigated by instructing the patient to perform the Valsalva maneuver.

The graft (same as that used with the trapdoor technique) containing tetracycline and collagen particles was pushed through the implant osteotomy using osteotomes. In the test group, MSCs were added to the synthetic graft material prior to graft placement.

The implants were placed immediately, and the flap was sutured using non-resorbable sutures that were removed 7 to 10 days after the surgery.

Group Descriptions

The graft used in Group 1 contained a mixture of beta-tricalcium phosphate (80%), hydroxyapatite (20%), tetracycline, and collagen particles. The graft used in Group 2 was composed of beta-trical- cium phosphate (80%), hydroxyapatite (20%), tetracycline, collagen particles, and MSCs, which were added to the synthetic graft material prior to graft placement. Both crestal and lateral approaches for sinus lift procedures have been described and were performed in patients of both groups.

Rigenera Protocol

MSCs were obtained using the Rigenera Protocol with the Rigeneracons device (Rigenera-HBW, C.so Galileo Ferraris, Torino). Rigeneracons is a hand-piece-activated centrifuge that can filter MSCs from tissue samples.

During surgical procedures, a piece of the periosteum (5 mm × 5 mm circa) was collected after the primary flap was elevated. The piece was placed in the device on the top of a grid that allowed only certain cells to pass through. A sterile saline solution (1.5 mL) was added, and the centrifuge was connected to a dental contra-angled hand-piece. The Adacons (Rigenera-HBW, C.so Galileo Ferraris, Torino) connector permits the Rigeneracons device to be attached to any dental hand piece, which was then activated at a speed of 80 rpm and 15 Ncm torque for 1 min. The holes of the grid allow only cells with a diameter of 50 μm to pass through, thereby allowing only MSCs to mix with the saline solution.

A syringe was connected to the device and used to collect the MSC-rich solution. The solution was added to the synthetic graft material (serving as a scaffold) prior to grafting.

Postoperative Procedure

All patients were prescribed oral amoxicillin and clavulanic acid (875 mg + 125 mg) and nimesulide 100 mg twice a day for 5 days and a chlorhexidine-based mouth rinse (0.12%) for 10 days after surgery to avoid the need for brushing trauma. The use of removable prosthetic appliances was not permitted during the healing period. The sutures were removed 15 days after the surgery.

Histology and Histomorphometric Analysis

The stability of the graft of all patients was evaluated after 90 days to determine whether implant placement or loading through the use of provisional abutments and crowns could be performed. Routine bone sampling with a core milling cutter (external diameter, 3 mm; internal diameter, 2.1 mm; Hager & Meisinger) was performed in the corono-apical direction to histologically evaluate whether the bone quality was appropriate for implant loading. The samples were taken from the central edentulous portion of the crestal bone when implants were not placed and from a distance of 2 mm from the implant if implants were inserted. To ensure that the samples were obtained from corresponding sites in all patients, we attempted to obtain the samples from areas most likely to have the greatest amount of graft, without compromising the clinical outcome of the rehabilitation. The obtained samples were kept in formalin for a minimum of 48 h. Before being processed and paraffin-fixed, the samples were decalcified for 8 days in a fume hood

using a solution of formic acid (4%), which was diluted in 156 ml of formic acid and concentrated in 1000 ml distilled and deionized water. The sample solution volume ratio was 1:10 and was changed every day. Descaling lasted for approximately 8 days. Before dehydration, the specimens were washed in a saline solution.

Once processed and embedded in paraffin, a sliding microtome was used to obtain consecutive sections of 3-μm thickness, which were placed on non-charged slides and subjected to the following histochemical stains: hematoxylin-eosin (HE), Goldner's trichrome (GLD), and Azan Mallory's (AZN) trichrome.

Hematoxylin-Eosin

HE stains all basophilic substances in rose gradations and nuclei in dark purple.

Staining Protocol

The slides were washed in distilled water, stained with ferric hematoxylin for 4 to 5 min, rinsed in running water for 10 min, soaked in distilled water, stained with eosin for 1 min, dehydrated with increasing concentrations of alcohol, and immersed in xylol.

Goldner's Trichrome

GLD stains the collagen tissues green, cytoplasm red, nuclei dark blue, erythrocytes orange-red, and mature bone magenta.

Staining Protocol

The slides were washed in distilled water, stained with hematoxylin of Weigert for 2 to 4 min, rinsed in running water for 10 min, soaked in distilled water, stained with acid Ponceau fuchsin for 5 min, rinsed in 1% acetic acid, immersed in Orange G and phosphomolybdic acid for 5 minutes, rinsed in 1% acetic acid, stained with green-light for 5 min, rinsed in 1% acetic acid, dehydrated quickly with increasing concentrations of alcohol, and immersed in xylol.

Azan-Mallory Trichrome

AZN stains the collagen fibers blue, mature bone red, erythrocytes orange-yellow, and nuclei dark violet. The osteoid tissue (immature, non-calcified bone tissue) is recognized by the red color of azocarmine, while calcified bone tissue is recognized by the blue color of aniline blue.

Staining Protocol

The slides were washed in distilled water, incubated with the azocarmine solution on a stove at 56°C for 30 min, removed from the stove, and allowed to rest for 5 min. The slides were then rinsed in distilled water, immersed for 1 min in 1% alcohol solution, immersed for 1 min in 1% acidulated solution, soaked in phosphotungstic acid for 30 min, dripped, stained with a polychromic mixture (according to Mallory) for 30 min, dehydrated with increasing concentrations of alcohol, and finally immersed in xylol.

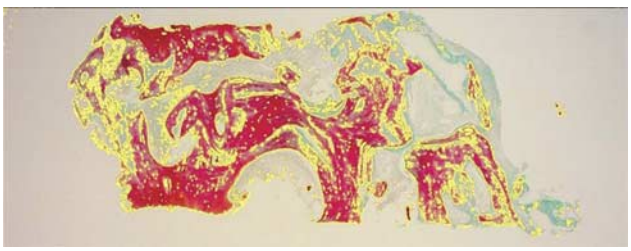

FIGURE 1. Sample tissue selection using image analysis software, ImageJ.

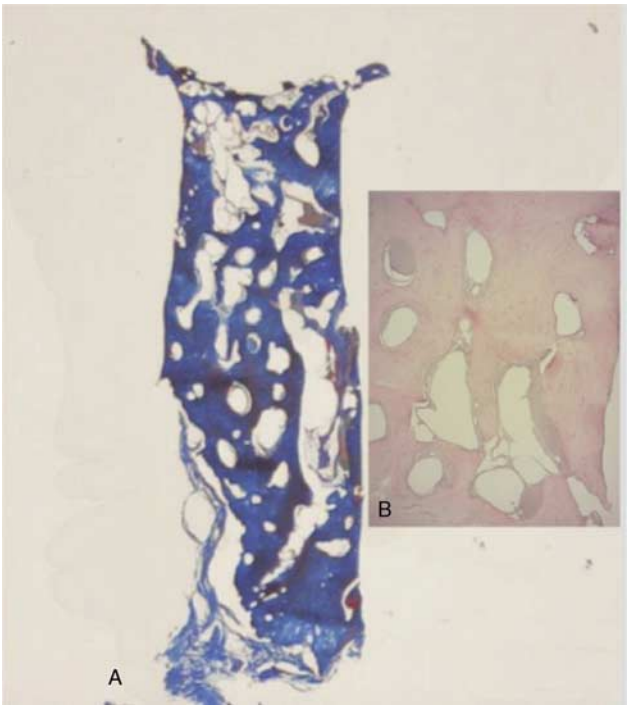

FIGURE 2. Trapdoor technique sinus lift group treated with mesenchymal stem cells and synthetic bone: (A) Panoramic view of the sample (Azan-Mallory trichrome 2.5×). (B) Detail in another color. Note the resorbing material and the significant amount of lamellar bone, medullary spaces, and vessels (Hematoxylin/Eosin 10×).

Histomorphometric Analysis

Blinded histomorphometric analysis was performed by an independent laboratory that was not a part of the research group. The stained slides were observed under an optical microscope (Zeiss, Axiophot), and pictures were taken using a digital camera (Moticam 5, Motic Asia, Kowloon Bay, Kowloon, Hong Kong) and processed using dedicated software (Motic Images Plus 2.0, Motic Asia, Kowloon Bay, Kowloon, Hong Kong).

The pictures were then sent to the personnel of Gemelli Policlinic microscopy laboratory and analyzed using the dedicated image analysis software ImageJ. The software was used to perform histomorphometric analysis and determine the percentage of newly formed bone and the total surface area of the histologic specimen (Fig. 1).

Sections of the bone areas were manually selected. To ensure a better understanding of tissue characterization, slices with different magnifications were analyzed for each sample. Tissue areas were observed under 2.5 × magnification to visualize the entire sample and avoid tissue-selection biases.

Two types of tissues were defined during histomorphometric analysis: Type 1, 'mature bone' comprising woven bone and osteo- cytes; and Type 2, 'osteoid bone' comprising a large number of osteoblasts and less organized fibers. The three staining techniques helped to distinguish the cells, analyze fiber organization, and provide evidence of tissue mineralization and the presence of blood vessels.

Cellular and non-cellular fibrous tissues were found in many samples and thoroughly analyzed while calculating the osteoid

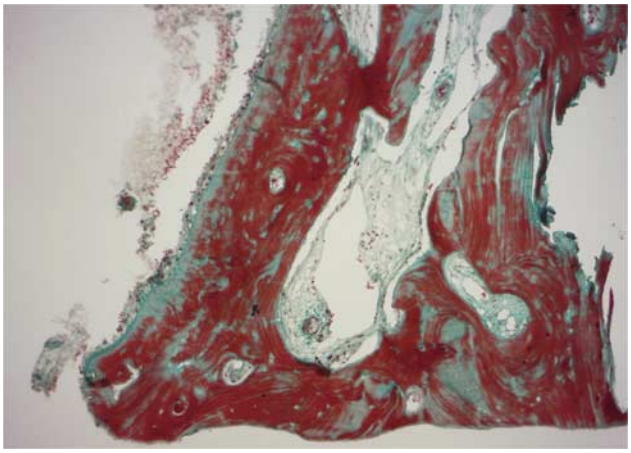

FIGURE 3. Trapdoor technique sinus lift group treated with mesenchymal stem cells and synthetic bone. Bone with intertwined lamellar fibers. Note the margin of bone neo-apposition on the left, which appears green/blue (Goldner's trichrome, 10×).

tissue areas. Fibrous tissue was not included in the statistical analyses.

The primary outcome of the study was the difference between the percentages of newly formed bone between the two groups.

Histomorphometry

For each sample, three slides stained with HE, AZN, and GLD were considered for tissue area selection. The percentage of newly formed bone was calculated on the HE-, AZN-, and GLD-stained slides to better identify the tissue characterization.

Different tissue areas were highlighted using ImageJ, and the tissue percentage over the entire sample area was calculated.

The areas of two types of bone tissue (Types 1 and 2) were delimited using ImageJ and calculated as a percentage of the total bone area on the slide (Figs. 2–6).

Statistical Analysis

The primary outcome of the study was the percentage of new bone formed three months after the surgery. The sample size

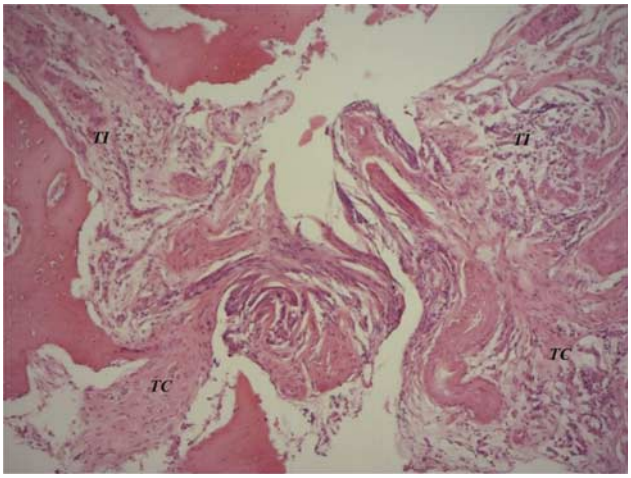

FIGURE 4. Trapdoor technique sinus lift group treated onlywith synthetic bone and without mesenchymal stem cells. Scarce bone cells immersed in connective tissue and inflammatory infiltrate (Hematoxylin/Eosin 10×). TC = Connective Tissue, TI = Inflammatory Tissue.

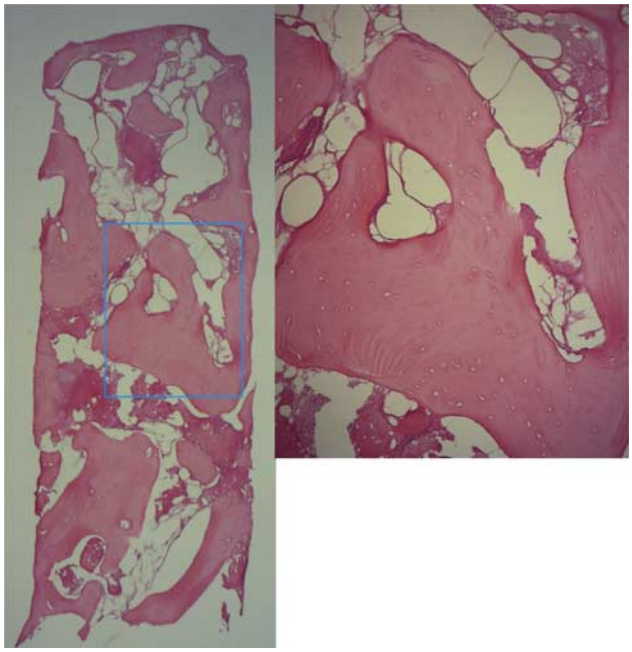

FIGURE 5. Crestal approach sinus lift group treated with mesenchymal stem cells and synthetic bone. (A) Panoramic view (Hematoxylin/Eosin, 2.5 ×) (B) Intertwined lamellar fiber bone with numerous osteocytes (Hematoxylin/Eosin 10x).

was calculated by considering the data reported by Iasella et al.[25] The study hypothesized that MSC suspension could increase the percentage of newly formed bone by 30% compared to the control group. Our study has a power of 80% and an alpha error of 0.05.

The quantitative variables were compared using the Kolmogorov-Smirnov test for normal distribution. The differences in groups compared to baseline were analyzed using the two-tailed variance analysis for parametric variables, while the Mann-Whitney or Kruskal-Wallis test was used for non-parametric variables. Binomials or discontinuous variables were evaluated using chi- squared tests and exact Fisher tests. The level of statistical significance was set at $P < 0.05$, and

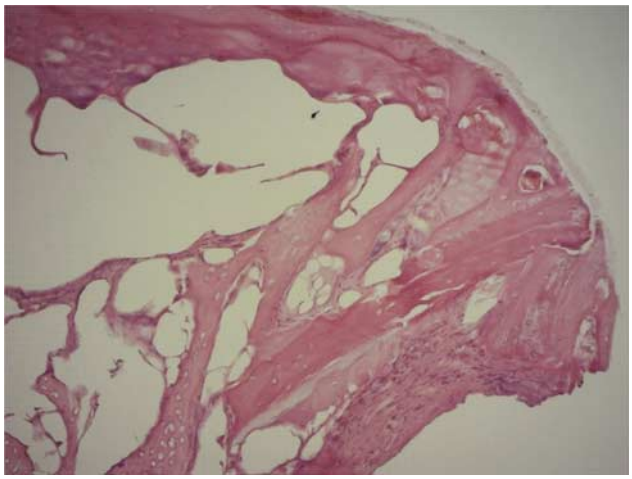

FIGURE 6. Crestal approach sinus lift group treated with synthetic bone only and no mesenchymal stem cells. Spicules of immature bone tissue with large and globular osteocyte gaps (Hematoxylin/Eosin, 10×).

statistical analysis was performed in the SPSS v. 21 for Macintosh program (IBM, Los Angeles, CA).

RESULTS

While some patients complained of mild and painless swelling on the first day after the surgery, no patient reported any major intraoperative or postoperative complications. There was no sign of inflammation in any patient one week after the surgery. The results are shown in Supplementary Digital Content, Digital Tables 1 and 2, http://links.lww.com/SCS/D513.

The mean ages of patients in Groups 1 and 2 were 59.75 years and 60.05 years, respectively.

The mean percentage area for Type 1 tissue was 27.24% in Group 1 and 44.45% in Group 2 ($P < 0.05$). The mean percentage area for Type 2 tissue was 10.86% in Group 1 and 7.04% in Group 2 ($P > 0.05$).

The mean percentage areas for Type 1 tissue after the crestal approach sinus lift were 24.52% for Group 1 and 50.78% for Group 2, while the mean percentage areas for Type 1 tissue after the trapdoor technique sinus lift were 29.18% for Group 1 and 39.92% for Group 2.

Statistical analysis showed no relevant significant differences in tissue regeneration according to sex or age.

DISCUSSION

The percentage area of Type 1 tissue in Group 2 (44.45%) was 63.18% higher than that in Group 1 (27.24%); the difference between the groups was statistically significant ($P < 0.05$). Our initial hypothesis of a ≥30% increase in Type 1 tissue in Group 2 was confirmed by histomorphometric data. The difference in the percentage of Type 2 tissue between the groups was not statistically significant.

To the best of our knowledge, this study is the first to evaluate bone formation after bone regeneration using graft containing MSCs during maxillary sinus lift procedures following a 3-month healing period; however, similar results were found in a previous study wherein bone marrow-derived MSCs were added to a hydroxyapatite (40%) and beta-tricalcium phosphate (60%) scaffold for sinus-lift procedures.[17] Evaluating six cases, the investigation collected and analyzed bone samples three months after the surgery and found a mean percentage of newly formed bone of 41.24%. Scanning electron microscope analysis showed MSC proliferation in the porous regions of the scaffold material.

A split-mouth study that performed sinus-lift procedures using allograft cellular bone matrix (ACBM) containing native MSCs and osteoprogenitor cells and conventional allograft (CA) revealed an average vital bone content of 32.5% ± 6.8% and residual graft content of 4.9% ± 2.4% in the 21 sinuses treated with ACBM after an average healing period of 3.7 ± 0.6 months.[26] The average vital bone content for cases treated using CA was 18.3% ± 10.6%, and the residual graft content was found to be 25.8% ± 13.4% after the same healing period.

The Rigenera protocol benefits from enabling the collection of MSCs without the need for a second surgical procedure. The processing of the tissue samples requires only a few minutes, and no manual manipulation of the sample is required. These features help to avoid unpredictable and expensive in vitro manipulations of cell cultures. Manipulation of samples could lead to excessive coagulation and a definite decrease in vital MSC numbers in the suspension.[27] The Rigeneracons device cannot be autoclaved and is intended for single-use only to avoid cross-contamination of the cellular suspension.

However, the relationship between age and Type 1 tissue percentage area in Group 1 patients treated using the trapdoor technique followed an inverse trend. This trend was not observed among Group 2 patients, whose results were consistent regardless of age (Supplementary Digital Content, Figure 1, http://links.lww.- com/SCS/D511 and Supplementary Digital Content, Figure 2, http://links.lww.com/SCS/D512). Our results suggest that while the benefits of autologous MSCs are unrelated to age, regular healing processes are slower in older patients when no MSCs are added to the scaffold.

Histological analysis showed the resorption of graft tissue in most cases. Only a few samples showed perfect integration of the synthetic material. Very few samples showed no synthetic graft material at all, probably due to the decalcification process that could have removed traces of tricalcium phosphate, thus leaving only hydroxyapatite granules.

To the best of our knowledge, this is the first time that the Rigenera protocol has been used in a relatively large group of patients. Our results showed that both the crestal and lateral sinus lift approaches significantly increased mature bone formation within 3 months of the surgery. Patients treated with MSCs showed a 63.18% increase in bone formation irrespective of sex and age. The relationship between the percentage of new bone formation and age in patients treated using the trapdoor technique and no MSCs added to the graft showed an inverse trend, highlighting the slowed healing processes in older patients.

The results of histomorphometric analysis confirmed the quality of the graft. MSCs can serve as the osteoinductive component in the graft, as the scaffold material does have an osteoconductive component, through a slow replacement mechanism. This effect can reduce the time required for bone graft maturation and therefore that required for insertion or loading of the implants. Furthermore, a faster and strong reconstruction of the maxillary bone can improve the biomechanical performance of dental implants.

Future prospective studies are needed to determine the longterm behavior of this graft protocol, especially to evaluate the possibility of delayed resorption. Further applications of this protocol should be explored through future research to demonstrate the potential of MSCs in bone regeneration.

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