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case report

CLINICAL AND HISTOMORPHOMETRIC OUTCOME IN FUTURE SITE DEVELOPMENT PERFORMED WITH A NANO-HYDROXYAPATITE THROUGH HYDRAULIC SINUS LIFT TECHNIQUE

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SUMMARY
Purpose. The crestal sinus lifting by means of hydraulic technique associated with a nano-cristalline hydroxyapatite in an aqueous medium is investigated.
Materials and methods. A specific purpose-made instrument is used, consisting of a syringe with a micrometrically controlled piston connected to a dispenser used to inject, in a calibrated and precise manner, noted volumes of graft material into the sub-schneiderian space. Adopting such a technique the Authors performed 7 future site developments, on 7 patients (2 males; 5 females; mean age 49.29±8.08 years), using a nanocrystalline hydroxyapatite. In the second stage, performed at 5.71±1.52 months, 7 implants were placed, after harvesting bone biopsies from the regenerated sites. The samples underwent histological and histomorphometric analysis.
Results. The average percentage of vital bone was of 33.27±5.98%, while the bone marrow and graft material were 60.84±5.93% and 5.87±3.05%, respectively.
Conclusions. The results confirm the effectiveness of this method in restoring bone volume in the sub-antral region. Furthermore nano-cristalline hydroxyapatite is a proper material for sinus floor augmentation.

Key words: maxillary sinus lift, hydraulic sinus lift, sinus floor augmentation, histomorphometric analysis.

Introduction

The crestal approach for sinus bone augmentation become increasingly popular in dental clinical practice (1-4) since its low morbidity respect to lateral approach (5-13).
Recently Andreasi Bassi and Lopez proposed a method that used graft material inserted by means of a hydraulic system to detach the antral mucosa and, simultaneously, fill the sub-schneiderian space (14-16).

The device designed for this "hydraulic sinus lift" is composed by three pieces: a syringe, a dispenser and a needle (Figure 1) as described in a previous report (14, 16).
The semi-spherical tip of the needle penetrates under the sub-schneiderian space without damaging the overlying mucosa allowing the uniform distribution of the graft material in a dome shaped way.
This technique is generally associated with simultaneous implant placement but if the thickness of the residual ridge is less than 4 mm or if the bone density is such that it does not allow for a suffi-

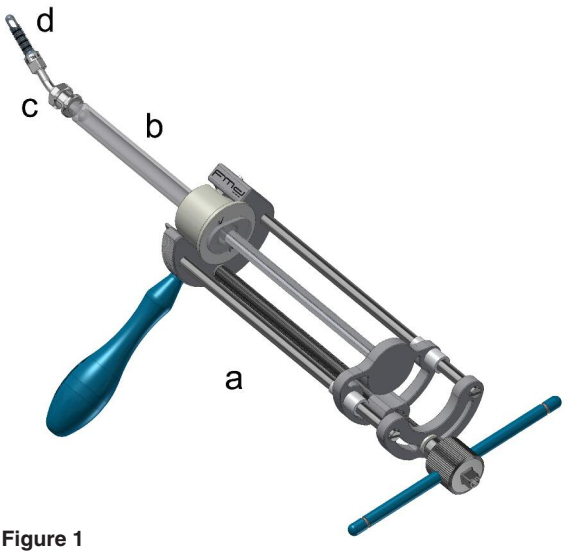

Figure 1
The Hydro-mab system assembled and composed of: a) Hydro-mab; b) syringe containing the biomaterial; c) needle; d) ML injector.

cient primary stability, a second staged implant placement is advisable. In this case it will be performed only the injection of the material and, after about 6 months, the implant insertion will be then executed.
Among the different bone substitutes it is possible to use a nano-cristalline hydroxyapatite in an aqueous medium (Nanogel, Teknimed. Vic en Bigorre, France) in order to verify if the system is suitable as further graft materials for Future Site Development (FSD).
In the present work an histological and histomorphometric analysis is carried out on bone biopsies harvested from the grafted sites filled with the above mentioned product.

Materials and methods

Patient selection and assessment

Protocol was similar to that previously reported (14, 16). Seven subjects, candidates for staged sinus lift, were screened according to the following inclusion criteria: controlled oral hygiene and absence of any lesions in the oral cavity.
The exclusion criteria were as follows: bruxism, consumption of alcohol higher than 2 glasses of wine per day, localized radiation therapy of the oral cavity, antitumor chemotherapy, liver, blood and kidney diseases, immunosuppressed patients, patients taking corticosteroids, pregnant women, inflammatory and autoimmune diseases of the oral cavity and maxillary sinuses.
The endoral X-ray exam of the seven edentulous sites showed a reduced thickness of the residual bone (Figure 2) which was then confirmed by the Denta Scan CT exam (NewTom 5G®, QR, Verona, Italy). Residual crestal bone levels were evaluated by the calibrated examination of digital intra-oral radiographs before the surgery (PSPIX, Sopro Acteon Imaging, France) measuring the lowest thickness of the edentulous crest. The measurement was rounded off to the nearest 0.1 mm. The same procedure was adopted to evaluate the graft increment measuring the vertical distance between the sinus floor and the top of the dome-shape elevation obtained after graft insertion.

Clinical procedure

All patients underwent the same surgical protocol as previously reported (14, 16): an antimicrobial prophylaxis was administered with amoxicillin clavulanate (Clavulin, GlaxoSmith Kline, Italy), 1g every 8h for 7 days, begun 3 hours before the operation. After an initial rinse with chlorhexidine digluconate 0.2% (Corsodyl Mouthwash, GlaxoSmithKline, Italy), for 1 minute to disinfect the mouth, loco-regional anesthesia was performed with articaine hydrochloride 4% with epinephrine 1:100000 (Citocartin, Molteni Dental, Italy). Having exposed the bone plane through an envelope flap, the bony tunnel and the subsequent controlled discontinuation of the sinus floor were performed using specifically designed 2.8 mm drills (Sinus Lift Drill, FAL-LIFT, FMD, Rome, Italy), followed by the positioning of the dispenser ML

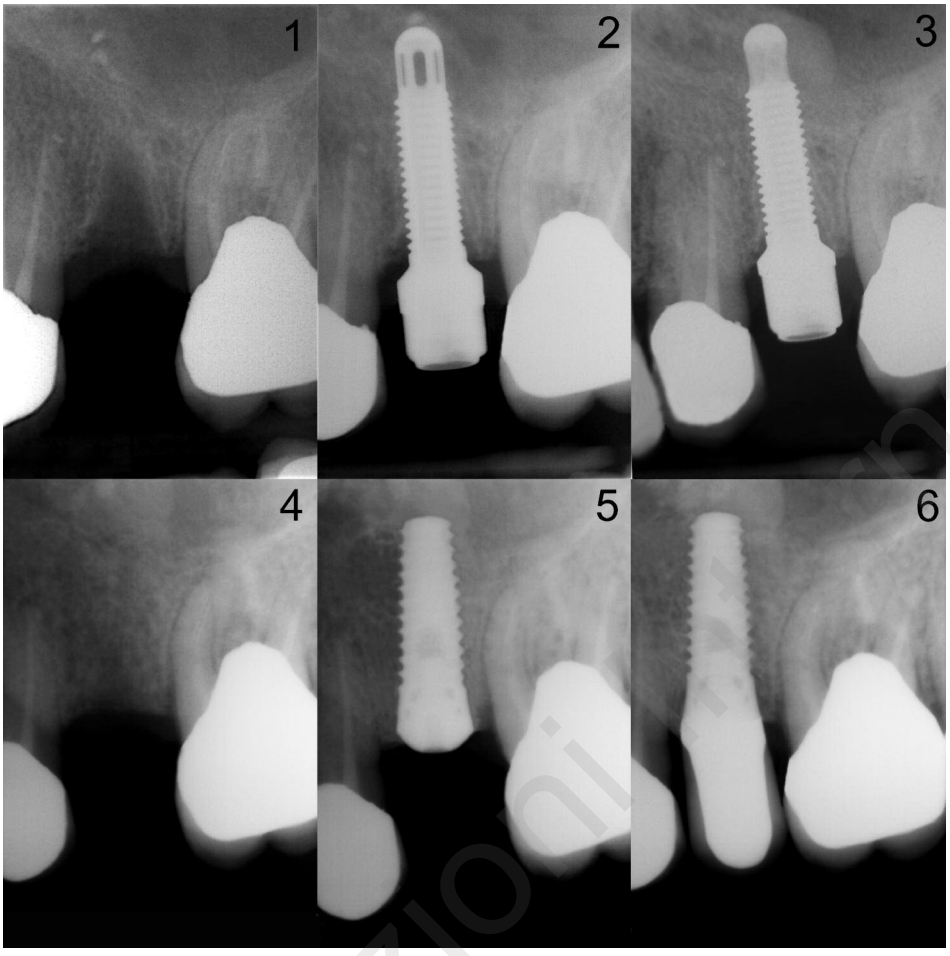

Figure 2
Intra-oral X-rays of a clinical case. 1) Pre-operatory; 2) intra-operatory showing, on site 2.5, the protrusion in the sub-antral space of the ML injector. 3) Verification of the dome elevation; 4) evaluation, of the graft integration, after 6 months post-operative; 5) Implant *in situ*; 6) one year after prosthetic finalization.

(3.2 cylindrical) preloaded with the graft material. Before connecting the ML to the Hydro-mab a radiographic check was performed in order to evaluate the protrusion of its nozzles in the lumen of the maxillary sinus. Progressively an amount, ranging from 0.5 to 1 ml, of material was injected, in a span of 3-5 minutes, followed by a radiographic check before the removal of the ML. The bony tunnel was completely filled with the same graft material used for the sinus floor elevation, and covered with a resorbable membrane (Evolution STD, Osteobiol by Tecnoss, Italy) prior to the suture of the flaps. Ibuprofen (Brufen 600 mg, Abbot, Italy), every 8-12 hours for 5 days was administered to control post-operatory pain and edema. Rinses with chlorhexidine digluconate 0.2% (Corsodyl Mouthwash, GlaxoSmithKline, Italy) were prescribed for the disinfection of the surgical wound, 2/3 times /day for 7 days. After 12 days the sutures were removed and oral hygiene instruction was provided. The clinical performance of the patients was evaluated analyzing the occurrence of some post-operative complications: bleeding; swelling; pain; hematoma; wound dehiscence; lack of graft integration.

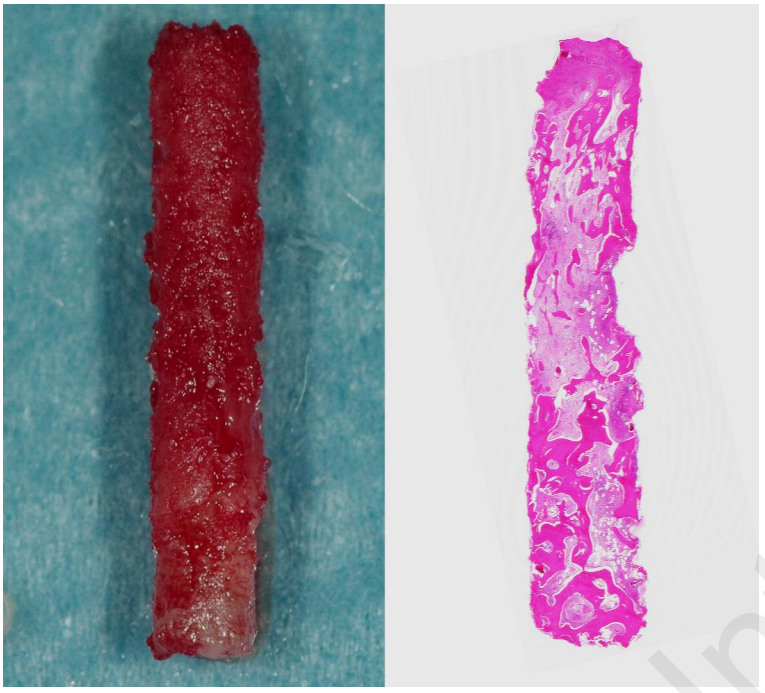

Figure 3
In each edentulous area, submitted to Future Site Development, a bone biopsy was harvested prior to the implant placement (left) and then processed for the histologic and histomorphometric analysis (right).

After a suitable period of time needed for the consolidation of the graft (mean value 5.71±1.52 months), the bone regeneration of the sites was assessed by means of endoral X-ray exams prior to the surgical procedure.

At re-entry the implant placement was performed with a flap elevation approach. The implant tunnel was executed by means of a trephine drill in order to harvest a bone biopsy of the regenerated area (Figure 3). Two-piece implants (FMD, Rome, Italy) were placed and healing screws were placed immediately prior the suture of the flaps. Drug treatments before and after surgery was identical to those of the first-stage surgery.

After 4 months, a progressive management of the prosthetic load, was carried out and protracted for a period of 4 months, through temporary resin crowns screwed onto preformed Peek abutments. The cases were finalized with cemented metal-ceramic crowns, on preformed titanium screwed abutments. All patients were included in a strict hygiene recall. A year after prosthetic finalization, a clinical and radiographic follow-up of the cases was performed in order to verify the condition of the soft and hard peri-implants tissues.

Samples processing and analysis

The biopsies as previously reported (14, 16), harvested from the sites of implant placement, were fixed in 10% formalin buffered with phosphate buffer (pH 7), demineralised with a descaling solution containing EDTA (Kaltek, Padua, Italy), dehydrated in a scale of increasing alcohol content, embedded in paraffin and, finally, sectioned along the major axis of the biological samples using a microtome (Leitz 1512, Germany). The sections obtained were stained with haematoxylin-eosin and observed under an optical microscope and polarized transmitted light (Leitz Dialux, Germany) (Figure 3). The aforementioned microscope was equipped with a digital camera (Nikon Coolpix 990, Japan)

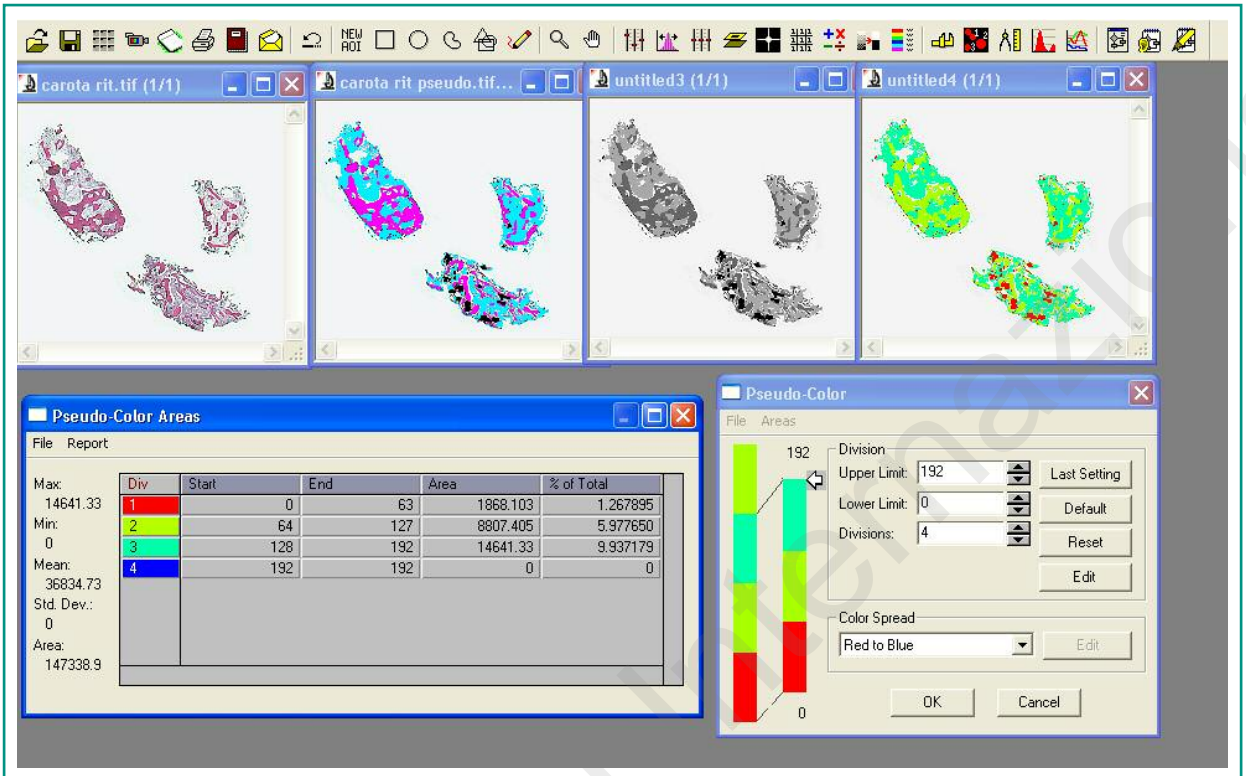

Figure 4
Histomorphometric analysis of a sample. After identification and discriminative colouring of the 3 components (bone, marrow, graft), the image is primarily converted into an 8-bit grayscale and then into pseudo-colours in order to calculate the percentage extension of each area.

making it therefore possible to photograph the samples, in uncompressed TIFF format, at different magnifications (10X-25X-40X-100X-250X). The images at lower magnification (10X-25X) were initially processed with digital image processing software (Adobe Photoshop CS3 Extended vers. 10.0.1, Adobe Systems Inc, U.S.) in order to allow discrimination between the mineralized bone, the bone marrow and the graft material. Subsequently, the images were subjected to histomorphometric analysis, using dedicated software (Image-Pro Plus 4.1, Media Cybernetics Inc., U.S.), first converted into a 8-bit grayscale and afterwards to pseudo-colours in order to assign a percentage to the areas occupied respectively by the three materials in question. The areas occupied by each colour were thus calculated and weighted on the total area of sampling (Figure 4).

Results

Clinical results

The thickness of the residual ridge and the elevation had a mean value of 4.74±1.47 mm and 6.06±1.22 mm, respectively (Table 1). In most of the cases the implant placement was in the area of the first molar (57.14%). The occurrence of post-operative complications, in the first stage, was mild and limited to swelling (14.3%) and pain (14.3%) (Table 2).

The graft material observed by means of an intraoral X-ray exam, executed immediately after the elevation procedure, showed an amorphous structure and a reduced radiopacity while, in the subsequent months, acquired a trabecular structure with an increase in opacity.

Table 1 - Table showing all the parameters analysed.

	Implants	Sex	Age (years)	Site	Residual Ridge (mm)	Elevation 1^ stage (mm)	Elevation 2^ stage (mm)	Elevation after 1y (mm)	Re-entry (months)	Bone (%)	Marrow (%)	Graft (%)
	1	F	43	1.6	3.0	5.7	5.2	4.9	7.5	27.4	68.0	4.6
	1	F	50	1.6	6.1	6.3	5.4	5.0	8.0	32.6	59.0	8.4
	1	M	54	1.5	7.1	8.1	7.6	6.8	6.0	35.1	63.4	1.5
	1	M	37	2.5	4.9	5.2	4.5	4.0	4.0	45.3	51.1	3.6
	1	F	45	2.6	3.8	4.7	4.2	3.8	5.0	30.5	62.6	6.9
	1	F	60	2.4	4.8	5.2	4.6	4.0	4.5	33.6	55.8	10.6
	1	F	56	2.6	3.5	7.2	6.3	5.5	5.0	28.4	66.0	5.6
Mean			49.286		4.743	6.057	5.4	4.857	5.714	33.271	60.843	5.886
St. Dev.			8.077		1.466	1.223	1.196	1.064	1.523	5.979	5.933	3.049

Table 2 - The occurrence of post-operative complications.

Post-operative complications	Cases
Bleeding	0
Swelling	1
Pain	1
Hematoma	0
Wound deiscence	0
Lack of graft integration	0

The intraoral X-ray analysis performed before the second stage of surgery showed that the volumes of the graft bone achieved in the first phase were preserved, presenting an average contraction of 10.8%. After one year the graft showed a further contraction (mean value 9%) probably due to bone remodelling process (Table 1).

At the time of re-entry no crestal defects were found as a result of the bone tunnel created for the ML application. At the 1 year follow-up, after prosthetic finalization, all implants were in place. The clinical appearance of the soft tissues was optimal and no pathological signs were recorded upon probing. Radiographic examination did not show substantial changes in the peri-implant bone volume in accordance with success rate parameters (17).

Histological and histomorphometric results

The histological examination of the samples shows spongy bone tissue in various stages of remodelling: there are areas of newly formed bone in which the cellular components are visibly active and dispersed areas in which the bio-material is integrated with the newly formed bone tissue or otherwise wrapped in connective tissue (Figure 5). They are no visible cellular elements indicating inflammation or immune reaction.

Histomorphometric analysis of all samples, examined in their entirety, shows that the mean percentage occupied by mineralized bone was 33.27±5.98%, while the bone marrow and graft material were 60.84±5.93% and 5.87±3.05%, respectively (Table 1).

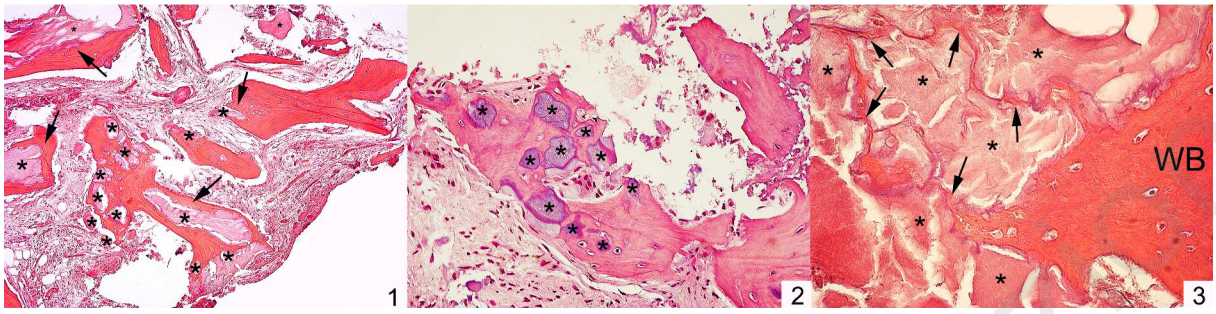

Figure 5
Histological analysis of the samples. 1) The graft material is dispersed in the form of islands of amorphous aspect (asterisks) and mainly surrounded by bone trabeculae formed by secondary osteons (arrows) (original magnification 100X); 2) spongy bone in various stages of remodelling. Several islands of graft material, surrounded by mineralized bone, can be observed (asterisks) (original magnification 250X); 3) woven bone (WB) in close contact with the graft material (asterisks). The biomaterial is separated by thin septa of newly formed bone (arrows) (original magnification 400X).

Discussion

Grafting the sub-schneiderian space of maxillary sinus floor is possible by means of hydraulic systems as previously reported (16). This is possible because this technique exhibits a reduced invasiveness, an high precision related to the micrometer control of piston progression, reduced operative time and a brief learning curve. The procedure is indicated both as a single or two stage surgical methods. In the first case sub-antral mucosal bone grafting and immediate implant placement through the hole of graft injection is performed, whereas the two stages surgery is ideal when the residual bone thickness is less than 4 mm or bone density does not guarantee the fixture primary stability (15, 16, 18-30).

In the present report the histological and histomorphometrical analysis of grafted bone indicates both the reliability of the surgical method and of the nano-cristalline hydroxyapatite in aqueous medium to increase bone volume of atrophic maxillary sinus.

The osteointegration process of nano-cristalline hydroxyapatite is histologically proved by the few residual amount of grafted material that is appreciable in the biopsies as well as the gradient of native bone progressively substituting the graft starting from the sinus floor (15, 31, 32) (Figure 6).

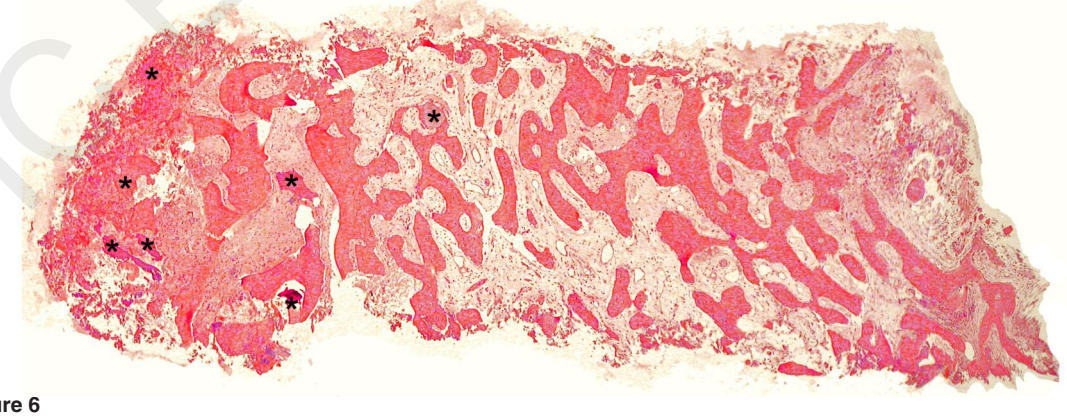

Figure 6
The amount of graft material (asterisks) seems to follow a gradient increasing from the residual ridge (right) towards the top of the dome (left). This phenomenon is likely attributable to the mitigation of the osteoconductive potential shown by the graft material.

The high mean rate of bone marrow observed (60.84±5.93) is attributable to reduced bone density in the posterior maxilla.

No major complications were observed during the execution of this surgical technique as a consequence that this technique is less invasive (Table 2).

The grafted material became progressively visible at X-rays controls since it reflects the mineralization process of the inserted material. This process confirms the clinical outcome where the implant is stabilized by the new bone in sinus floor.

Bone regeneration should be performed with adequate material after controlling the periodontal disease since it can have an impact on peri-implantitis onset (10, 33).

In conclusion, the present report demonstrated that both crestal sinus lift by means of hydraulic system is a reliable surgical procedure as well as nano-cristalline hydroxyapatite in an aqueous medium is a good material for sinus floor augmentation.

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