Case Series

Sinus Floor Augmentation With Simultaneous Implant Placement Using Choukroun's Platelet-Rich Fibrin as the Sole Grafting Material: A Radiologic and Histologic Study at 6 Months

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Background: *Sinus augmentation with simultaneous implant placement without bone graft material is a hotly debated technique. This technique could be improved and secured by the use of an autologous leukocyte- and platelet-rich fibrin (PRF) (Choukroun's technique) concentrate. The objectives of this study were to assess the relevance of PRF clots and membranes as the sole filling material during a lateral sinus lift with immediate implantation using radiologic and histologic analyses in a case series.*

Methods: *Twenty-five sinus elevations with simultaneous implantation were performed on 20 patients with Choukroun's PRF as the sole filling biomaterial. For each patient, a presurgical exam and a 6-month postsurgical radiologic exam were performed with a panoramic x-ray and three-dimensional volumetric computed radiography (VCR) to evaluate the subsinus residual bone height and the final bone gain around the implants. In nine patients, 6 months after the sinus lift, bone biopsies were collected on the buccal wall of the alveolar ridge at the level of the osteotomy window, and evaluated by histomorphometry.*

Results: *In this study, 41 implants from three different systems with different screw designs (Biomet 3I Nanotite, MIS Seven, Intra-Lock Ossean) were placed. All implants were inserted in residual bone height between 1.5 and 6 mm (mean ± SD: 2.9 ± 0.9 mm). The final bone gain was always very significant (between 7 and 13 mm [mean ± SD: 10.1 ± 0.9 mm]). No implant was lost. After radiologic analyses, the position of the final sinus floor was always in the continuation of the end of the implant. All biopsies showed well organized and vital bone.*

Conclusions: *From a radiologic and histologic point of view at 6 months after surgery, the use of PRF as the sole filling material during a simultaneous sinus lift and implantation stabilized a high volume of natural regenerated bone in the subsinus cavity up to the tip of the implants. Choukroun's PRF is a simple and inexpensive biomaterial, and its systematic use during a sinus lift seems a relevant option, particularly for the protection of the Schneiderian membrane.* J Periodontol 2009;80:2056-2064.

KEY WORDS

Blood platelets; bone regeneration; dental implants; fibrin; maxillary sinus; wound healing.

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A sinus lift for implant placement is considered one of the most predictable procedures for augmenting bone in the maxilla. Several approaches have been developed and are currently used.

The lateral approach using a Caldwell-Luc osteotomy is historically the first main technique, where the maxillary sinus floor is grafted to provide a sufficient quantity of bone for the placement of endosteal dental implants. A current issue is the definition of the best filling material for the sinus cavity after lifting the sinus membrane.[1] Considering the high osteogenic potential of the Schneiderian membrane and its periosteum-like behavior, the consensual approach is to consider that most materials, bone substitutes or autologous bone, are efficient in this situation.[2-5] Using this approach, implant placement can be performed in one or two surgical stages depending on the residual alveolar bone height. A minimum of 4 to 5 mm was recommended for a one-stage surgical procedure (simultaneous implant placement), but data published since 1998[6-8] have shown that using an appropriate implant design (tapered or microthreaded) and/or an optimal surgical technique (to reach implant stability),[7,8] sinus floor augmentation with simultaneous implant placement can be performed in cases of 1 to 2 mm of residual alveolar bone height with predictable results during a follow-up >10 years.

The axial approach using the Summers osteotomy was developed to simplify the sinus-lift procedure using simultaneous sinus floor elevation and implantation without the surgical opening of the sinus cavity.[9,10] The objective of this approach is to use the natural osteogenic properties of the Schneiderian membrane to gain the missing millimeters of bone around the tip of the implants. This less invasive technique is an attempt to reduce the grafting volume to the strict minimum and generate only the required bone volume needed for the adequate osseointegration and anchorage of the implants.[11] Implant stability in the residual bone height is a key issue, just as in the one-stage lateral sinus lift, and the use of implants with a microthreaded and/or tapered collar may be a relevant option to stabilize implants in a limited bone volume.[12-14]

The choice of the technique, a lateral approach using the Caldwell-Luc osteotomy or an axial approach using the Summers osteotomy, is mainly dependent on the residual bone height of the alveolar ridges. Currently, most simple cases can be treated with the Summers osteotomy technique, which implies less pain and no waiting time between grafting and implantation.[11] However, the lateral approach offers a better control of the surgical site, particularly in a severely resorbed maxilla or when extensive implantation is needed. Both approaches showed similar results in the literature.[6-8,11-14]

Recently, a third approach was developed based on the concept of guided bone regeneration.[15] Several authors showed that a full sinus lift can be performed using the lateral approach with whole blood as the sole filling material.[16,17] This strategy requires the implants to be stabilized in the residual bone height (particularly by using implants with tapered and microthreaded collars) and to maintain the Schneiderian membrane pushed in the highest possible position using implant tips as tent pegs. This concept of bone regeneration leads to a very natural bone reconstruction around implants. However, this technique requires a very skilled surgeon because a perfect sinus membrane lifting without tears is necessary to maintain its osteogenic potential. Filling the sinus cavity with a stabilized blood clot remains quite difficult to control. The use of blood preparations such as platelet concentrates or fibrin glues might seem an interesting option to improve this sinus-lift approach, but such preparations are often expensive and complicated to prepare.[18]

Choukroun's platelet-rich fibrin (PRF) was first described by Choukroun et al.[19] in France in 2001. It is a simple, natural, and inexpensive technique for the production of leukocyte- and PRF (L-PRF) concentrates:[18] blood is collected without anticoagulant and immediately centrifuged.[20] Coagulation starts during the centrifugation, and three parts quickly appear in the tube: a red blood cell base at the bottom, acellular plasma as a supernatant (platelet-poor plasma), and the PRF clot in between. The clot can be transformed into a membrane by compression between two sterile gauzes or preferentially by using a specific tool¶ for clot collection and membrane standardization. Moreover, the protocol is very simple, and many PRF clots can be produced in <20 minutes.

PRF is a consistent fibrin biomaterial and not an improved fibrin glue from the platelet-rich plasma (PRP) family.[21] Each PRF membrane concentrates most platelets and more than half of the leukocytes from a 9-ml blood harvest.[22,23] Platelets are merged within the fibrin meshes like a cement, but enmeshed leukocytes are alive and functional into the dense fibrin network.[24] Moreover, PRF releases high amounts of growth factors (such as transforming growth factor-β1 [TGFβ-1], platelet-derived growth factor-AB [PDGF-AB], vascular endothelial growth factor [VEGF]), and matrix glycoproteins (such as thrombospondin-1) during at least 7 days in vitro.[25] Thus, this biomaterial presents a specific biology.

Some PRF applications were already described in oral and maxillofacial surgery,[26-29] ear, nose, and

¶ PRF Box, Process, Nice, France.

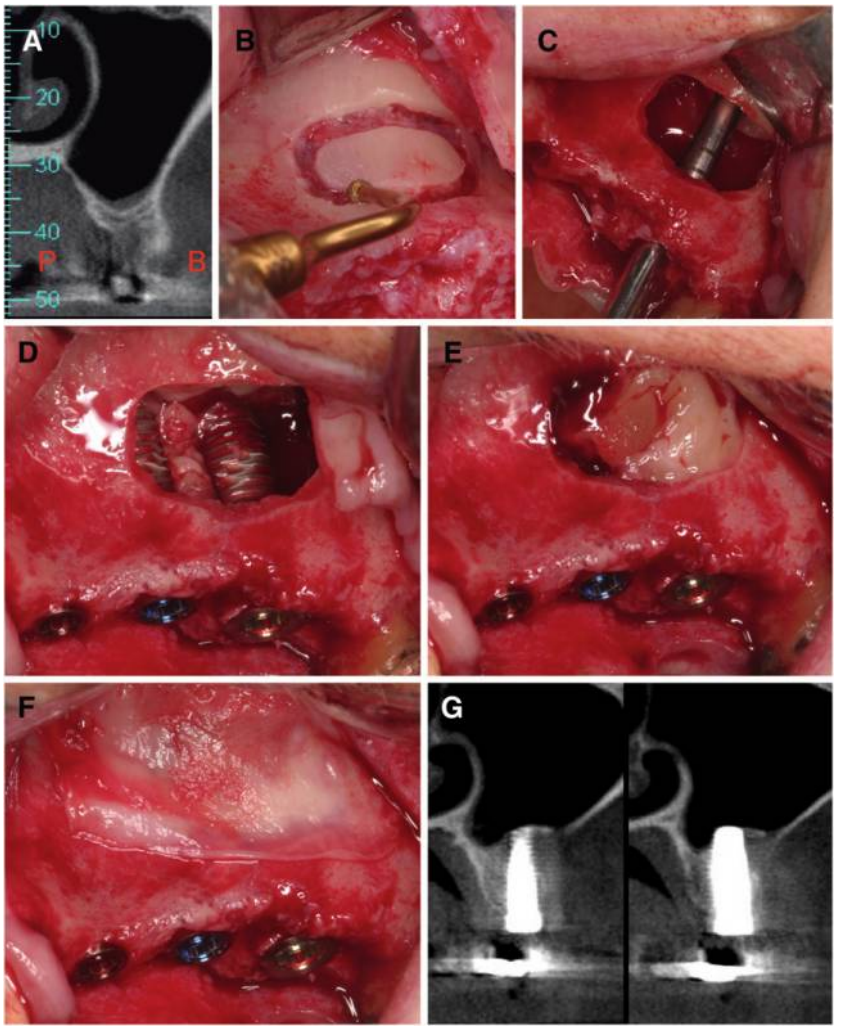

Figure 1.
A) *X-ray three-dimensional (3D) examination showing ~2 mm residual bone height in the posterior maxilla. P = palatal; B = buccal.* ***B)*** *Lateral osteotomy was performed using an ultrasonic lancet.* ***C)*** *Implant sites were drilled and checked with a manual osteotome.* ***D)*** *PRF membranes covering the sinus membrane. Implants were placed and blocked in the residual bone height with their tapered collar. Implant tips were positioned to maintain the PRF-patched sinus membrane in a high position and served as tent pegs.* ***E)*** *The subsinus cavity was filled with compressed PRF clots.* ***F)*** *A final PRF membrane was used to cover the lateral osteotomy window.* ***G)*** *Six months after surgery, the x-ray 3D examination showed implants surrounded with a bone-looking dense tissue up to the tip of the implant.*

throat and plastic surgery,[30,31] and in preimplant and implant surgery.[32-34] PRF stimulates many different kinds of cells, particularly the proliferation and differentiation of osteoblasts.[24] The use of PRF during sinus-lift procedures has been advocated for many years during lateral sinus-lift[28,33,34] or vertical osteotome augmentation.[13]

The objectives were to assess the relevance of PRF clots and membranes as the sole filling material during a lateral sinus lift with immediate implantation using radiologic and histologic analyses in a case series.

MATERIALS AND METHODS

Patient Selection and Study Design

This case series consists of 25 sinus elevations performed on 20 patients between June 2007 and June 2008 in a private practice in Ra'anana, Israel, with Choukroun's PRF as the sole filling biomaterial. As the literature[15] does not contraindicate this approach for a sinus lift, no ethical problems were raised. The study was conducted in accordance with the standards of the Declaration of Helsinki. The patients were informed about the aim and design of the study, and written consent was obtained.

Patients with immunologic diseases, unstable diabetes mellitus, ongoing chemotherapy or radiotherapy, or other contraindicating systemic conditions were excluded. The inclusion criteria included having a blood concentration of thrombocytes within the normal range and an absence of acute maxillary sinus inflammation. Patients had to be compliant during their preliminary periodontal treatment, to accept the required follow-up, and to show no or a minor smoking habit (less than five cigarettes per day). The clinical examination and preoperative radiographs showed atrophy of the maxilla in the premolar/molar area that required a sinus lift before implantation. All of the cases in this preliminary series needed relatively small sinus lifts, with only one or two implants required per sinus. For each patient, a presurgical radiologic exam was performed using a first panoramic x-ray and low-dose volumetric computed radiography (VCR)# to evaluate the subsinus residual bone height (Fig. 1A).

The patients included 14 females (70%) and six males (30%) with a mean age of 54.1 ± 5.2 years (range: 41 to 65 years). Two patients were smokers but smoked less than five cigarettes per day. Presurgical standard blood analyses showed normal blood variables, particularly platelet and leukocyte concentrations.

The subsinus residual bone height was evaluated between 1.5 and 6 mm (mean ± SD: 2.9 ± 0.9 mm): most implant sites (75%) showed between 1.5 and 3 mm of residual bone height, and the other sites (25%) showed >3 mm residual bone height. The width

ICAT, Imaging Sciences International, Hatfield, PA.

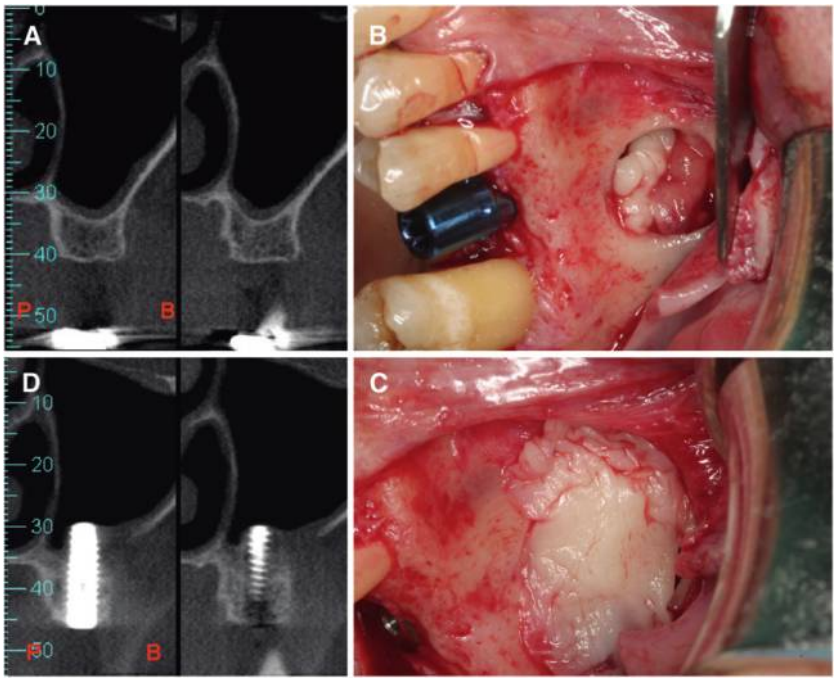

Figure 2.
A) *X-ray 3D examination before surgery showed a 6-mm residual bone height in the first molar region. P = palatal; B = buccal.* ***B)*** *A sinus was elevated and filled with PRF clots. A 15-mm long-screw implant with a tapered and microthreaded collar was easily blocked in the residual bone height and kept the PRF-patched sinus membrane in a high position.* ***C)*** *Two PRF membranes were used to cover the lateral osteotomy window.* ***D)*** *Six months after surgery, the x-ray 3D examination showed the implant surrounded with a bone-looking dense tissue up to the tip.*

of the alveolar bone ridges was considered a non-interfering parameter because the width was always sufficient for a secure implantation.

Three different implant systems were used in this study to evaluate the more adequate implant shape and design for this specific application, where implants had to be inserted and stabilized in a very limited residual bone height. Six patients were treated with a total of nine tapered screw implants** (Fig. 1), nine patients were treated with a total of 19 tapered screw implants with a microthreaded collar†† (Fig. 2), and five patients were treated with a total of 13 tapered screw implants with a microthreaded collar and a different screw design.‡‡ Implants were inserted under clean, but not sterile, conditions as defined by Scharf and Tarnow.[35]

PRF Preparation

PRF clots and membranes were prepared as described by Choukroun et al.[19] During surgery, 72 ml whole blood was drawn into eight glass-coated plastic tubes without anticoagulant and was immediately centrifuged at ~400 × g for 12 minutes using preparation kits and a centrifuge specifically designed for this application.§§[36] The coagulation cascade lead to the formation of a natural fibrin clot in the middle of each tube. This PRF clot gathered most platelets and more than half of the leukocytes from the initial blood harvest. This clot was removed from the tube and prepared as previously described. Clots and membranes were stored in metal cups before sinus filling. Five clots and three membranes were produced for the treatment of each sinus.

Surgical Technique and Postoperative Management

Surgery was performed with local anesthesia. Access to the buccal maxillary wall was achieved via a mucosal crestal incision, anterior and posterior releasing vestibular incisions, and full-thickness flap elevation. A bone window was outlined using a diamond insert in an ultrasonic lancet|||| with constant saline irrigation (Fig. 1B).[37,38] After careful elevation of the Schneiderian membrane without perforation, the bone window was left attached to the membrane and served as a new sinus floor. The size of the window was dependent on the number of implants required for the treatment, but it was always kept as small as possible to protect the osteogenic potential of the sinus cavity.

One or two PRF membranes were placed on the Schneiderian membrane to patch and heal all visible or invisible holes and tears of the sinus membrane. Implant sites were prepared with careful undersized drilling. The final stage of osteotomy was performed with the implant serving as a manual osteotome. The implant is thus inserted in compression within the residual alveolar bone (Figs. 1C and 1D). Implant stability was always obtained due to the tapered profiles and, when available, the microthreaded collars of the implants. The end of the implants always touched the PRF-patched sinus membrane, and served as tent pegs. Considering the sinus cavity morphology, the nasal side of the implant surface was in direct contact with the PRF patching membranes (Fig. 1D).

Five PRF clots were inserted in compression inside the sinus cavity to fill all of the volume stabilized with the implants (Fig. 1E). Finally, one or two PRF membranes were used to cover the osteotomy window and protect the filled sinus from potential mucoinvagination (Fig. 1F).

** Nanotite Certain, Biomet 3i, Palm Beach Gardens, FL.
†† MIS Seven, Shlomi, Israel.
‡‡ Intra-Lock Ossean, Intra-Lock, Boca Raton, FL.
§§ Process, Nice, France.
|||| Piezosurgery, Mectron, Carasco, Italy.

For postoperative management, medications were prescribed, including chlorhexidine rinses twice a day for 14 days, 1 g amoxicillin two times daily for 6 days (pristinamycin, 500 mg × 2, two times daily, was used for penicillin-sensitive patients), ibuprofen (400 mg) four times daily unless medically contraindicated, and pain medication as needed for pain. Patients were not allowed to use any removable prosthesis. The sutures were removed 8 to 10 days postoperatively, and a panoramic x-ray was taken to check the position of the implants.

Radiographic Follow-Up, Prosthetic Rehabilitation, and Bone-Sample Harvesting

For each patient, 6 months after sinus-lift surgery, a radiologic exam was performed using a panoramic x-ray and low-dose VCR[¶¶] to evaluate the sinus bone gain around each implant (Fig. 1G) and validate the next step of the treatment. After surgical uncovering, all implants had healing screws placed at 25 Ncm. At a later date, impressions were taken, and implant-supported metal-ceramic crowns were placed within 2 to 4 weeks thereafter. During placement of healing screws in nine patients, bone biopsies were collected using a trephine on the buccal wall of the alveolar ridge at the level of the bony window used for the sinus lift.

The aim of the radiographic analysis was to determine, on the reconstructed x-ray pictures, the final bone gain around each implant 6 months after sinus-lift surgery. Thus, each VCR was analyzed using the proprietary VCR software. Before surgery, three measurements of the residual bone levels were performed on each implant site. Six months after treatment, three measurements of the bone levels were performed per implant (1 mm mesial, 1 mm distal, and in the center of the implant). For each implant, the mean bone gain was calculated. In two cases, the first VCR exam was performed with residual teeth before avulsion. Thus, the evaluation of the residual bone height before the sinus lift in these patients was slightly overestimated due to these teeth.

Histologic Preparation and Histomorphometry of Bone Biopsies

Bone biopsies were harvested during the uncovering and placement of healing screws 6 months after the sinus-lift procedure. The trephine drilling was perpendicular to the bone wall in the center of the regenerated osteotomy window of the sinus lift. The trephines containing the bone were fixed in 10% neutral buffered formalin. Upon receipt in the laboratory (Minneapolis, Minnesota and Gothenburg, Sweden)., the specimens were immediately dehydrated with a graded series of alcohols for 9 days. After dehydration, the specimens were infiltrated with a light-curing embedding resin.[##] After 20 days of infiltration with constant shaking at normal atmospheric pressure, the specimens were embedded and polymerized by 450 nm light with the temperature of the specimens never exceeding 40°C. The specimens were prepared by the modified cutting/grinding method.[39,40] The specimens were cut to a thickness of 150 μm on a cutting/grinding system.[***] The slides were polished to a thickness of 45 μm using the microgrinding system followed by an alumina polishing paste. The slides were stained with Stevenel's blue and Van Gieson's picro fuchsin. After histologic preparation, the cores were evaluated morphometrically. All of the cores were digitized at the same magnification using a microscope[†††] and a digital camera.[‡‡‡] Histomorphometric measurements were completed using a combination of picture-treatment software[§§§] and a public-domain image program.[‖‖] Two slides of each core were evaluated. The parameters evaluated were the total area of the core and the percentage of new-bone formation.

RESULTS

This case series consists of 25 sinus elevations performed on 20 patients who fulfilled the inclusion criteria and were treated with 41 implants. No clear sinus membrane perforation was observed, probably due to the soft sinus-lifting procedure with an ultrasonic lancet. After surgery, healing was uneventful for all patients. Six months after surgery, all implants were clinically stable during abutment tightening.

This study was designed for the validation of the PRF as a filling material. Thus, it was important to discard any implantation-related parameters. To simplify as much as possible the data analysis, most implants showed similar lengths and widths. Globally, 37 implants were 13 mm long, two implants were 11.5 mm long, and two implants were 15 mm long. In diameter, five implants were 3.25 mm wide, 18 implants were 3.75 mm wide, three implants were 4 mm wide, 13 implants were 4.3 mm wide, and two implants were 5 mm wide.

All implants were inserted in a residual bone height between 1.5 and 6 mm (mean ± SD: 2.9 ± 0.9 mm). Early postoperative panoramic radiographs (8 to 10 days after surgery) showed implants inserted in the sinus cavity without dense tissue around them, PRF filling being radiotransparent. However, 6 months after the sinus lift, the sinus cavity around the implants was filled with a dense bone-like tissue. Radiographic analysis showed that the final bone gain was always

¶¶ ICAT, Imaging Sciences International.
Technovit 7200 VLC, Kulzer, Wehrheim, Germany.
*** EXAKT Technologies, Oklahoma City, OK.
††† Axiolab, Carl Zeiss MicroImaging, Thornwood, NY.
‡‡‡ Coolpix 4500, Nikon, Melville, NY.
§§§ Photoshop, Adobe Systems, San Jose, CA.
‖‖ NIH Image, National Institutes of Health, Bethesda, MD.

very significant with these quite long implants (bone gain: between 7 and 13 mm [mean ± SD: 10.1 ± 0.9 mm). In this case series, no implant was lost, leading to a 100% success rate after 6 months.[41]

No statistical comparison between the different implant systems was performed to define which implant system was more efficient for bone gain around implants. Indeed, after radiologic analyses, the position of the final sinus floor was for all cases in the continuation of the end of the implant (Fig. 2). In this technique, implants were used as tent pegs to define the required bone volume, and the implant shape did not seem to influence the position of the new sinus floor.

All biopsies showed well organized and vital bone (Fig. 3), often with >30% bone matrix (mean ± SD: 33% ± 5%). No bone substitutes were used in this case series, and the biopsies were taken in the center of the regenerated osteotomy window of the sinus lift. Therefore, all of the observed bone must be considered new bone built starting from the sole PRF fibrin matrix. At a low magnification, the general architecture of the bone looked natural, with structured trabeculae and a dense collagen matrix. At a high magnification, osteoblasts were easily identified, and osteocytes in the lacunae demonstrated the vitality of this bone sample.

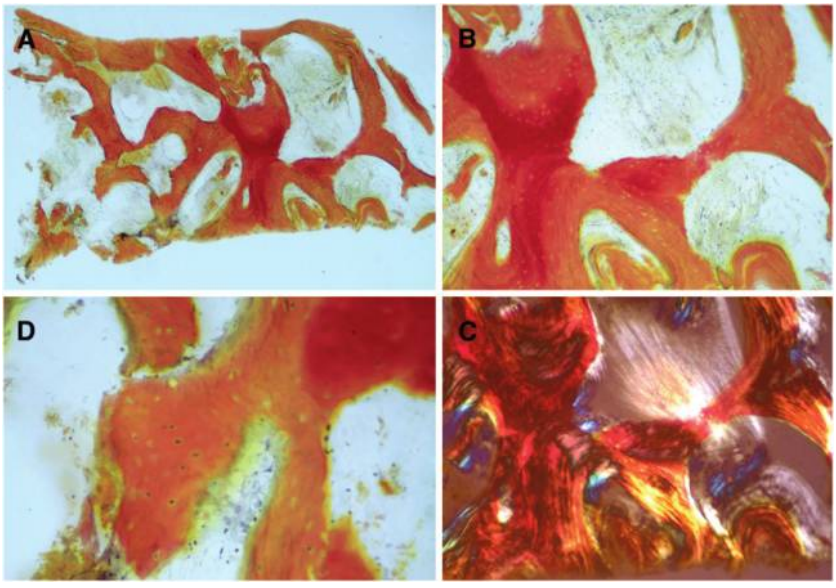

Figure 3.
Histologic evaluation of bone samples collected 6 months after surgery on the buccal wall of the elevated sinus at the level of the sinus-lift window. ***A)*** *The core shows 33% vital bone. As no grafting bone material was used, the bone sample was completely regenerated starting from the PRF clots and membranes used to fill the sinus cavity.* ***B)*** *At a higher magnification, the bone architecture seemed already well structured and quite mature, with good connectivity of the bone trabeculae, surrounded by remodeling areas.* ***C)*** *This finding was confirmed by examination under polarized light, where the collagen network appeared very clearly.* ***D)*** *With the highest magnification, the osteoblastic remodeling looked even more active, with clear osteocytes embedded in their lacunae (dark-blue points) and a very active osteoid border (yellowish). (Van Gieson's staining; original magnification: A, ×40; B and C, ×100; D, ×200).*

DISCUSSION

Many studies[11,12,15-17] discussed the relevance of using a biomaterial during a sinus lift to reconstruct a significant bone volume for implantation or at least maintain space for bone regeneration. The sinus cavity shows a high osteogenic potential and is a very strong model of an osteogenic chamber for bone regeneration. Thus, a sinus lift without grafted bone material is a very natural and attractive approach[15,16] and is the natural consequence and evolution of the quantitative and qualitative success of the crestal sinus lift with an osteotome (the Summers technique), using no grafting material even in residual bone height <5 mm. However, if the clinical results are relevant and the survival rate high, some authors[42,43] showed that the true final bone gain may be limited, and implant ends may be enmeshed in a thick sinus connective tissue and, thus, not osseointegrated.

Because the present study was performed without a control group, our interpretation is only based on observations of a series of relevant cases. Thus, it is difficult to be sure that similar results could not be reached with the physiologic blood clot as the sole filling tissue. However, many arguments maintain the use of PRF as the partial or sole filling material during sinus-lift procedures.

The main issue of the concept of a simultaneous sinus lift and implant placement without grafted bone material is to increase the predictability and security of this procedure without denaturing the underlying concept of natural bone regeneration.[15] Using PRF as the sole filling material seemed the relevant solution. PRF is a natural and optimized blood clot and is used during a sinus lift for protection of the sinus membrane or improvement of the bone graft maturation.[33,34] In the first international publication[28] on this subject, it was assessed that a sinus grafting material built with an allograft and PRF in equal volume was suitable for implantation after only 4 months and potentially even more mature than a sole allograft after 8 months. Another study[13] showed that PRF membranes were easy to use during a Summers osteotomy and offered a good compromise as a filling material and shock absorber during sinus floor

elevation and provided healing support for the damaged Schneiderian membrane.

In this case series, three-dimensional x-ray pictures showed that new bone around implants did not look very dense or cortical. However, this bone was clinically and histologically very dense and mature. This situation is exactly the opposite of what occurs during a sinus lift when using a calcified bone substitute such as bovine porous bone mineral, for example, where the mixture of the non-resorbed graft and vital bone is radiologically very dense but clinically still fragile. This finding means that the new bone formed around implants, starting from the PRF fibrin matrix, already showed a strong matrix architecture but without final calcification. The analysis of bone density on an x-ray exam is useless in such circumstances.

The precise effects of PRF membranes on Schneiderian membranes have not been investigated. However, a PRF membrane may improve the healing of a Schneiderian membrane and stimulate its periosteum-like behavior and perhaps increase or stabilize the bone volume around the implant end.[24,25,44,45] From a practical point of view, the use of a PRF membrane on a Schneiderian membrane is a very simple mechanical and biologic protection that can be used in daily practice. It could even be a key element for success when clear sinus-membrane perforations occur, as tears and holes can be easily patched with PRF membranes.

The lateral window of a sinus should be protected with a membrane (such as collagen membranes) to avoid invagination of the mucogingival tissues.[5] The general explanation about this phenomenon is that the sinus cavity must be protected with a barrier like a guided bone regeneration area. In this case series, PRF membranes were used as the sole protection membrane for sinuses, with a PRF layer covering each sinus window. The x-ray analysis of this case series showed no invagination. In the nine cases where bone biopsies were harvested in the area of the window, no connective tissue invagination was observed, and bone samples were all very dense. This result seems to indicate that PRF membranes were able to protect the sinus-graft area. PRF is an inexpensive autologous biomaterial with a significant slow release of growth factors and can easily replace xenogenic and expensive collagen membranes in some situations.

In this study, all implants achieved primary stability, and implant stabilization was obtained with the tapered profile and/or microthreads of the implant neck. The implant design seemed a relevant parameter as the stability of the implant is a key parameter for osseointegration and bone regeneration. Thus, the use of tapered and microthreaded implants might be a more secure and simple choice than the use of cylinder-type implant.[16] However, alternative profiles might lead to similar results, if used with the adequate careful surgical procedure.[6]

Finally, this preliminary study was performed with small sinus lifts only, with one or two implants per sinus. Thus, it would be interesting to discover if PRF might be as effective as an osteoconductive biomaterial in larger sinus grafting cavities and to follow the evolution of the bone levels and quality after several years.

CONCLUSIONS

Choukroun's PRF is a simple and inexpensive technique, and the systematic use of this biomaterial during a sinus lift seems a very interesting option, particularly for the protection of the Schneiderian membrane. From a radiologic and histologic point of view 6 months after surgery, the use of PRF as the sole filling material during a simultaneous sinus lift and implantation was able to stabilize a high volume of natural bone in the subsinus cavity up to the tip of the implants. Thus, PRF, as a natural and optimized blood clot, seemed the adequate adjuvant to secure this technique and to improve the natural bone regeneration around implants.

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