Case Report

Using Extracted Teeth as a Novel Graft Material in Atrophic Ridge Augmentation: A Report of Two Cases with Histology and Cone-Beam Computed Tomography

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Abstract

After a dental extraction procedure, the extracted teeth are discarded disregarding their properties as an excellent graft material. The use of extracted teeth as a graft material was first performed by Dr. Marshall Urist in 1967, where he discovered and verified that the decalcified dentin matrix can induce bone formation, but his decalcification method took at least 5 days to accomplish before being able to use it as a graft material. Currently, an ultrasonic technology with temperature and vacuum control, named VacuaSonic® System reduces the decalcification time dramatically (≤80 min) converting the tooth into graft material at chairside immediately after the dental extraction with the result being an autogenous tooth graft material (ATG). The aim of this article is to introduce two clinical cases: one case of alveolar ridge augmentation, and the other, socket preservation using ATG mixed with liquid-phase concentrated growth factors (LP-CGF) prepared at chairside on the same day of the dental extraction procedure. LP-CGF is obtained from the same patient, which was collected in blood collection tubes and processed by a special centrifuge device. The result obtained by mixing ATG with LP-CGF is a graft matrix named "Gummy Tooth Graft."

Keywords: Atrophic ridge, decalcification, extracted teeth, sinus augmentation, socket preservation, tooth graft

Introduction

Several techniques have been proposed for bone regeneration in patients with atrophic ridges. Bone grafting is commonly applied in dentistry to induce bone formation, resulting in increased bone volume and quality.[1]

Autogenous bone is still considered the gold standard among graft materials in guided-bone regeneration, but it has limitations such as added operative time for graft harvest, donor-site morbidity, graft resorption, molding/adoption challenges, and limited availability.[2]

These issues have spurred on researchers and clinicians to look for novel graft solutions, more specifically, the use of tooth-derived materials.[3]

Yeomans and Urist were the first to use extracted teeth as a graft material in 1967, where he discovered and verified that decalcified dentin matrix can induce bone formation, but his decalcification method took at least 5 days to achieve a graft material that could be reimplanted.[4]

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The tooth is composed of hydroxyapatite and type I collagen, and it is the most similar hard tissue to autogenous bone in two respects: it is both osseo-compatible and osteoconductive, thereby providing a physical matrix for the deposition of new bone.[5]

Teeth and bones share many similarities because they embryologically originate in the neural crest, sharing identical origin.[6] An extracted tooth from the same patient can be used as an excellent bone graft material after appropriate decalcification and sterilization processes, in block or particulate form and it has been widely used for ridge and sinus augmentation.[7]

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Another novel biological material that is currently being used in guided-bone regeneration is concentrated growth factors, especially liquid-phase concentrated growth factors (LP-CGF), which when mixed with the graft material allows the particles not to disperse during molding and the healing period.[8] These growth factors are proteins which regulate the complex processes of wound healing. The growth factors are mainly located in blood plasma and platelets and play the main role on cell migration, cell proliferation, and angiogenesis in the tissue regeneration phase.[8] The protocol to obtain LP-CGF is very simple. The venous blood is taken through phlebotomy using sterile 10 ml tubes without anticoagulants and immediately centrifuged in a special centrifuge device (Medifuge, Silfradent Srl, Sofia, Italy) for 12 min.[9]

After centrifugation, the tube demonstrates two different layers: the top layer is LP-CGF and the bottom layer is the red blood cells which will be discarded.[10] LP-CGF is obtained from the upper layer using a syringe and mixed with autogenous tooth graft (ATG) particles and allowed to sit for 5–10 min for polymerization, in order to produce gummy tooth graft.

The main objective of this report is to introduce the effectiveness of using extracted teeth processed and turned into ATG which is then mixed with LP-CGF to obtain a bone matrix termed "Gummy Tooth Graft" for alveolar ridge augmentation in clinical cases that show excellent outcomes through cone-beam computed tomography (CBCT) and histological analysis.

Preparation of the gummy tooth graft

1. Processing of the extracted tooth: residual soft tissue is removed from the extracted tooth by using a scalpel blade no. 15 and handpiece with carbide bur. The clean extracted tooth is then crushed to create a particulate graft material which is processed using the VacuaSonic® System following the manufacturer's instructions using reagents (DecalSi® PDM; CosmoBioMedicare, Seoul, Korea). The processing of the extracted tooth consists of demineralization, sterilization, and washing [Figures 1-4][11,12]
2. Obtainment of LP-CGF: Before start of the surgical procedure, four tubes of 10 ml without anticoagulant (one tube with yellow cap and three tubes with red cap) were used to obtain venous blood from the patient's antecubital fossa. Those tubes with venous blood were then immediately centrifuged from 2,400 to 2,700 rpm using a special centrifuge with a rotor turning at alternated and controlled speeds for 12 min (Medifuge; Silfradent Srl, Sofia, Italy). After centrifugation, the yellow cap tube presented with two different layers: the top layer is LP-CGF that will be obtained with a syringe and mixed with ATG to fabricate the gummy tooth graft [Figure 5].

The bottom layer corresponds to the red blood cells which will be discarded. The tubes with the red cap presented after the centrifugation process with three blood fractions: (1) the upper platelet-poor plasma layer, (2) the middle fibrin-rich gel with aggregated platelets and CGF, and (3) the lower red blood cell layer [Figure 6].

The middle fraction corresponding to CGF, which is taken from the tube and placed over metal storage box and compressed with the metal cover to turn it into a CGF membrane to be used to cover the osseous graft.[13]

Once obtained, the ATG particles and LP-CGF are mixed in a recipient sterile dish and allowed to rest for 5–10 min with the final outcome being the gummy tooth graft [Figure 7].

Figure 1: Cleaning and eliminated any contaminated soft tissue from the extracted tooth using the high-speed hand piece

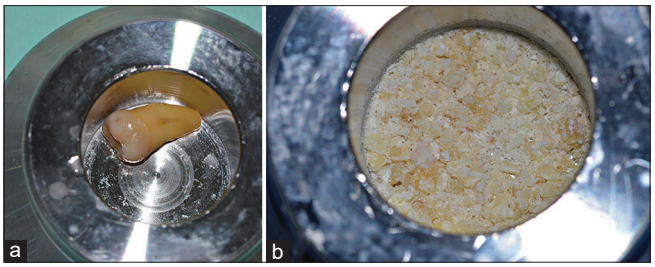

Figure 2: Tooth ready to be processed, clean of any soft tissue (a), pulverized tooth (b)

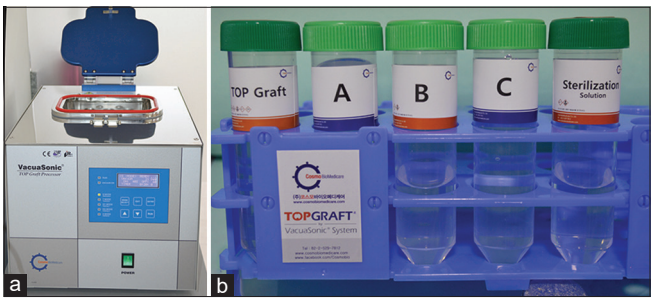

Figure 3: The VacuaSonic® machine to process the extracted teeth into graft material (a), reagent kit to demineralize, sterilize, and wash (b)

Figure 4: Tooth demineralized, sterilized, and then turned into an autologous tooth graft material ready to use

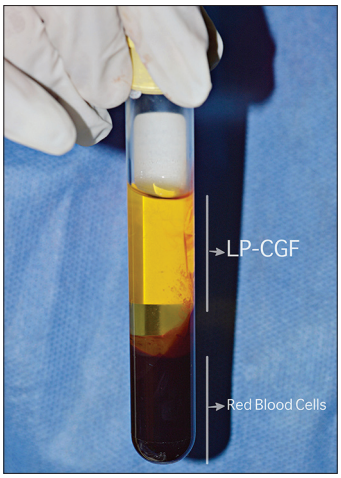

Figure 5: Blood collection tube with yellow cap demonstrates two different layers after centrifugation: with the top layer corresponding to liquid-phase concentrated growth factors and the bottom is the red blood cells layer

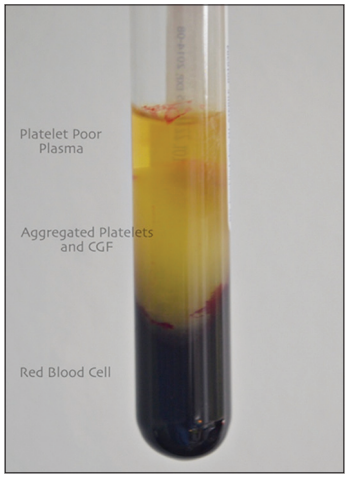

Figure 6: Blood collection tube with red cap demonstrates three different layers after centrifugation: the top layer is platelet-poor plasma; the middle layer is concentrated growth factor; and the bottom layer corresponding to red blood cells

Cases Reports

Case 1: Horizontal ridge augmentation using gummy tooth graft with immediate implant placement

A 68-year-old female patient presented with missing tooth #12. She did not want to use a conventional fixed prosthesis because she would want to preserve the integrity of the adjacent teeth to the edentulous space and being able to floss them individually, therefore, a screwed implant-supported single crown was proposed to the patient and was accepted as the treatment.[14,15] Medical history indicated a nonsmoking and healthy patient. Radiographic examination using a CBCT demonstrated an atrophic ridge with 3 mm width at the crestal, 1.5 mm width at the middle, and 3 mm width at the apical, making it impossible for a conventionally placed dental implant in the existing ridge [Figures 8 and 9].

Onset of the treatment

Surgery was performed under local anesthesia using 2% lidocaine with 1:100.000 epinephrine. A #15 scalpel blade was used to make a crestal incision with two vertical-releasing incisions, and a full-thickness mucoperiosteal flap was raised to expose the narrow alveolar ridge in the zone of the missing tooth corresponding to #12. At the same surgical appointment, tooth #32 which had no antagonist tooth was extracted to prepare the gummy tooth graft [Figure 10].

An osteotomy for a 3.75 mm × 11.5 mm dental implant (Neo, AlphaBio, Israel) was prepared at the site, and then, the implant was placed with good initial stability but with severe exposure of implant surface on the buccal due to dehiscence [Figure 11].

The gummy tooth graft was prepared mixing ATG with LP-CGF, and it was placed over the exposed implant surface and horizontal bone defect, and three CGF membranes were used to cover the gummy tooth graft for three-dimensional ridge augmentation [Figures 12 and 13]. The flag was repositioned over the graft to achieve the primary closure.

The flap was sutured with 4/0 monofilament polypropylene nonabsorbable suture (Prolene, Ethicon). The sutures were removed 10 days postoperatively. CBCT was ordered immediately after the surgery [Figure 14].

The dental implant was uncovered after 6 months of healing, and a favorable three-dimensional ridge augmentation was observed. A CBCT was performed to evaluate the ridge augmentation in relation to the implant. A sample from the buccal wall of the graft site was taken and prepared for histologic sections [Figure 15].

Implant stability was measured using a radio-frequency device (Penguin RFA, Integration Diagnostics Sweden AB). The implant-stability quotient (ISQ) was measured twice in two directions (buccal and palatal), and the measure obtained was 76 ISQ what means the implant is already so stable, osseointegrated, and ready to receive the final restoration [Figure 16].

Figure 7: Autologous tooth graft material mixed with liquid-phase concentrated growth factor giving as final result a bone matrix named gummy tooth graft

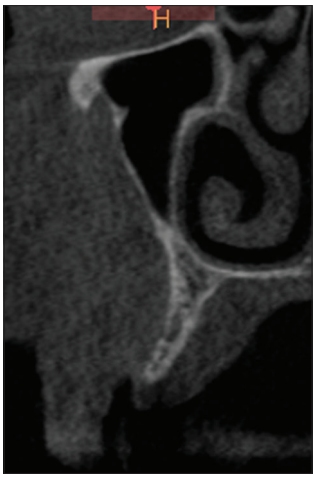

Figure 8: The cross-sectional image of cone-beam computed tomography reveals an alveolar atrophic ridge

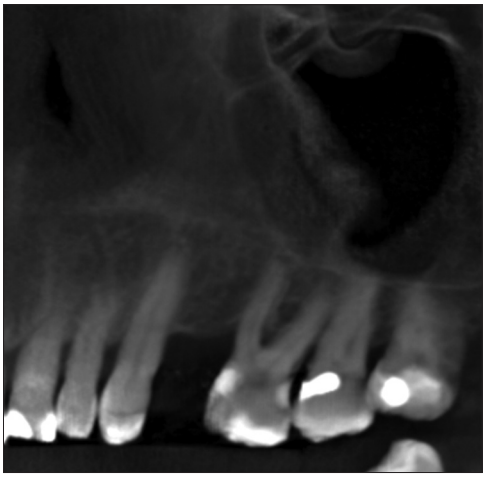

Figure 9: The preoperative radiograph reveals absent tooth #12

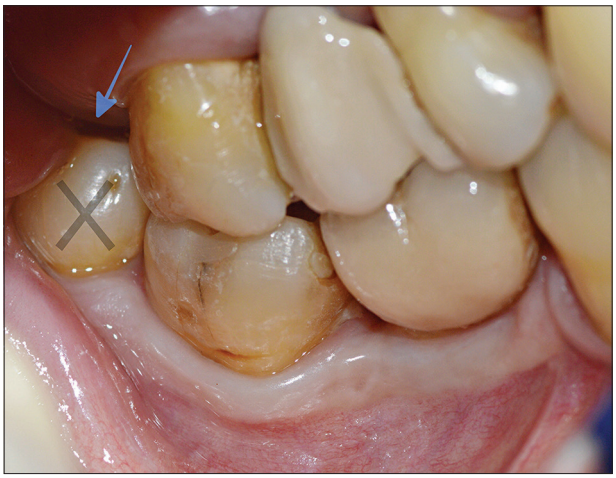

Figure 10: The photograph shows tooth #32 without the antagonist tooth

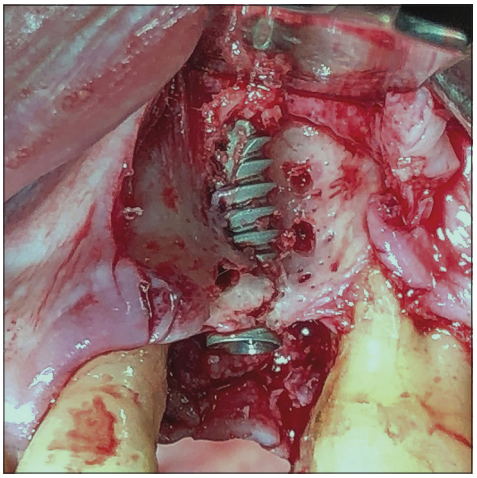

Figure 11: The photograph shows a dental implant placed in the atrophic ridge with its exposed threads and decortication to receive the gummy tooth graft

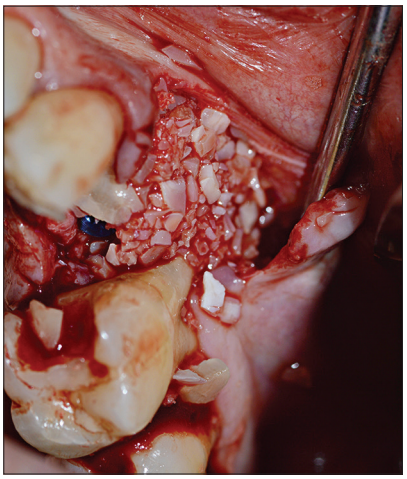

Figure 12: Gummy tooth graft covering the dental implant and bone defect

A final restoration consisted of an implant-supported single crown was screw retained to the dental implant, and it has been in function during 2 years loading [Figure 17]. The treatment for this case was started in February 2017, and the final rehabilitation was finished and delivered in October 2017 [Figure 18].

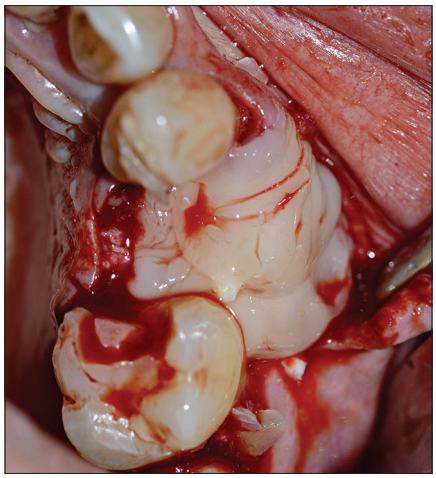

Figure 13: Concentrated growth factor membranes covering the tooth graft

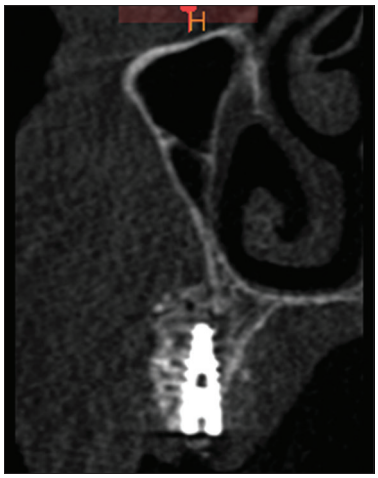

Figure 14: The cross-sectional image of cone-beam computed tomography revealing the dental implant in position covered with particles corresponding to the autologous tooth graft material

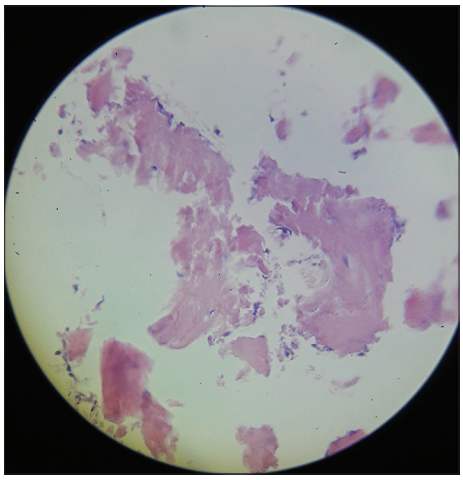

Figure 15: Biopsy shows favorable new bone formation along with tooth graft without the sign of inflammation. Mineralized bone matrix fragments and the presence of osteocytes are observed. The section was stained with H and E, ×10

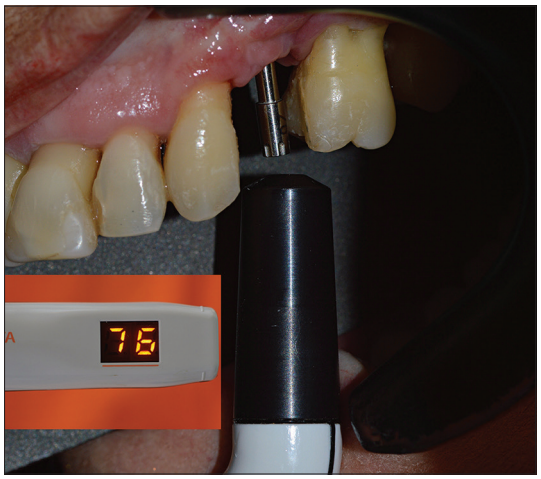

Figure 16: Measuring the implant stability quotient

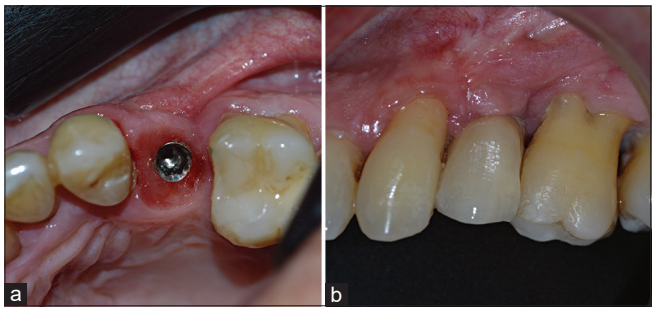

Figure 17: Final outcome, gingival contour healthy (a) and implant-supported single crown in 2 years follow-up (b)

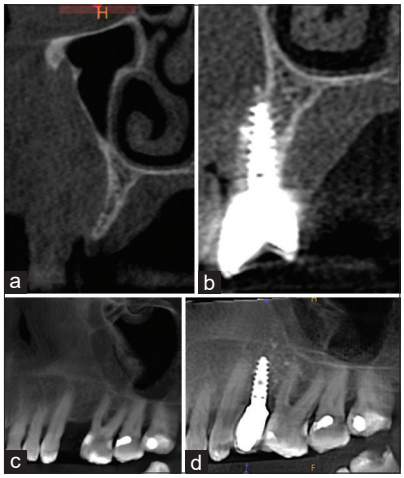

Figure 18: The cross-sectional image of cone-beam computed tomography previous the treatment (a) and after the treatment showing the screwed crown on the dental implant with good adaptation (b). The preoperative radiograph (c) and after the treatment with a dental implant in position and its respective screwed crown (d)

Case 2: Using gummy tooth graft in molar socket preservation with sinus lifting and immediate implant placement

A 72-year-old male patient presented with a vertical fracture of tooth #15. Medical history noted controlled hypertension,

nonsmoking, and controlled diabetes. Radiographic examination using a CBCT demonstrated a bone height of 6 mm from the bone crestal in the septum among the three alveoli to the maxillary sinus floor and a radiopaque image into the left maxillary sinus corresponding to a mucocele.[16]

Onset of the treatment

The patient was placed on antibiotic prophylaxis with amoxicillin (Amoxal®; GlaxoSmithKline Lab, Bogotá, Colombia) 500 mg each 8 h beginning 1 day before the procedure and continuing for 6 days postsurgically. The surgery was performed under local anesthesia using 4% Pricanest, (Ropsohn Therapeutics SAS, Bogotá, Colombia). As the vertical fracture ran from mesial to distal, an odontosection was performed to separate the two buccal roots and the upper left second molar's three roots were extracted individually. The three extracted roots were placed into the crusher container to be pulverized and then processed (demineralization and sterilization) using the VacuaSonic® System.

Immediately, the venous blood from the patient was drawn and centrifuged to develop the CGF clots and LP-CGF. Alveoli curettage was performed using Lucas' curette to remove granulation tissue and any contaminated tissue. Once the socket was exposed and cleaned, the surgical procedure continues performing one pilot perforation into the septum between the three root alveoli where the dental implant was planned using a 1.6-mm round carbide insert with external irrigation (SO16; BukBu Dental Co., Daegu, Korea), connected to the ultrasonic piezoelectric device (Surgybone; Silfradent Srl, Sofia, Italy). This allowing for deepening and perforation of the maxillary sinus floor without tearing of the sinus membrane. After perforating the sinus floor with the round carbide insert, a 2.8-mm wide cylindrical carbide insert (HPISE insert, SO28i; BukBu Dental Co.) was used to enlarge the osteotomy site and elevate the Schneider membrane using hydraulic pressure by internal irrigation at the same time according to the Sohn's protocol [Figure 19].[17,18]

The hydraulic pressure produced by the internal irrigation of saline solution through the HPISE insert penetrates the sinus floor and gently elevates the sinus membrane, creating a secluded compartment between the sinus floor and Schneiderian membrane.

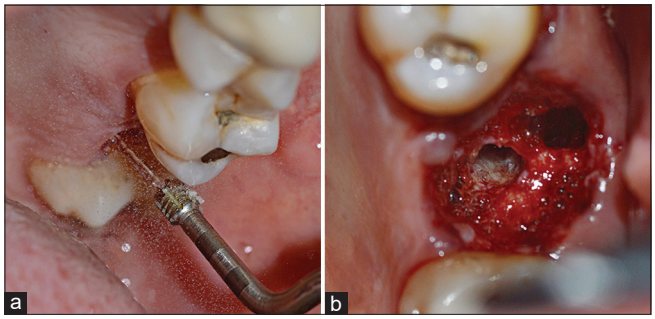

Figure 19: HPISE insert SO28i (a), sinus membrane intact without tearing (b)

The membrane perforation was evaluated using the Valsalva maneuver that consisted of performing a moderately forceful attempted exhalation with closed nose as if blowing up a balloon, which permitted the direct visualization of the sinus membrane confirming that it was not perforated. A twist drill of 3.2 mm diameter of the dental implant system was used to finalize the osteotomy procedure. Three CGF clots were inserted into the new compartment of the maxillary sinus as an alternative to bone grafting and to accelerate the new bone formation in the sinus [Figure 20].

One dental implant of 4.20 mm × 11.5 mm (SPI AlphaBio, Israel) was placed in the maxillary sinus into the osteotomy that was created. The dental implant achieved primary stability due to undersizing the osteotomy and lateral bone condensing when the wide implant was placed into the site. Each of the residual root alveoli was filled with gummy tooth graft previously prepared, and the graft material was covered using the CGF membrane. The buccal flap on its internal side was brushed using the soft-brushing kit (PRF Process Choukroun, Nice, France) to be stretched and covering the surgical site tension-free to achieve primary closure. The flap was sutured using 4/0 monofilament polypropylene nonabsorbable sutures (Prolene, Ethicon). The patient was instructed not to blow his nose for 2 weeks after the surgery and to cough or sneeze with open mouth to help prevent accidental disruption of the sinus area at the implant site during the primary healing phase. The sutures were removed 10 days postoperatively. Panoramic radiography was ordered immediately after the surgery and before dental implant uncovery. The implant was

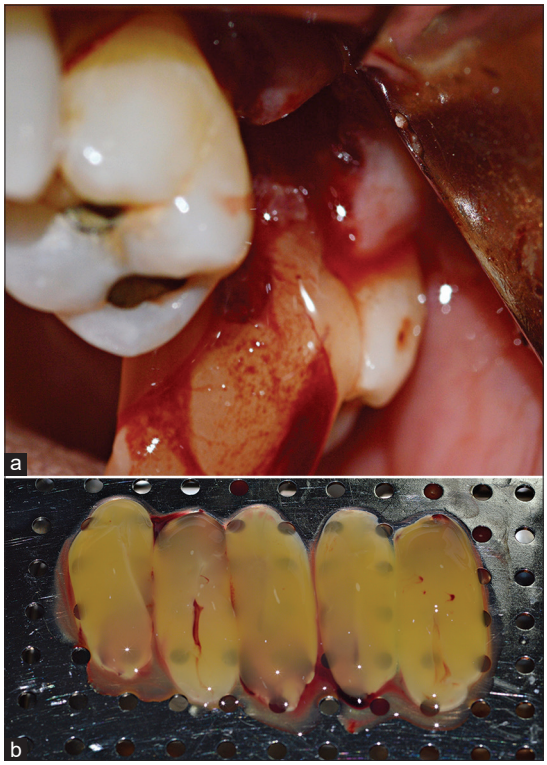

Figure 20: Inserting concentrated growth factor clots into the maxillary sinus (a), concentrated growth factor clots ready to be used (b)

uncovered after 32 weeks of healing. Once uncovered the dental implant, small bone segments were acquired from the graft over the cover screw to be analyzed in histology [Figure 21].

Histologically, osteoid matrix, mineralized bone matrix with the presence of osteocytes, and a strong fusion between new bone and tooth graft were observed at 8 months on cross-sectional slides.

The final restoration consisted of an individual screw-retained crown placed over the dental implant has been in function during 2 years loading [Figure 22].[19]

The treatment for this case was started in March 2017, and the final rehabilitation was finished and delivered in November 2017.

Discussion

There are currently four kinds of bone graft materials: autograft, allograft, xenograft, and alloplast wherein the autograft is the gold standard thanks to its osteoinductivity, osteoconductivity, and osteogenicity, and it can also integrate into the host tissue most rapidly and completely.[7,20] Although autograft does not trigger foreign-body reaction, the donor site can present some complications after harvesting. With regards to intraoral donor sites, it may result in decreased sensitivity in the soft tissue by the damage to the mental nerve, decreased sensitivity in the buccal mucosa corresponding to the innervation of the buccal nerve, and decreased sensitivity in the symphysis. With regards the extraoral donor sites: walk disturbances in the 1st week due to iliac and tibial bone harvests, high pain postoperatively and neurological complications have been reported after calvarial bone harvest.[21-23] Many researchers have paid attention to the human tooth as one of the intraoral donor due to its chemical similarities to the bone. Enamel consists of 96% inorganic substances and 4% water, whereas dentin has inorganic and organic components that are very similar to those of human bone. In dentin, the inorganic content is 70%–75%, whereas the organic content is about 20%. In alveolar bone, the inorganic content is 65%, and the organic content is 25%. At least 90% of the organic content of dentin is type I collagen, which plays an important role in bone formation and mineralization. Dentin and cementum include various growth factors such as bone morphogenic proteins (BMPs), which promote the differentiation of mesenchymal stem cells in chondrocytes and consequently enhance bone formation.[7,21] Enamel also contains BMP-2, which has the capacity for osteoinduction, important in bone formation.[24]

Other biomaterials included in this report are LP-CGF. LP-CGF is a fibrin biomaterial that contains CD 34-positive cells (presents in vascular maintenance, neovascularization, and angiogenesis) and the growth factors such as: transforming growth factors β-1, platelet-derived growth factor, epithelial growth factor, insulin growth factor-I, and vascular endothelial growth factors, which is indispensable in creating intercellular communication and neo-angiogenesis during bone regeneration and healing, stimulates cell proliferation, and they are obtained by special centrifugation of venous blood using Silfradent device. CGF is 100% autologous and biocompatible, it does not need the addition the others substances to polymerize and has a high concentration of platelets in a fibrin network. In addition to platelets, CGF contains fibroblasts, leukocytes, and endothelial cells for angiogenesis, tissue remodeling, and provides a matrix for cell migration.[7,10,25-27]

LP-CGF is mixed with autologous tooth graft to help the particles that do not disperse during molding at the bone defect thanks to the strong interconnection of each tooth particle entrapped by the fibrin network. Gummy tooth graft can adapt to the different shapes of bone defects.

When mixing autologous tooth graft with LP-CGF, the result is a graft matrix 100% autologous with the three properties: osteoinduction, osteoconduction, and osteogenesis, and it does not produce reject.

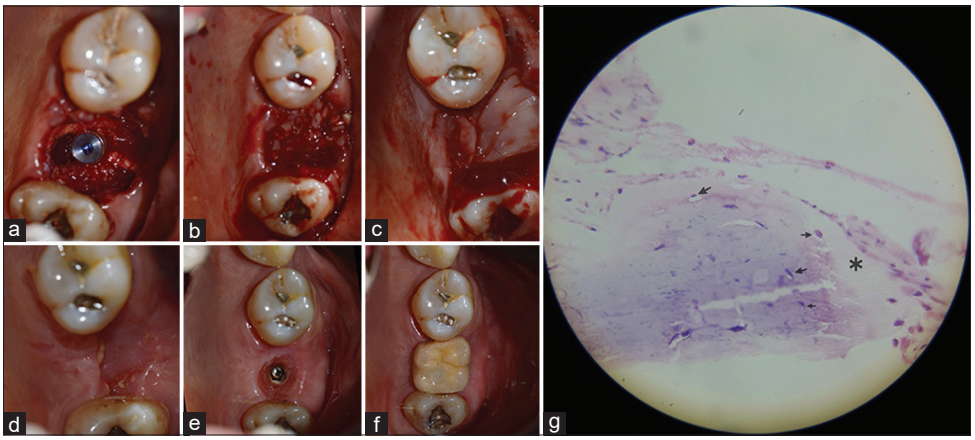

Figure 21: Dental implant placed in the septum among the three alveoli (a), socket and dental implant covered filled with gummy tooth graft (b), Covering the graft material using concentrated growth factor membranes (c), healing 8 days after surgery (d), gingival contour 32 weeks after surgery (e), final restoration: An individual screw-retained crown placed over the dental implant (f), and the histological section at 8 months after the surgery was stained with H and E staining (×100) showing osteocytic inclusion in tooth graft material (arrows) and a strong fusion between new bone and tooth graft material (asterisk) (g)

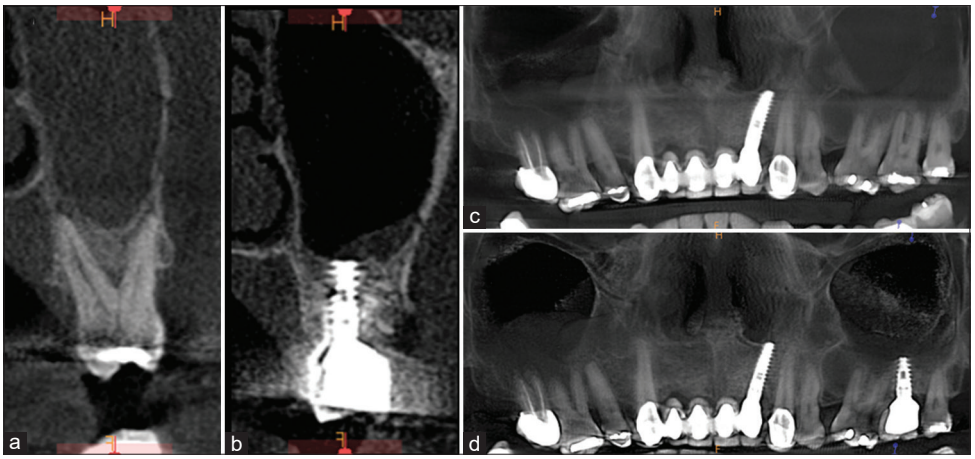

Figure 22: Radiographic description: Axial slice: Mucocele and vertical fracture (a), maxillary sinus free of mucocele (b), the new bone around of the dental implant into the maxillary and the buccal wall is kept (c and d)

Conclusion

ATG and LP-CGF utilized together are called in this report gummy tooth graft, and it can be a good alternative to autologous bone grafts. Gummy tooth graft is a biomaterial safe that does not produce cross transmission of some kind of illness or rejection because it is obtained from the same patient. It is prepared the same day as dental extraction at the chairside. It is easy to make, manipulate, and mold to any shape of bone defect. This present report suggests that gummy tooth graft can be an effective graft material for alveolar ridge augmentation and socket preservation.

Acknowledgments

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Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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