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Collagen membrane and L-PRF with Xenogeneic bone block for vertical ridge augmentation: An Experimental study in a canine model

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Abstract

Background: Guided bone regeneration has been tried using a variety of barrier membranes, and it is thought to be achieved when osteoprogenitor cells are allowed to repopulate the area of the bone defect solely while non-osteogenic tissues are prevented from entering. PRF, have the advantage that without anticoagulants, a fibrin matrix that incorporates the full set of growth factors trapped within its matrix can slowly release these growth factors over time. Furthermore, L-PRF contains white blood cells, which are key contributors to wound healing. The aim of this study was to evaluate the benefits of using L-PRF in bone regeneration when utilizing GBR technique.

Methods: The study involved six mature mongrel dogs, each weighing at least 18 kg. In the first phase, four standardized saddle-type defects were prepared. After a two-month recuperation period, a xenogeneic block graft was utilized in the two groups. In group (1), block graft was covered by a collagen membrane (Block + C M), while in group (2), two L-PRF membranes were added first before top coverage by collagen membrane (Block + L PRF + C M). Animals were subjected to surgical reentry after a three-month healing period following grafting for clinical observation and then euthanized for histological processing.

Results: In group 1, there was a statistically significant difference between (New bone apical side) and each of (New bone periosteum side) and (New bone intermediate) ($p \leq 0.001$). In group 2 no statistically significant difference between (New bone periosteum side), (New Bone intermediate) and (New Bone apical side) ($p = 0.225$).

Conclusion: With the inherent limitations of this study, the usage of the (L PRF) in ridge augmentation appears to enhance the quality of regenerated bone.

Keywords: Ridge augmentation; leukocyte and platelet-rich fibrin; collagen membrane; tissue engineering.

Running title: Benefits of (L PRF) for ridge augmentation

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Introduction:

Due to anatomical constraints and technical challenges, vertical alveolar bone loss in partly dentate patients is a substantial difficulty. The amount of bone available for the implant procedure is limited due to the nasal cavity, maxillary sinus, and inferior alveolar nerve. (1).

Despite the fact that autogenous bone in block or particle form has long been thought to be the gold standard, and the best augmentation material due to its homogeneity and regenerative abilities, bone harvesting often necessitates an additional surgical site, so morbidity, healing period, and chairside time all rise as a result of this. Aside from that, the amount of bone that can be collected is limited.

To circumvent these restrictions, bone substitutes (Allograft and Xenograft) were developed. They also allow the selection of blocks with a predetermined structure as well as cortico-cancellous composition(2). Data on the use of xenogeneic block grafts in different ridge augmentation therapy has shown considerable bone regeneration, with dense, well-vascularized tissues; high bone-to-implant contact; and replacement of graft particles with new bone in a short amount of time (3).

Vertical ridge augmentation aims to achieve bone regeneration without osseous wall containment and for this reason, it is biologically demanding, as angiogenesis must reach a certain distance from existing bone for new bone to be formed. In the proof of principle study, utilizing xenogenic equine block graft for vertical ridge augmentation, a little to no bone regeneration was evident coronal to the apical native bone. While when combining this block with recombinant human platelet-derived growth factor-BB (rhPDGF-BB), significant vertical bone regeneration resulted, with a dense, well-vascularized bone; high bone to implant contact; and accelerated replacement of graft particles with newly formed bone(4).

The use of growth factors to promote wound healing and bone volume has advanced periodontal regenerative therapy dramatically. The effect of growth factors on bone and tissue

regeneration has been one of the key topics of periodontal research. In experimental and clinical trials, platelet-derived growth factors (PDGF), insulin-like growth factors (IGFs), and bone morphogenic proteins (BMPs) have all been used to treat significant ridge and alveolar bone defects. (5).

With the right instruments, platelet-rich fibrin (PRF) clots can be easily converted into dense fibrin membranes or cylinders. PRF membranes release enormous amounts of proteins (such as fibronectin, thrombospondin-1 and vitronectin) and growth factors (such as platelet-derived growth factor PDGF-AB, vascular endothelial growth factor VEGF and transforming growth factor TGFb-1,). The major feature of leukocyte- and platelet-rich fibrin (L-PRF) is that it releases biologic mediators slowly over a period of 7 days or longer. Leukocytes in LPRF affect cell responses, growth factor release, the proliferation of a variety of cell types. (6).

One primary proposed reason for a slower release of growth factors over time is the ability of the fibrin matrix to hold proteins within its fibrin network as well as cells capable of further release of growth factors into their surrounding microenvironment. Leukocytes are highly important immune cells capable of directing and recruiting various cell types during the wound healing process. Since high centrifugation forces are known to shift cell populations to the bottom of collection tubes (whereas PRF is collected from the top one-third layer), it was recently hypothesized that by reducing centrifugation speed (G-force), an increase in leukocyte numbers may be achieved within the PRF matrix(7).

It was since shown that with decreased centrifugation G-force (now termed advanced PRF [A-PRF]), an increase in total leukocyte numbers within PRF matrix scaffolds was observed. Furthermore, and in agreement with this hypothesis, it was shown that the release of several growth factors, including platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor (TGF)-b1, epidermal growth factor (EGF), and insulin-like growth factor (IGF), were significantly higher

in A-PRF compared with conventional PRF and PRP(8).

Concerning the aforementioned benefits and characteristics of (L-PRF), especially in allowing more profitability role during bone augmentation procedure, the present study is aimed to assess its effectiveness when combined with xenogeneic block graft in ridge augmentation therapy.

METHODOLOGY:

Six mature mongrel dogs, each weighing at least 18 kg and with fully erupted permanent dentition, were used in this study. The dogs were provided with a soft food meal once a day during the experiment. The institutional of the animal house committee, Faculty of Medicine, Cairo University, approved routine standardized protocols for animal selection, management, and surgical protocol. The research was carried out in compliance with section 1 of NF EN ISO 10993.part 2 .

The mandibular 2nd, 3rd, and 4th premolars (P2–P4) were extracted in the initial procedure. In the lower jaw of each dog, a total of two standardized saddle-type defects (mesiodistal width: 10mm; height: 8mm) were created (i.e. n=2 defects per animal in the lower jaw). Following a healing period of 2 months, a xenogeneic block graft was utilized in all groups. In group (1), block graft was covered by a conventional collagen membrane (Block + C M), while in group (2), two L-PRF membranes were added first before top coverage by collagen membrane(Block + L PRF + C M).

Venous blood was drawn in dry glass tubes and then spun for 12 minutes at low speed (2,700 rpm) (708 g) in a specific centrifuge for (L PRF) preparation. Platelet activation and fibrin polymerization were activated quickly in the absence of anticoagulants. Following centrifugation, three layers emerged: a PRF clot in the center, an acellular plasma top layer, and an RBC base layer. The PRF clot collected the majority of the platelets and leucocytes from the removed blood, resulting in a strong fibrin matrix with complex three-dimensional architecture. The PRF clots were squeezed using a specialized box, and PRF membranes were ready for application.

3 months following augmentation, animals were sacrificed by IV injection of a lethal dose of (Thiopental sodium) . Each mandible was dissected and fixed in buffered formalin and processed for histological evaluation.

Individual blocks including the fixation screw and surrounding hard tissues were dehydrated in a series of graded ethanol solutions after being fixed in a 4 % formaldehyde solution. The specimens were decalcified by immersion in EDTA solution. A diamond band saw fitted into a precision slicing machine was used to cut the blocks in a buccolingual plane. Two histology slides were taken from the centre region of the screwed-on area (central slide). In each slide, the area of interest was separated into three parts (periosteal, intermediate and apical). The sections were then reduced to a thickness of approximately 50 microns using a cutting–grinding technique and stained with haematoxylin and eosin stain (H&E).

Haematoxylin and eosin stain (H&E)was utilized to reveal new bone and differentiate it from graft remnants. The new bone is somewhat dense, well organized and perfused with bone marrow spaces of varying sizes and shapes. Using higher magnification, the new bone manifests all signs of viability, in terms of cellularity and vascularity, as there are osteocytes within their lacunae and a marrow space embracing a dilated blood vessel and lined by osteoblasts. The bone graft remnant, on the other side, has empty lacunae and is sharply demarcated from the surrounding viable newly formed bone.

Microscopic inspections and histomorphometric assessments were carried out. A colour CCD camera was installed atop of binocular light microscope for image acquisition. Digital images with different magnifications were evaluated using an image analysis software program.

The percentages of new bone were assessed for a coronal/apical extension of 6 mm from the bony crest. The area of interest was also divided into three parts. The upper area corresponds to the first two threads of the fixation screw (new bone periosteal side), the

third and fourth threads determine the intermediated area (new bone intermediate) and threads no five and six determine the apical bone area (new bone apical side).

Two sections were taken for each specimen, with four randomly selected fields within each section, totaling eight measurements for each specimen, with the mean value representing the final value for statistical analysis. The mean values and standard deviation values were computed for each group. Repeated measure ANOVA was used to compare samples that were related. An independent t-test was used to compare two groups in unrelated samples. $P \leq 0.05$ was used as the significant level. IBM SPSS Statistics Version 20 for Windows was used to accomplish the statistical analysis.

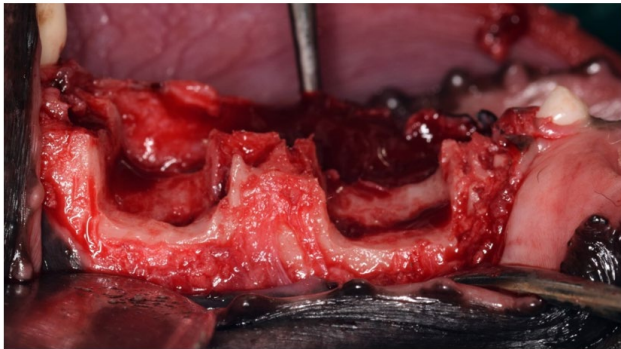


Figure 1: Defect creation in the first surgical phase

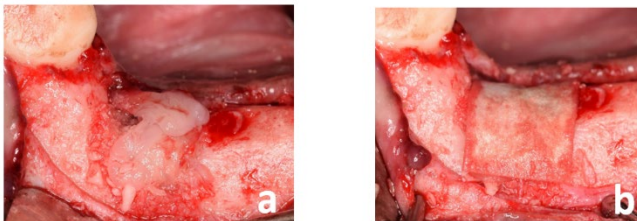


Figure 2: Two layers of (L-PRF) bandages plus resorbable collagen membrane utilized in group 2

Results:

During the two months following the formation of the mandibular defects, all 12 surgical sites healed without complications. The mandibular alveolar ridges resembled atrophic defects, resembling atrophic posterior mandibles with regional atrophies. Healing was uncomplicated for 11 of the 12 sites three months after grafting, with one site in the first group (Block + C M) showing mild soft tissue exposure with the shadow of the fixation screw head exposed.

The amount of new bone in group 1 and 2 was 58.32 and 65.58 respectively with no statistical significant between the groups (Table 1).

Table 1: shows the mean and standard deviation (SD) of new bone values in both groups.

Variables	New Bone	
	Mean	SD
Group 1 (Block + C M)	58.32 ^b	3.46
Group 2 (Block + LPRF + C M)	65.58 ^b	11.44
p-value	0.2735	

There was a statistically significant difference between (New bone apical side) and each of (New bone periosteum side) and (New bone intermediate) in Group 1 specimens. ($p=0.001$) and ($p<0.001$) (Table 2).

There was no statistically significant difference between (New bone periosteum side) and (New Bone intermediate) ($p=0.168$).

Table 2: shows the mean and standard deviation (SD) values for the new bone in Group 1.

Variables	Group 1	
	Mean	SD
New bone periosteum side	56.00 ^b	5.54
New Bone intermediate	58.32 ^b	3.46
New bone apical side	63.19 ^a	4.34
p-value	0.003*	

Significant differences are indicated by different small letters in the same column, *, significant ($p<0.05$)

The difference between (New bone periosteum side), (New Bone intermediate) and (New Bone apical side) was not statistically significant in Group 2. ($p=0.225$) (Table 3).

Table 3: shows the mean and standard deviation (SD) values for the new bone in Group 2.

Variables	Group 2	
	Mean	SD
New bone periosteum side	56.83 ^a	12.55
New Bone intermediate	65.58 ^a	11.44
New Bone apical side	64.81 ^a	9.48
p-value	0.225ns	

Significant differences are indicated by different small letters in the same column, *, significant ($p<0.05$)

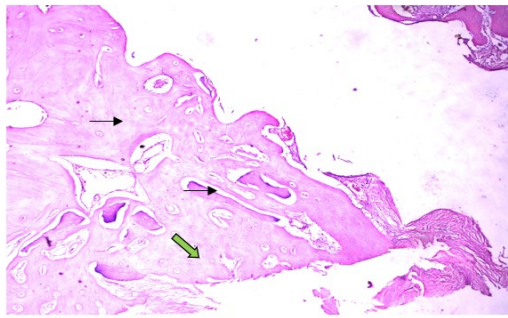


Figure 3. A photomicrograph of a G1 case stained with H&E showing the fixation screw/bone interface (black arrows), where the inter-thread spaces of the screw are filled by a newly formed cancellous bone. Basophilic graft remnants are evident within the marrow spaces (green arrows) (Original magnification x4).

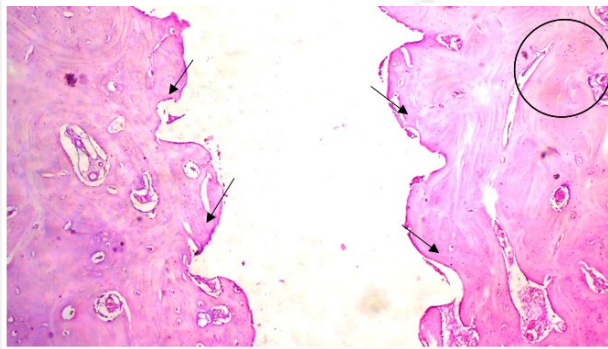


Figure 4. A photomicrograph of a G2 case stained with H&E showing the profile of the fixation screw threads, as well as the newly bone formed along each side (black arrows). The new bone trabeculae are strikingly thick, coherently connecting with nearly normal architecture. The circle highlights a primary attempt toward organization into mature compact bone, through the formation of osteon complexes.

Discussion:

The surgical model used in this study is well-known for evaluating bone grafts on their own or in conjunction with membrane application and growth factors. (9,10). In similar surgical models, the effects of osteoinductive elements and growth factors on bone regeneration have recently been investigated. (11).

The present study was conducted on a total of six adult mongrel dogs each weighing a minimum of 20 kg and exhibiting a fully erupted permanent dentition. All attempts were made so the dogs were close in age, weight and health. The concept of critical size defect (CSD) was developed more than 25 years ago as an attempt to standardize research on bone regenerative materials. Originally, it referred to the smallest

defects that would not heal by natural process during the lifetime of the animal(12). The reviewed literature showed that animal age, weight, and sex influenced bone mineral concentration (BMC) and bone mineral density (BMD), therefore affecting the healing potential of any bony defect. In general, middle-aged dogs (3–10 years) revealed the highest BMC and BMD levels. Moreover, in ageing dogs, the skeletal exchange of calcium falls to a very low level with an increase in osteoclastic bone resorption and loss of skeletal mass. On the other side, bone defects in skeletally immature dogs heal at a faster rate than skeletally mature individuals, which could result in misleadingly high potentials of a tested material if skeletally immature dogs have been used(13).

To treat significantly absorbed alveolar ridges, surgery for vertical alveolar ridge augmentation is required. No clear conclusions about the superiority of any particular VRA technique can be drawn based on the total of scientific evidence from the eligible research included in a recent systematic review on the effectiveness of vertical ridge augmentation treatments. (14). One of the most effective procedures is onlay block grafting. (15). Despite the fact that an autologous bone graft is a gold standard, it has drawbacks such as restricted availability, donor site morbidity, and unpredictably high resorption (16,17).

These restrictions have prompted the search for alternative bone substitutes. In clinical dentistry, one of the most commonly used bone alternatives is xenograft (bovine, equine, porcine), which is accessible in unlimited quantities. It has a physicochemical structure that is comparable to natural bone, and when it comes into proximity with newly formed bone, it exhibits osteoconductive capabilities (18).

In the present study, L PRF was used as a growth factor and wound healing enhancer in group 2. Numerous preclinical studies have demonstrated the capacity of PRF-matrices, especially those prepared according to the low-speed centrifugation concept (LSCC) to release high concentrations of different growth factors. These factors include epidermal growth factor (EGF), which is essential for vascularization and regeneration, platelet-derived growth factor

(PDGF), which plays a significant role in osteogenesis, as well as a vascular endothelial growth factor, which plays a major role in angiogenesis(19). Additionally, benefits of PRF-treated osteoblasts and fibroblasts include significantly enhanced proliferation, cell migration, and metabolic activity when compared with untreated osteoblasts and fibroblasts(20).

In the present study, the percentage of regenerated bone was higher in (group 2) in which the(L- PRF) was utilized. While (group 2) show 65% of new bone and (group 1) was 58%, these figures still not statistically significant. When we look at the amount of regenerated bone in each group, there was a statistical difference between different zones in (group1)(statistical difference between apical bone and both intermediate and coronal bone). This difference between the zones was disappeared in (group 2) in which (L PRF) was utilized so the nourishment was enhanced from the periosteum side.

In a recent retrospective study by **Valladão et al**, leukocyte and platelet-rich fibrin were used in conjunction with bone grafts for staged vertical and horizontal ridge augmentation utilizing the GBR technique. Twenty-nine sites with horizontal bone deficits were recruited, while twenty-three sites with vertical bone deficiencies were selected. When all regenerated locations were considered, the thickness gain for horizontal bone defects was 5.9 ± 2.4 mm on average. This finding is larger than the 3.5 ± 1.18 , and 2.2 ± 1.68 mm mean gain reported in other research and systematic reviews(21,22). However, in terms of surgical procedures and biomaterials, these reviews revealed a greater degree of variation. The authors hypothesised that adding PRF to bone grafting would promote osteogenic differentiation, angiogenesis, and stem cell migration throughout the graft, favouring graft integration and clinical outcomes. (23).

According to **Lorenz et al.**, applying L PRF-based matrices to bone graft may lead to osteoprogenitor cells rapid migration in the augmentation bed due to bioactive growth factor release, hence improving the graft's regeneration capacity. (24). This finding corroborates the

results of the current study in which the percentage of total bone in the area of interest were higher in group 2 which combine L PRF bandages on top of the block graft with the perforated collagen membrane.

Conclusion:

With the inherent limitations of this study, the usage of the (L PRF) in ridge augmentation appears to enhance the quality of regenerated bone.

Conflict of interest: The authors confirm that the study's contents are free of any conflicts of interest. The authors have funded this research on their own.

References:

1. Rocchietta I, Fontana F, Simion M. Clinical outcomes of vertical bone augmentation to enable dental implant placement: A systematic review. *J Clin Periodontol.* 2008;35(SUPPL. 8):203–15.
2. Leong DJM, Oh TJ, Benavides E, Al-Hezaimi K, Misch CE, Wang HL. Comparison between sandwich bone augmentation and allogenic block graft for vertical ridge augmentation in the posterior mandible. *Implant Dent.* 2015 Feb 6;24(1):4–12.
3. Simion M, Trisi P, Piattelli A. Vertical ridge augmentation using a membrane technique associated with osseointegrated implants. *Int J Periodontics Restorative Dent.* 1994 Dec;14(6):496–511.
4. Simion M, Rocchietta I, Fontana F, Maschera E, Nevins M, Kim DM, et al. Vertical ridge augmentation using an equine block infused with recombinant human platelet-derived growth factor-BB: A histologic study in a canine model. *Int J Periodontics Restor Dent.* 2009;29(3):245–55.
5. Giannobile W V., Hernandez RA, Finkelman RD, Ryarr S, Kiritsy CP, D'Andrea M, et al. Comparative effects of platelet-derived growth factor-BB and insulin-like growth factor-I, individually and in combination, on periodontal regeneration in *Macaca fascicularis*. *J*

- Periodontal Res. 1996 Jul 1;31(5):301–12.
6. Dohan Ehrenfest DM, de Peppo GM, Doglioli P, Sammartino G. Slow release of growth factors and thrombospondin-1 in Choukroun's platelet-rich fibrin (PRF): A gold standard to achieve for all surgical platelet concentrates technologies. *Growth Factors*. 2009;27(1):63–9.
 7. El Bagdadi K, Kubesch A, Yu X, Al-Maawi S, Orłowska A, Dias A, et al. Reduction of relative centrifugal forces increases growth factor release within solid platelet-rich-fibrin (PRF)-based matrices: a proof of concept of LSCC (low speed centrifugation concept). *Eur J Trauma Emerg Surg*. 2019;45(3):467–79.
 8. Fujioka-Kobayashi M, Miron RJ, Hernandez M, Kandalam U, Zhang Y, Choukroun J. Optimized Platelet-Rich Fibrin With the Low-Speed Concept: Growth Factor Release, Biocompatibility, and Cellular Response. *J Periodontol*. 2017;88(1):112–21.
 9. Schenk RK, Buser D, Hardwick WR, Dahlin C. Healing pattern of bone regeneration in membrane-protected defects: a histologic study in the canine mandible. *Int J Oral Maxillofac Implants*. 1997;9(1):13–29.
 10. Stavropoulos F, Dahlin C, Ruskin JD, Johansson C. A comparative study of barrier membranes as graft protectors in the treatment of localized bone defects: An experimental study in a canine model. Vol. 15, *Clinical Oral Implants Research*. Clin Oral Implants Res; 2004. p. 435–42.
 11. Teng F, Wei L, Yu D, Deng L, Zheng Y, Lin H, et al. Vertical bone augmentation with simultaneous implantation using deproteinized bovine bone block functionalized with a slow delivery of BMP-2. *Clin Oral Implants Res*. 2020;31(3):215–28.
 12. Schmitz JP, Hollinger JO. The critical size defect as an experimental model for craniomandibulofacial nonunions. *Clin Orthop Relat Res*. 1986;NO. 205:299–308.
 13. Marei HF, Mahmood K, Almas K. Critical size defects for bone regeneration experiments in the dog mandible: A systematic review. Vol. 27, *Implant Dentistry*. 2018. p. 135–41.
 14. Urban IA, Montero E, Monje A, Sanz-Sánchez I. Effectiveness of vertical ridge augmentation interventions: A systematic review and meta-analysis. *J Clin Periodontol*. 2019;46(S21):319–39.
 15. Tamimi F, Torres J, Gbureck U, Lopez-Cabarcos E, Bassett DC, Alkhraisat MH, et al. Craniofacial vertical bone augmentation: A comparison between 3D printed monolithic monetite blocks and autologous onlay grafts in the rabbit. *Biomaterials*. 2009 Oct;30(31):6318–26.
 16. Tinti C, Parma-Benfenati S, Polizzi G. Vertical ridge augmentation: what is the limit? *Int J Periodontics Restorative Dent*. 1996;16(3):220–9.
 17. Pape HC, Evans A, Kobbe P. Autologous bone graft: Properties and techniques. Vol. 24, *Journal of Orthopaedic Trauma*. J Orthop Trauma; 2010.
 18. Accorsi-Mendonça T, Conz MB, Barros TC, de Sena LÁ, Soares G de A, Granjeiro JM. Physicochemical characterization of two deproteinized bovine xenografts. *Braz Oral Res*. 2008;22(1):5–10.
 19. Dohle E, El Bagdadi K, Sader R, Choukroun J, James Kirkpatrick C, Ghanaati S. Platelet-rich fibrin-based matrices to improve angiogenesis in an in vitro co-culture model for bone tissue engineering. *J Tissue Eng Regen Med*. 2018 Mar 1;12(3):598–610.
 20. Wang X, Zhang Y, Choukroun J, Ghanaati S, Miron RJ. Behavior of gingival fibroblasts on titanium implant surfaces in combination with either injectable-PRF or PRP. *Int J Mol Sci*. 2017 Feb 4;18(2).
 21. Naenni N, Lim HC, Papageorgiou SN, Hämmerle CHF. Efficacy of lateral bone augmentation prior to implant

- placement: A systematic review and meta-analysis. *J Clin Periodontol*. 2019 Jun 1;46(S21):287–306.
22. Wessing B, Lettner S, Zechner W. Guided Bone Regeneration with Collagen Membranes and Particulate Graft Materials: A Systematic Review and Meta-Analysis. *Int J Oral Maxillofac Implants*. 2018 Jan;33(1):87–100.
23. Amaral Valladão CA, Freitas Monteiro M, Joly JC. Guided bone regeneration in staged vertical and horizontal bone augmentation using platelet-rich fibrin associated with bone grafts: a retrospective clinical study. *Int J Implant Dent*. 2020;6(1).
24. Lorenz J, Al-Maawi S, Sader R, Ghanaati S. Individualized titanium mesh combined with platelet-rich fibrin and deproteinized bovine bone: A new approach for challenging augmentation. *J Oral Implantol*. 2018;44(5):345–50.



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