Sustainability and Release Pattern of Growth Factors from Bone Grafts Prepared with Platelet-Rich Fibrin

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Background: Platelet-rich fibrin (PRF) is used to prepare "sticky bone" by combining it with bone graft material. The present study investigated the ability of different bone grafts to absorb growth factors from the PRF and release them over time. *Materials and Methods:* Human blood was collected from 10 healthy volunteers for liquid PRF preparation. Bovine bone, allograft (mineralized and demineralized), and synthetic bone were each mixed with the PRF to prepare a sticky bone. All sticky bone samples were incubated for up to 4 days. The absorption and release pattern kinetics of two selective growth factors within the PRF—platelet-derived growth factor (PDGF) and bone morphogenetic protein-2 (BMP-2)—were quantified with immunofluorescence staining and enzyme-linked immunoassay (ELISA) testing. *Results:* All bone graft materials adsorbed the examined growth factors from the PRF. β-TCP showed the highest adsorption levels, followed by the xenograft, and the allografts showed the lowest adsorption levels. Furthermore, PDGF showed a fast-release pattern from the grafts, whereas BMP-2 was released at a later stage. Similar to the adsorption pattern, the β-TCP and xenograft were better able to sustain the release of the PRF growth factors from the graft than the allografts. *Conclusions:* The adsorption of PDGF and BMP-2 differ between graft materials, with superior results for β-TCP, followed by xenograft, then allograft materials. *Int J Oral Maxillofac Implants 2024;39:473–478. doi: 10.11607/jomi.10529*

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Platelet-rich fibrin (PRF), introduced by Choukrun, is a second-generation autologous platelet concentrate produced by centrifuging whole vein blood without additives.¹ The PRF contains a significant amount of platelet-derived growth factor (PDGF), insulin-like growth factor, vascular endothelial growth factor (VEGF), transforming growth factor (TGF) beta, and leukocytes.¹ PRF also includes cytokines, such as interleukin (IL) 1 β , IL-6, and IL-4, and tumor necrosis factor, which are critical in the immune system and therefore may play a supporting role in PRF's pro-healing capacity.² The growth factors and cytokines are trapped within the fibrin network and are released slowly, exerting a gradual, long-standing effect on tissue repair.³ Those biologic traits are independent of the PRF preparation protocols.⁴

Lack of sufficient bone volume at implant recipient sites is one of the main challenges in daily dental practice. Guided bone augmentation using graft materials such as autografts, allografts, xenografts, and alloplastic grafts is widely accepted to increase bone volume, allowing implant placement. Although autogenous bone grafts are still considered the gold standard due to their osteoinduction, osteogenic, and osteoconduction properties, their enhanced bone resorption, limited

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Submitted March 8, 2023; accepted May 19, 2023. ©2024 by Quintessence Publishing Co Inc. volume, and the need for an additional wound site are major obstacles.⁵ Consequently, allografts, xenografts, and synthetic grafts have been used as alternative graft materials. Allografts have osseoinductive properties, but their freeze-drying process (to reduce immunogenicity and cross-contamination) also decreases its osteoinductive potential and structural strength.⁶ Xenografts, mostly of bovine origin, are osteoconductive and have a low absorption rate.⁷ Alloplastic bone grafts such as beta-tricalcium phosphate (β-TCP) have an osteoconductive effect and fast resorption rate.⁸ As none of these graft materials share the biologic advantages of autogenous bone, an alternative bone substitute has been sought.

Growth factors with osteoinductive potential, such as PRF or recombinant human bone morphogenetic protein-2 (BMP-2), were introduced as additional or replacement materials in bone augmentation procedures. The main concern regarding the effective use of these growth factors is the need for a solid structural scaffold, owing to the lack of osteoconductive capability of the growth factors and the need for them to reach the target tissue while retaining their bioactivity during the therapeutic time frame.9 Sticky bone is a recent concept that utilizes a bone graft matrix enriched with growth factors with autologous fibrin glue.10 The advantages of this material include versatile moldability, good structural stability, and selectivity for osteogenic progenitors through the prevention of soft tissue cell migration via fibrin interconnections. Furthermore, the fibrin network also allows rapid cell adhesion and

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accelerated healing.11 Yet, PRF sticky bone application shows conflicting results. Some animal studies report the superior effect of PRF combined with bovine bone compared with bovine bone alone12 and that PRF with bovine graft increases angiogenesis and osteogenesis compared with bovine graft alone,13 while other studies revealed limited bone formation with the addition of PRF, such as Zhang et al, who found that bovine graft alone and PRF-bovine graft following sinus floor augmentation had equal results.14 Additionally, Knapen et al found no additive effect on the kinetics, quality, and quantity of bone by using L-PRF for guided bone regeneration.15 Nonetheless, no study examined the combination of PRF with graft substitutes other than bovine bone.

It was hypothesized that PRF with various bone graft materials would produce different biologic sticky bone, which might affect its clinical efficacy in guided bone regeneration. Specifically, it was hypothesized that growth factors from the PRF would adsorb differently onto the different bone graft materials. Two representative growth factors (PDGF and BMP-2) were selected to test this hypothesis.

MATERIALS AND METHODS

Study Population

The study was approved by the Internal Review Board for Human Studies of the Hadassah Medical Center (HMO-18-0578), and the participants signed a consent form. Pregnant women, chronic medication intake (including aspirin or any other blood-thinning drugs), and smokers were excluded from the study. A total of 10 healthy blood donors were included, with 50% women and a mean age of 23.3 years.

PRF Preparation

Blood was drawn from each subject's antecubital fossa veins into two 10-mL PRF tubes (PRF Process). One tube was used for liquid PRF preparation by centrifugation at 1,300 rpm (maximum relative centrifugal force [RCF]: 170 g)¹⁶ for 5 minutes at room temperature (RT) in a fixed-angle centrifuge (PRF Process). The other PRF tube was used to prepare a serum by centrifugation at 3,000 rpm for 10 minutes at RT in a swing-out centrifuge (Hermle).

Sample Preparation

The following bone grafts were used: (1) mineralized allograft (OraGraft, LifeNet Health); (2) demineralized allograft (OraGraft, LifeNet Health) with particle sizes of 0.25 to 1 mm; (3) bovine xenograft (Bio-Oss, Geistlich) with particle sizes of 0.25 to 1 mm; and (4) alloplastic synthetic material (β -TCP, Sigma-Aldrich) with particle sizes of 0.5 to 0.7 mm.

Two black 96-well plates (Corning) were prepared for each subject, with two wells per plate each containing 10 mg of graft material mixed with 100 uL liquid PRF, and wells containing only the PRF served as control. Following a 5-minute incubation at RT, 125 uL of phosphate-buffered saline (PBS) and 50 uL of serum from the same subject were both added to each well. The plates were incubated at 37°C with 5% CO₂ for 2 hours and 4 days (one plate for each time point). A supernatant was collected from each well (100 µL) every 24 hours, and the medium was refreshed by adding 100 uL fresh PBS. The extracted supernatants were stored at -80°C until use.

Immunofluorescence Staining

Plates containing the grafts within the wells were washed three times with 200 uL of double-distilled water (DDW), blocked with serum from the same patient, and incubated with specific BMP-2 and PDGF antibodies (Abcam). Control staining samples were incubated with DDW. After 24 hours of incubation at RT, all wells were washed with DDW, and fluorescent-labeled specific secondary antibodies (each targeting one of the primary antibodies and labeled with a different fluorochrome) were added to each well, then incubation was continued for 1 hour at RT. Samples were washed with DDW and analyzed with a fluorescence plate reader. The results are expressed as mean relative fluorescent units (RFU).

Microscopic and ELISA Examinations

The 2-hour samples were analyzed under a fluorescence stereomicroscope (Olympus).

Supernatants were collected after 2, 24, 48, and 96 hours from graft preparations. The levels of PDGF and BMP-2 were quantified with enzyme-linked immunoassay (ELISA) kits according to the manufacturer's instructions (R&D systems).

Statistical Analysis

With an estimated effect size of 20% change, an 80% power to detect a significant difference between the groups will require a cohort of 10 cases. Data were analyzed with a statistical software package (SigmaStat, Systat Software). Differences between treated groups were evaluated with one-way repeated-measures ANO-VA. If insignificant, intergroup differences were tested for significance with Student *t* test with Bonferroni correction for multiple tests.



Fig 1 Quantitative analysis of BMP-2 and PDGF adsorption onto the graft materials after (*a*) 2 hours and (*b*) 4 days. Mineralized allograft, demineralized allograft, xenograft, and β -TCP were used to make sticky bone with liquid PRF. The adsorption of BMP-2 and PDGF after preparation was investigated with fluorescence staining and a plate reader. Data are presented in RFU as mean ± SD. Asterisks indicate a statistically significant difference: **P* < .05. ***P* < .01.

RESULTS

Growth Factor Adsorption onto Graft Materials

Two hours after preparation, PRF alone (sham, without graft material) showed the lowest growth factor values compared to all other groups (Fig 1a; P < .05). This is likely because the growth factors were washed out with the PRF during sample preparation. Of the graft materials, β -TCP showed the highest PDGF and BMP-2 levels, whereas both allografts (mineralized and demineralized) showed the lowest level of adsorption from the PRF. The xenograft displayed an intermittent level between the allograft and the alloplastic materials. The

levels of PDGF and BMP-2 in the β -TCP samples were significantly higher than those in the xenografts and the allografts (P < .05).

The retention of the growth factors at 4 days after sticky bone preparation was also examined. Similar to the 2-hour results, both β -TCP and the xenograft showed the highest PDGF and BMP-2 levels compared with the PRF sham, with no differences between the allografts and PRF sham (Fig 1b).

Microscopic Analysis of Growth Factor Adsorption

The microscopic images revealed an even distribution of the growth factors in all tested graft materials Polak et al



Fig 2 Fluorescence microscopy analysis of BMP-2 and PDGF adsorption onto graft materials after 2 hours. Scale bar = 500 μm.

after 2 hours of incubation with the PRF (Fig 2). Further, the staining intensity corroborates the quantitative results, with the highest staining for β -TCP followed by the xenograft and the allograft (mineralized and demineralized).

Growth Factor Release Pattern

The release pattern of the two growth factors from the graft materials differed. The PDGF release was high in the first 2 hours after preparation, then decreased over time (Fig 3). Alternatively, the BMP-2 release was low in the early stage and increased in the following days, with the highest release at 4 days of incubation (see Fig 3). An intragroup comparison for the different graft groups found that the demineralized allograft, xeno-graft, and β -TCP showed significantly higher releases at day 4 than the levels obtained after 2, 24, and 48 hours.

In contrast, the PDGF levels in the supernatants were significantly higher after 2 hours compared with the levels at 24, 48, and 72 hours for the same graft.

DISCUSSION

The present study shows that combining bone graft with PRF to prepare sticky bone enriches the graft material with growth factors that can **bolster** their osteoinductive properties. Those factors are released from the graft preparation in a time-dependent manner. Of the tested materials, β -TCP and xenograft showed a superior ability to adsorb BMP-2 and PDGF compared with the allografts. This, in turn, influenced the release pattern of these factors from the grafts into their surrounding environment.

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Fig 3 Quantitative analysis of BMP-2 and PDGF release from graft materials. The BMP-2 and PDGF concentrations in the graft supernatants were quantified with ELISA at 2, 24, 48, and 72 hours after sticky bone preparation. Data are presented as mean ± SD. An *asterisk* (*) indicates a statistically significant difference between marked bars. A *pound sign* (#) indicates a statistically significant difference from all other groups with different grafts at the same time point. A *double asterisk* (**) indicates a statistically significant difference from all other groups with the same graft at different time points.

PRF is endogenous material rich in pro-healing proteins. Indeed, Dohan et al showed that the slow polymerization occurring during PRF formation leads to intrinsic integration of PDGF-BB and TGF-1 growth factors and IL-1β, IL-4, IL-6, and TNF-a cytokines into the fibrin meshes.² Serafini et al found that various growth factors, such as TGF-1, PDGF-AB, PDGF-BB, VEGF, BMP-2, and fibroblast growth factor-2 are released from the liguid fibrinogen up to 14 days after preparation.¹⁷ In that study, PDGF-BB showed the highest levels, whereas BMP-2 presented a low but continuous release pattern. The present results agree with these findings in terms of growth factors present within the PRF and also show that growth factors adsorb to the different graft materials. Several factors could influence the adsorption rate, such as material porosity and hydrophilicity. Granules of bovine xenograft and alloplast (β-TCP) have a low hydrophilicity compared with the other xenogeneic and alloplastic materials,¹⁸ which may impact the observed growth factor adsorption pattern.

The adsorption of growth factors onto the grafts leads to their release, and indeed, the PDGF release from the graft shows an opposite pattern to their absorption, with a greater release for PRF without a graft and for mineralized and demineralized freeze-dried bone allograft. On the other hand, most PDGF absorptive materials (β -TCP and xenograft) showed a reduced early release and a prolonged phase of sustained release. BMP-2 showed a different pattern, with delayed release from all bone grafts. Yet, the highest levels of released BMP-2 were from materials also displaying the greatest BMP-2 absorption (β -TCP and xenograft). These findings are in accordance with Castro et al, who found that BMP-2 shows high levels of sustained release from xenograft with PRF compared to PRF alone.¹⁹

There is no consensus regarding the osteogenic properties of PRF alone. This may be because PRF lacks rigidity and has a fast degradation,²⁰ which culminates in limited osteogenic ability compared with other materials,²¹ even though the PRF entraps many osteogenesis factors. On the other hand, Choukroun et al²² described the potential ability of PRF to induce angiogenesis and osteoinductivity following sinus elevation, with faster bone formation and maturation. The results of the present study support this notion by showing the ability of bone-forming growth factors to be adsorbed and released from graft materials, which may enhance the PRF osteogenic properties.

CONCLUSIONS

The present study shows that the release kinetics of PDGF and BMP-2 from different graft materials vary considerably. The results suggest that combining PRF with the right graft material for the formation of sticky bone may improve treatment outcomes.

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The research is original, not under consideration for publication elsewhere, and free of conflicts of interest.

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