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Comparison of platelet-rich plasma (PRP), platelet-rich fibrin (PRF), and concentrated growth factor (CGF) in rabbit-skull defect healing

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ABSTRACT

Objectives: The objective of this study was to evaluate the effect of platelet-rich plasma (PRP), platelet-rich fibrin (PRF), and concentrated growth factor (CGF) on bone healing.
Study design: Twelve rabbits were included in this randomized, blinded, prospective study. 15-mm × 10-mm-sized defects were created in the parietal bone, filled with PRP, PRF, CGF, and void. The bone mineral density and bone volume were analyzed with microscopic computed tomography (micro-CT) and histomorphometrics at the 6th and 12th week.
Results: In micro-CT analysis, bone mineral density and bone volume were greater in the experimental group than in controls at both 6th and 12th week, but not among the experimental groups. Similarly, histomorphometric examination revealed that more bone formation was seen in the experimental group.
Conclusion: The addition of PRP, PRF, and CGF had significantly increased bone formation at the 6th week. The effect of PRP, PRF, and CGF was similar and may be useful in the future to increase the success rate of bone grafting.

1. Introduction

In order to achieve successful dental implant placement, one of the essential conditions is the presence of sufficient residual bone at the edentulous site. Much research has been done to improve the efficiency of alveolar bone grafting. One approach is to ameliorate bone graft healing by growth factor enhancement. Growth factors are bioactive proteins which control the process of wound healing. Growth factors have a critical role in cell migration, cell proliferation, and angiogenesis for tissue regeneration.[1] These growth factors are present in blood, within platelets and in plasma. Platelet concentrates such as platelet-rich plasma (PRP), platelet-rich fibrin (PRF), and concentrated growth factor (CGF) have been used for reconstruction of bony defects.[2]

In 1998, Marx reported that bone grafting with a gel-type PRP stimulates ossification in patients with mandibular defects.[3] PRP consists of blood plasma enriched with platelets so that the graft contains more platelets than is present in normal plasma (150–400 × 1000/dl).

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In the research setting, PRP has been reported to facilitate angiogenesis, hemostasis, osteogenesis, and bone growth and has been found to have an anti-infective effect.[4–10] Recently, researchers introduced PRF and CGF and their production methods, which are different from PRP and contain growth factors. The production method of PRF, introduced by Choukroun, is simpler than that of PRP and does not require the addition of thrombin and calcium chloride.[11] Likewise, CGF, introduced by Sacco, is produced through centrifugation, and platelets are concentrated in a gel layer containing fibrin matrix.[12,13]

Growth factor-containing preparations such as PRP, PRF, and CGF are now widely used with the goals of shortening the interval between bone graft placement and implant insertion, thus increasing the success rate of bone grafting and implant therapy. Therefore, the aim of the experiment was to place PRP, PRF, and CGF into bony defects of the rabbit skull in order to compare and evaluate their osteogenic effects.

2. Study design

2.1. Research materials

Preoperative intravenous samples of 5–10 cm^3 of blood were taken from the rabbit ear veins of 12 New Zealand white rabbits and the blood samples were centrifuged to produce autologous PRP, PRF, and CGF.

2.1.1. PRP production

To concentrate platelets from autologous blood, a double centrifugation technique is required. The first spin (called the hard spin) separates the red blood cells from the plasma that contains the platelets, the white blood cells, and the clotting factors. The second spin (called the soft spin) delicately separates the platelets and white blood cells together with a few red blood cells from the plasma. This soft spin produces the PRP and separates it from the platelet-poor plasma (PPP) free from the interference associated with large numbers of red blood cells.[10] In this study, two sample tubes were centrifuged using a PRP centrifuge (GYRO416, Gyrozen, Korea) at 3000 rpm for 4 min. Then, the 4.5 cm^3 of blood plasma was decanted into new tubes, and 4.5 cm^3 of distilled water was added to each new tube. The new tubes were centrifuged at 2200 rpm for 5 min. Then, the blood plasma was divided into the PRP fibrin layer and plasma layers. The pellet was collected as the PRP.

2.1.2. PRF production

PRF is a second-generation platelet aggregation fibrin-rich gel produced from the venous blood by single centrifugation. After centrifugation, the middle layer is obtained from the lowest level of red blood cells, and contains almost no platelets, while above there is a layer of plasma. PRF contains clotting factors that form a fibrin network that traps various cytokines in the PRF. It is not necessary to artificially delay PRF formation with an anticoagulant because it does not begin immediately. It is also not necessary to promote the natural blood-clotting process and platelet activation as the fibrin network structure is formed by centrifugation with large amounts of biological factors such as cytokines being captured. In this study, blood was taken in exactly the same way as for PRP and put into two different tubes. The sample tubes were centrifuged with a PRF centrifuge (GYRO416, Gyrozen, Korea) at 3000 rpm for 10 min. During centrifugation, the hemostasis phenomenon divided the blood sample into layers, and one of these layers was PRF, a fibrin layer containing platelets and plasma.

2.1.3. CGF production

CGF was first developed by Sacco. CGF is produced by the centrifugation of the venous blood as with PRF. However, the technique differs by the centrifugation speed. Unlike PRF, CGF uses variable speeds from 2400 to 2700 rpm to separate cells in the venous blood. This results in fibrin-rich blocks that are much larger, denser, and richer in growth factors than PRF, which in turn results in a better regenerative capacity and greater versatility when using the fibrin-rich block.[23] In this study, an intravenous blood sample was placed into two different tubes. The sample tubes were centrifuged with a CGF centrifuge (MEDIFUGE™, Silfradentsrl, S. Sofia, Italy) at 3000 rpm for 12 min. Centrifugation divided the blood into four layers, and the second layer or buffy coat and the third layer, also called growth factor layer, were made up of the CGF.

Each of the collected products was applied to the bone defects of the rabbit (Fig. 1D–F).

2.2. Animal experiment

The animals were operated while under intravenous general anaesthesia. A 5-cm longitudinal incision was made on the central scalp and the periosteum was elevated from the skull. Then oval-shaped full-thickness bone defects with a diameter of 15 mm × 10 mm were made on each side of the midline sagittal suture (Fig. 1A–C). PRP, PRF, and CGF were randomly selected for grafting of the bone defects (Table 1). The periosteum and the muscle were sutured using 4/0 polyglycolic acid, and the skin with 3/0 silk. Antibiotics (Gentamycin 5 mg/kg, Ajupharm, Korea) were injected intramuscularly to all rabbits to prevent infection.

Those rabbits grafted with PRP, PRF, and CGF made up the experimental group and the rabbits with void defects without any graft constituted the control group. Six rabbits were sacrificed at the 6th week after surgery, and the other six at the 12th week postoperative time point. The samples were fixed for 6 weeks in 10% formalin.

2.3. Analysis on osteogenic effects

2.3.1. Radiologic examination analysis

X-rays of the grafted regions of the cranial bones were taken (65 kvp, 7.5 mA, and 0. 25 s). In order to set a benchmark for the examination, a three-level (0.5, 1.5, and 2.5 mm thickness) aluminium step wedge was used for X-rays of cranial bones. Adobe Photoshop (CS5 extended ver. 12.0, San Jose, CA, USA) was used to evaluate grayscales of the step wedge and five spots around the bone defects within a range of 1 mm. Grayscales were assumed to indicate the density of the new bone (Fig. 2).

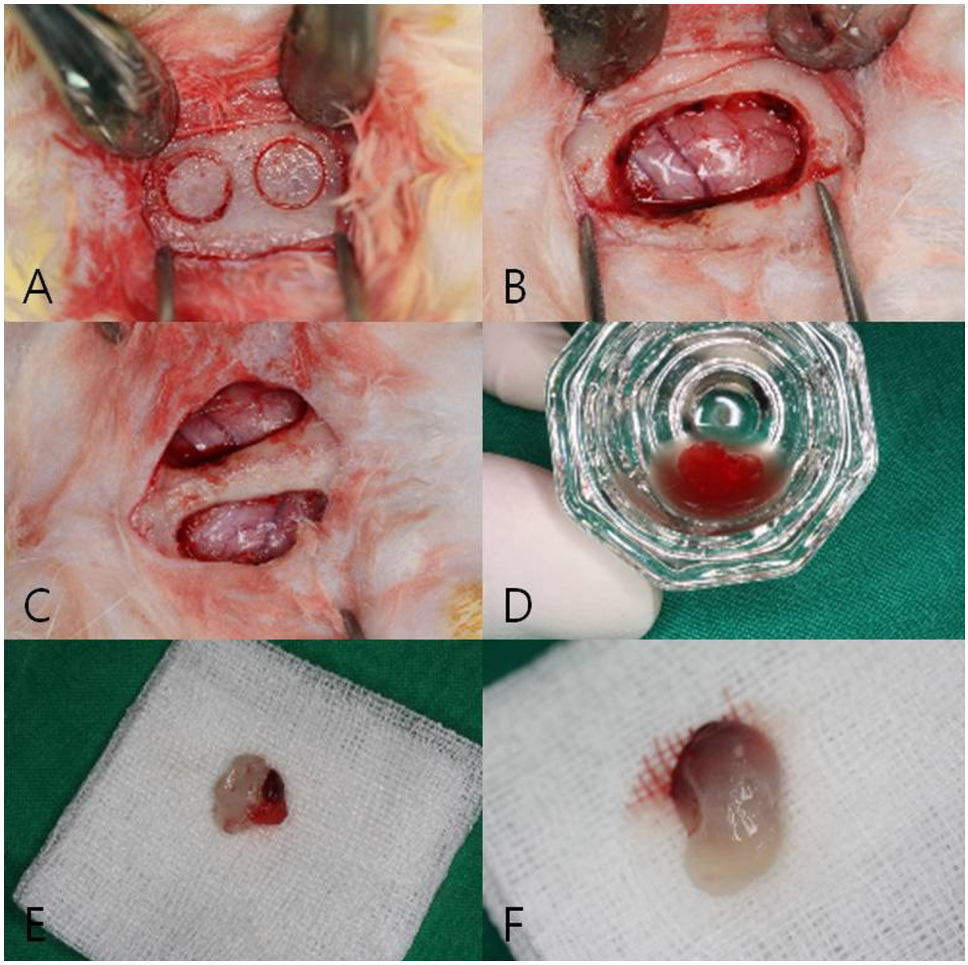

Fig. 1 – (A) Incision is made on the midline of rabbit calvaria with subperiosteal dissection. One side of calvaria is indentated by a 5-mm trephine bur. (B) Critical-(12 mm) and supra-cortical-sized defects created on the right and left rabbit parietal bones. (C) The two critical defects are visible. (D) PRP mixed with thrombin and calcium chloride after centrifugation. (E) CGF after centrifugation of blood. (F) PRF after centrifugation of blood.

2.3.2. *Microscopic computerized tomography analysis*

To evaluate bone repair, a microscopic computerized tomography (micro-CT) (FLEX TM for flatform X-O TM, GMI) was used (Fly mode in 2 × 2 binning, 1184 × 1120 pixels the size of image, 80 kV, 210 μA, 64 mm X-ray detector) and a total of 512 slices were analyzed. As seen from horizontal, sagittal, and coronal views, bone formed within the boundary line of the defects was considered to be a new bone, and the density and volume of the new bone were measured. The bone defects were re-created three dimensionally with 170 pixel size and 512 matrix size.

2.3.3. *Histomorphometric analysis*

Tissue samples were fixed and decalcified for 48 h. Next, the tissue was dehydrated. The sample was embedded in paraffin and cut into 4-μm-thick serial sagittal sections. The sections were stained with haematoxylin and eosin (H–E) stain, and analyzed with an optical microscope. Using digital image analysis, the percentage of the new bone area from of the total defect area was measured. A single investigator traced all the images and measured the percentage of the new bone area relative to the total defect

Table 1 – Grayscale of the defect of the rabbit skull measured by plain film.

		Void	PRP	PRF	CGF	*p* value
Grey scale (Mean ± SD)	6 week	0.2 ± 0.007	0.4 ± 0.006	0.4 ± 0.010	0.2 ± 0.006	0.000*
	12 week	0.4 ± 0.007	0.47 ± 0.011	0.47 ± 0.010	0.4 ± 0.008	0.000*

Data represent mean ± SD.
* Statistical significance was considered to be $p < .05$.
PRP, platelet-rich plasma; PRF, platelet-rich fibrin; and CGF, concentrated growth factor.

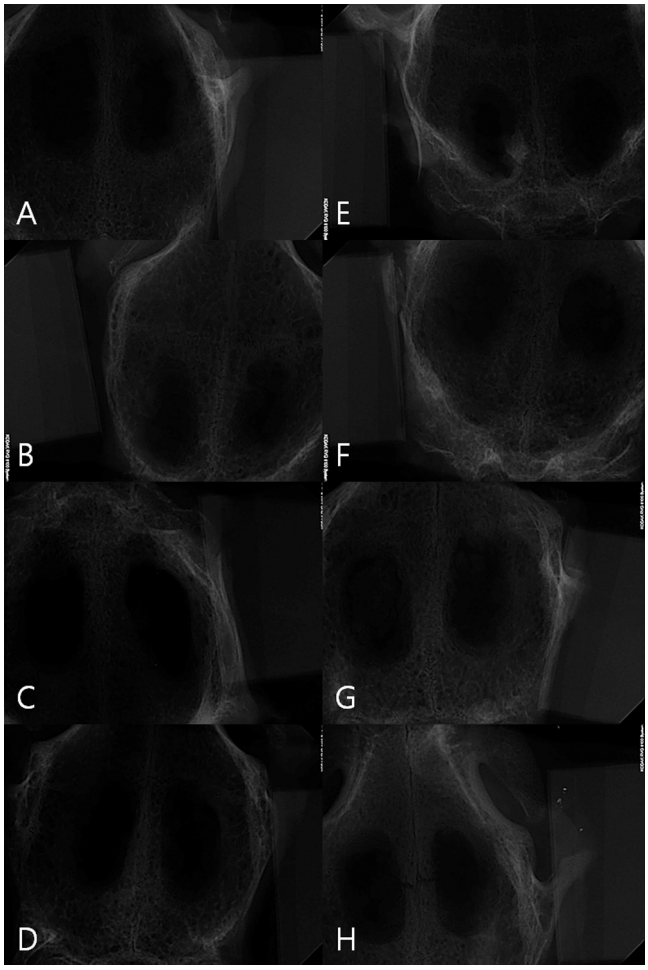

Fig. 2 – Postsacrifice radiograph showing defects bilaterally in the parietal bones. (A) A control group radiograph: grayscale is high. (B) Experimental group: grayscale is low. Therefore, in the experimental group, there is greater new bone formation.

area. In addition, Masson's trichrome stain was used to detect new bone formation.

2.4. *Statistical analysis*

In order to determine the osteogenic differences between the experimental group and the control group and the differences within the experimental groups of growth factors, data from the 6th week postoperative time points and the 12th week postoperative time points were evaluated statistically using one-way analysis of variance (ANOVA). Grayscale values from the radiologic examination, volume and density from the micro-CT scan analysis, and the new bone area from the histomorphometric analysis were tested using one-way ANOVA (SPSS Window18.0, SPSS Inc., Chicago, IL, USA) to determine statistical significance. When a p-value was $< .05$, it was considered to be statistically significant. Turkey test was also performed to check for significant differences between groups after postmortem examination.

Table 2 – Post hoc test after one-way ANOVA results: plain X-ray film ($p < .05$).

		p value	
		6 week	12 week
Void	PRP	.011*	.000*
	PRF	.000*	.000*
	CGF	1.000	.926
PRP	PRF	.000*	.824
	CGF	.004*	.000*
PRF	CGF	.000*	.000*

* Statistical significance was considered to be $p < .05$.
PRP, platelet-rich plasma; PRF, platelet-rich fibrin; and CGF, concentrated growth factor.

3. Results

3.1. *Clinical evaluation*

There was no unexpected mortality among the rabbits and all the surgical sites healed well. When the surgical sites were reexposed after sacrifice, both groups showed formation of new bone at the parietal bone defects.

3.2. *Radiologic analysis*

Table 2 shows grayscale values of five spots around the bone defects within a range of 1 mm. The PRP group showed higher grayscale values for the 6th week and the highest grayscale values for the 12th week. At the 6th week postoperative time point, the PRF group showed the highest grayscale values. At the 6th week and 12th week postoperative time points, differences between the control group and the PRF group were statistically significant. In addition, the CGF group showed the lowest grayscale values for the 6th week postoperative time points ($p < .05$) (Fig. 2, Table 3).

Table 3 – Bone mineral density and bone volume on the defect of the rabbit calvaria measured by micro-CT ($p < .05$).

		Void	PRP	PRF	CGF	p value
Bone volume (Mean ± SD)	6 week	27.49 ± 2.21	43.70 ± 1.93	45.39 ± 2.17	45.35 ± 7.36	0.022*
	12 week	37.74 ± 2.76	57.36 ± 5.22	59.58 ± 10.23	59.52 ± 5.22	0.011*
Bone mineral density (Mean ± SD)	6 week	202.14 ± 1.28	245.09 ± 15.14	251.92 ± 59.22	251.42 ± 4.37	0.248
	12 week	250.79 ± 14.36	264.21 ± 44.48	256.92 ± 11.51	257.68 ± 12.36	0.749

BMD – mg/ml, Bone volume – mm^3.
Data represent mean ± SD.
* Statistical significance was considered to be $p < .05$.

Table 4 – Post hoc test after one-way ANOVA results – Micro-CT.

		p value			
		BV		BMD	
		6 week	12 week	6 week	12 week
Void	PRP	.015*	.049*	.463	.931
	PRF	.015*	.029*	.279	.978
	CGF	.043*	.022*	.235	1.000
PRP	PRF	1.000	.969	.963	.697
	CGF	.232	.965	.947	.903
PRF	CGF	.240	1.000	1.000	.956

* Statistical significance was considered to be $p < .05$.
PRP, platelet-rich plasma; PRF, platelet-rich fibrin; and CGF, concentrated growth factor.

3.3. *Micro-CT analysis*

The values of volume and density of the new bone are shown in Table 4. Larger amounts of the new bone were formed in the experimental group than those in the control group ($p < .05$). The value of the new bone density of the experimental group was higher than that of the control group although not statistically significant ($p = .248$). For the 12th week postoperative time point, the volume of bone formed in the experimental group was larger than that of the control group ($p < .05$) (Figs. 3 and 4).

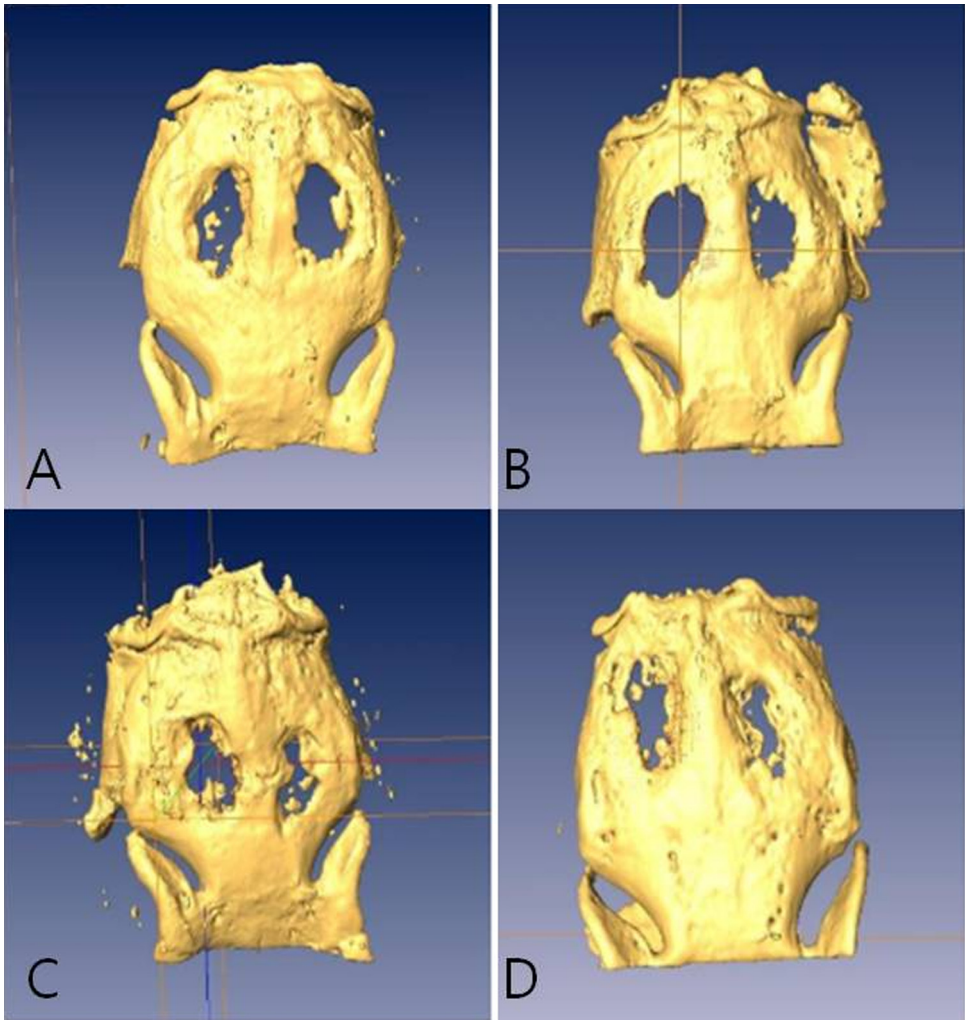

Fig. 3 – Micro-computed tomography (μCT) analysis – 3D reconstruction. (A) 3D reconstruction image in an experimental group at 6 weeks after sacrifice. (B) 3D reconstruction image in a control group at 6 weeks after sacrifice. (C) 3D reconstruction image in an experimental group at 12 weeks after sacrifice. (D) 3D reconstruction image in a control group at 12 weeks after sacrifice.

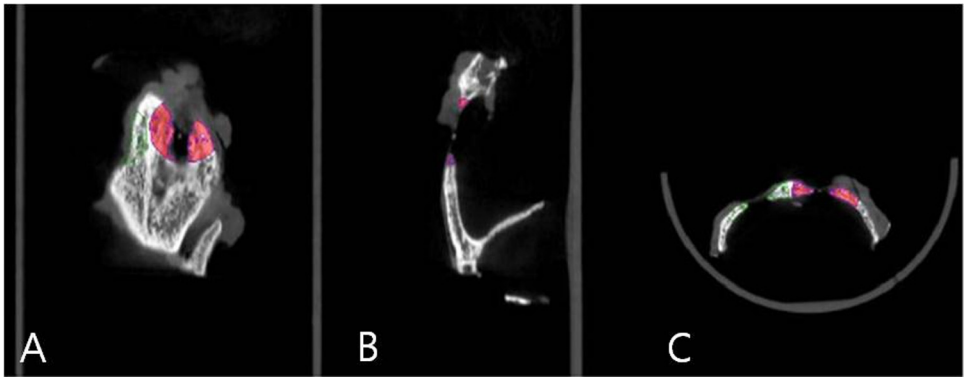

Fig. 4 – Micro-computed tomography: (A) Coronal plane. (B) Sagittal plane. (C) Coronal plane.

Among the experimental groups, for the 6th week postoperative time point, the PRP group showed the lowest values for volume and density of new bone but with no statistical significance ($p > .05$). For the 12th week postoperative time point, the PRP group showed the lowest value for volume and the highest value for density but with no statistical significance ($p > .05$). In the control group, the void should have the lowest values for volume and density of new bone, but at the 12th week postoperative time point, the bone mineral density of the void was high like the values of the experimental group (Table 5).

3.4. *Histomorphometric analysis*

The data from histomorphometric analysis are summarized in Table 6. At the 6th week, each of the experimental groups formed a larger amount of new bone than the control group, and at the 12th week was like the 6th week, but without any statistical significance. Among the experimental groups, the PRP group formed a smaller amount of new bone than the other two groups. However, there was no statistical significance (Figs. 5 and 6).

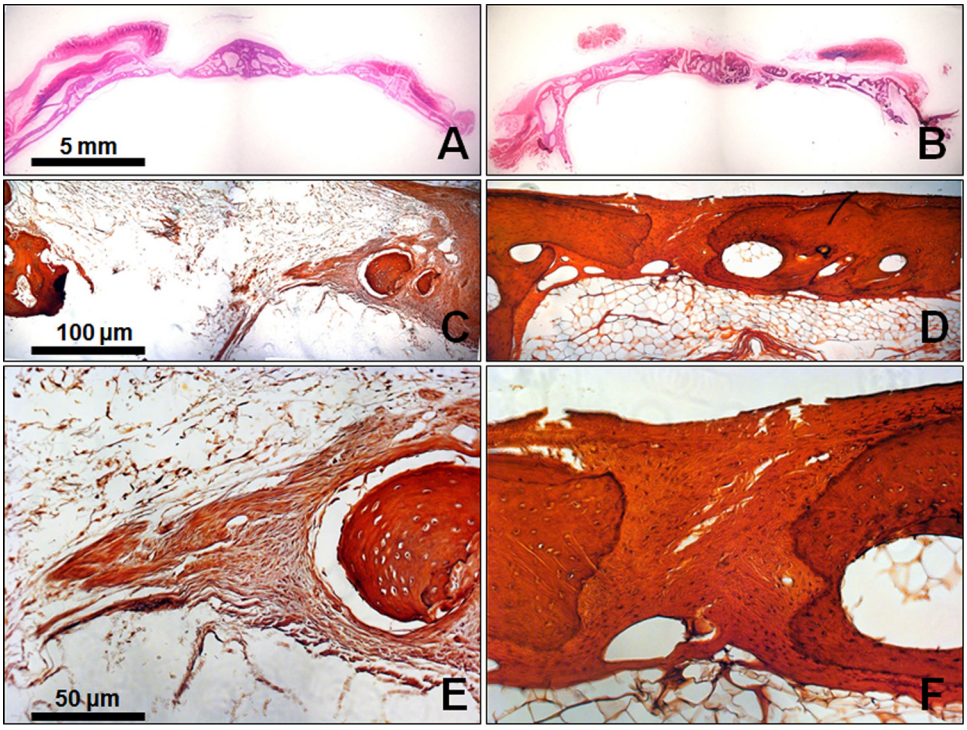

Fig. 5 – (A) Microphotograph at week 6 after sacrifice in control site. H–E. × 10. (B) Microphotograph at week 6 after sacrifice in experimental site. H–E. × 10. (C) Microphotograph at week 6 after sacrifice in control site. H–E. × 100. (D) Microphotograph at week 6 after sacrifice in experimental site. H–E. × 100. (E) Microphotograph at week 6 after sacrifice in control site. H–E. × 200. (F) Microphotograph at week 6 after sacrifice in experimental site. H–E. × 200. In nongrafted defect (control group), central ingrowth of new bone from the marginal bone was relatively formed but was not fused at centre. In grafted defect (experimental group), the defect was completely covered with newly formed bone.

Table 5 – Ratio (%) of new bone volume on the defect of the rabbit calvaria measured by histomorphometry.

		Void	PRP	PRF	CGF	p value
Bone volume (Mean ± SD)	6 week	26.63 ± 2.47	36.86 ± 4.66	37.85 ± 3.40	39.18 ± 2.46	0.001*
	12 week	48.14 ± 9.33	52.69 ± 2.16	50.70 ± 4.60	57.52 ± 2.48	0.618

Data represent mean ± SD.
* Statistical significance was considered to be $p < .05$.
PRP, platelet-rich plasma; PRF, platelet-rich fibrin; and CGF, concentrated growth factor.

Table 6 – Post hoc test after one-way ANOVA results: Histomorphometry.

		p value	
		6 week	12 week
Void	PRP	.002*	.671
	PRF	.003*	.961
	CGF	.043*	.677
PRP	PRF	.961	.876
	CGF	.068	1.000
PRF	CGF	.057	.888

* Statistical significance was considered to be $p < .05$.
PRP, platelet-rich plasma; PRF, platelet-rich fibrin; and CGF, concentrated growth factor.

Each experimental group formed more new bone than the control group. Among the experimental groups, the PRF group formed the least new bone, while the CGF formed the most new bone although the difference was not statistically significant ($p > .05$).

4. Discussion

Implant treatment has become an important part of dentistry, and much research is concentrated on alveolar atrophy that makes dental implant placement difficult. One approach is the use of concentrated platelets. Concentrated platelets contain many growth factors including: platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β), insulin-like growth factor (IGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), and bone morphogenic protein (BMP). PDGF, which Ross first described in 1974, exists in platelet alpha granules or giant cells and stimulates angiogenesis, osteoblastic proliferation and differentiation, and mesenchymal cell division. PDGF also facilitates cell proliferation and collagen synthesis in fibroblast.[14] TGF-β affects osteoblast in an early stage of development and stimulates collagen synthesis by fibroblasts, which facilitates regeneration of bone and cartilage. IGF helps differentiation and stimulates osteogenesis. PDGF and TGF-β are especially known to ameliorate tensile strength and callus formation with effects

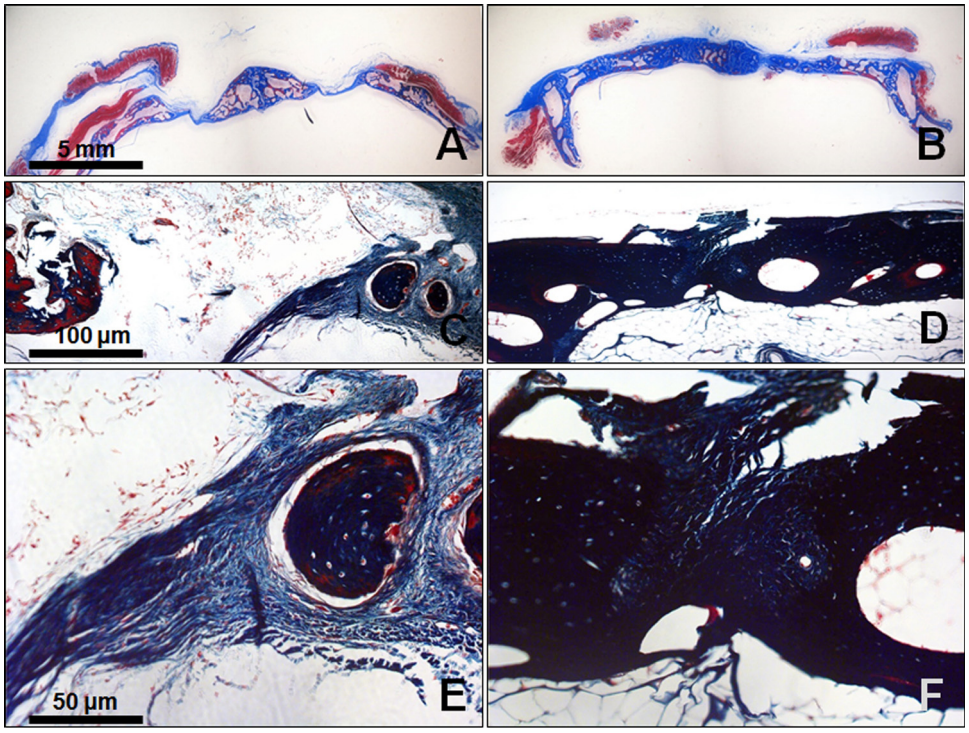

Fig. 6 – (A) Microphotograph at week 6 after sacrifice in control site. Masson's trichrome. ×10. (B) Microphotograph at week 6 after sacrifice in experimental site. Masson's trichrome. ×10. (C) Microphotograph at week 6 after sacrifice in control site. Masson's trichrome. ×100. (D) Microphotograph at week 6 after sacrifice in experimental site. Masson's trichrome. ×100. (E) Microphotograph at week 6 after sacrifice in control site. Masson's trichrome. ×200. (F) Microphotograph at week 6 after sacrifice in experimental site. Masson's trichrome. ×200.

on healing of soft tissue and bone.[15–17] Tsay has suggested that growth factors such as PDGF and TGF-β cause chemotaxis of precursor cells of osteoblast to sites where bone regeneration is needed, and the chemotactic process is followed by proliferation and differentiation of osteoblasts.[18] Numerous methodologies have been suggested which use concentrated platelets containing such growth factors. As a result, PRP, PRF, and CGF have been developed.

PRP contains highly concentrated platelets created through centrifugation with approximately 1 million platelets per 1 mm^3. PRP has been reported to increase the density of trabecular bone at bone graft sites.[18]

PRF resembles PRP but PRF has, in addition to PRP, its own natural fibrin network. The number of concentrated platelets in PRF is same as that of PRP, but PRF has its own natural fibrin network that protects growth factors from proteolysis.[19] Furthermore, PRF does not require additives such as thrombin as it possesses the previously mentioned inherent fibrin network.

CGF, first introduced by Sacco, has recently become popular. CGF forms richer layers of growth factors and provides an enriched fibrin clot.[12] This fibrin clot has a high cohesion because of the agglutination of fibrinogen, factor XIII, and thrombin. Factor XIIIa, which is activated by thrombin, causes fibrin to clot. This provides protection from plasmin degradation, resulting in higher fibrin tensile strength and stability.[20]

In the radiographic analysis of this study, each experimental group had a larger volume and higher density of new bone than the control group for both the 6th week and the 12th week time points. In histological analysis, the experimental groups have more new bone formation. However, comparison among the experimental groups showed little difference in bone density and the percentage of new bone. In micro-CT analysis, there was a statistically significant difference between the experimental groups and the control group ($p = .022$), but no statistically significant differences were seen within the experimental groups. For bone density in micro-CT analysis, the PRP group showed the lowest value for the 6th week time point but the highest for the 12th week time point. The result implies that higher bone density does not necessarily mean larger volume of new bone. In addition, the control group showed a large increase in density between the 6th week and the 12th week time points, while the experimental groups showed little difference. The result indicates that the growth factors in PRP, PRF, and CGF have large effects on nearly every stage of bone graft healing. This result is in agreement with other studies on effects of growth factors.[3,21]

Additionally, comparisons of two-dimensional radiographs, micro-CT, and histological observations did not show statistical significance among the measured values of the experimental groups. For the 6th week time point, the grayscale value of the CGF group was lower than that of other experimental groups. For the 12th week time point, there was little or no difference in the grayscale value among the experimental groups ($p = 1.000$). However, this result does not agree with the result from the micro-CT analysis. Therefore, if the micro-CT is considered more credible and the two-dimensional radiography is considered less credible, then it is understandable that the bone density inside the newly formed bone graft may be low even if two-dimensional radiographs show similar grayscale results of new and mature bone. Thus, two-dimensional radiography is not a dependable imaging technique to determine bone graft healing. Micro-CT is far more precise. Accurate assessment of bone graft healing time before starting prosthetic treatment such as dental implant may lead to better results.

The results which indicate that the experimental group formed more new bone agree with the results of other PRP studies that used PRP as the only experimental group in assessing osteogenic effects.[10,13]

However, comparison of the osteogenic effects among the experimental groups showed no statistically significant differences, and the result does not support the results of other studies suggesting that CGF and PRF have more profound osteogenic effects. In this current study, unlike previous studies, the osteogenic effects of PRF and CGF were not greater than those of PRP.[22]

Marx suggests that it takes 5–7 days for growth factors to directly affect cells in bone grafting. Ling suggests that PRP factors last 7 days and have no protection effects, while PRF factors have greater effects in stimulating proliferation and differentiation of osteoblast for longer time periods.[3,21] Exudates containing growth factors from PRF stimulate differentiation and proliferation of osteoblast to accelerate inorganic synthesis. This effect is maximized at the 14th day. Lundquist argued that enriched fibrin in PRF protects the growth factors from proteolysis.[19] Ling also stated that TGF-β1 of PRF synthesized more collagen than PDGF-AB of PRP and created more extracellular matrix. The extracellular matrix ameliorates calcification of osteogenesis.[21]

In research performed by his group with sinus augmentation, Sohn suggests that CGF alone has sufficient inherent osteogenic effects. PRP, PRF, and CGF were applied and observed for 5–30 days. These results suggested that CGF formed new bone more effectively when used with guided bone regeneration (GBR) and guided tissue regeneration (GTR) with membranes.[23] CGF and PRF consist of almost identical components; however, CGF protects the growth factors better from proteolysis due to its higher tensile strength and viscosity.

However, the placement of dental implant requires more than a 12-week-long interval after bone grafting. Thus, the growth factors that cause differences in osteogenic effects at early stages may have little or no effect in the long term. The experimental groups for both the 6th week and the 12th week time points showed more new bone formation than the control group; hence, it can be inferred that applying growth factors to bone defects results in greater osteogenic effects than applying nothing. Moreover, the efficacy of growth factors in bone regeneration depends on many parameters including the animal species, the concentrations of growth factors, and the individual itself of the same species. Based on the methods of PRP, PRF, CGF preparation used in the current study, the number of animals, and the size of defect, our results showed that applying growth factors as well as other treatments such as a bone graft to an area where bone regeneration is required may accelerate the bone graft healing and shorten the time to dental implant placement, shortening the time period in which patients remain in an edentulous state.

5. Conclusion

Based on these results, the use of PRP, PRF, and CGF facilitates new bone formation in the early stage of bone graft healing. After the 12th postoperative week, there was no difference in osteogenesis among growth factors. Thus, the authors consider that further studies about the long-term effects of PRP, PRF, and CGF must be done.

Author's contribution

Tae-Hoon Kim involved in writing manuscript, performing animal surgery. Sung-Hee Kim contributed in performing animal surgery, analysis of results. George K Sandor involved in design animal model, reviewing the manuscript and Yong-Deok Kim contributed in study design, writing manuscript.

Funding

None.

Conflict of interest

There is no conflict of interest.

Ethical approval

This study was approved by the Animal Care Ethics Committee of Pusan National University Hospital, Korea.

REFERENCES

1. Clark RAF. Fibrin and wound healing. *Annals of the New York Academy of Sciences* 2001;**936**:355–67.
2. Bhanot S, Alex JC. Current applications of platelet gels in facial plastic surgery. *Facial Plastic Surgery* 2002;**18**:27–33.
3. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma-Growth factor enhancement for bone grafts. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontology* 1998;**85**:638–46.
4. Schaberg SJ, Petri WH, Geory FW, Auclar PL, Jacob E. A comparison of freeze dried allogenic and fresh autogenous vascularized rib graft in dog radial discontinuity defects. *Journal of Oral and Maxillofacial Surgery* 1985;**43**:932–7.
5. Ross R, Raines EW, Bowen-Pope DF. The biology of platelet derived growth factor. *Cell* 1986;**46**:155–69.
6. Antoniades HN, Williams LT. Human platelet-derived growth factor: structure and function. *Federation Proceedings* 1983;**42**:2630–4.
7. Canalis E, McCarthy TL, Cetrella M. Effect of platelet-derived growth factor on bone formation in vitro. *Journal of Cellular Physiology* 1989;**140**:530–7.
8. Pierce GF, Mustoe TA, Altrock BW, Deuel TF, Thomason A. Role of platelet-derived growth factor in wound healing. *Journal of Cellular Biochemistry* 1991;**45**:319–26.
9. Kassolos JD, Rossen PS, Reynolds MA. Alveolar ridge and sinus augmentation utilizing platelet rich plasma in combination with freeze dried bone allograft: case series. *Journal of Periodontology* 2000;**71**:1654–61.
10. Marx RE. Platelet-rich plasma (PRP). What is PRP and what is not PRP? *Implant Dentistry* 2001;**10**:225–8.
11. Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part IV: clinical effects on tissue healing. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontology* 2006;**101**:e56–60.
12. Rodella LF, Favero G, Boninsegna R, Buffoli B, Labanca M, Sacco L, et al. Growth factors CD34 positive cells, and fibrin network analysis in concentrated growth factors fraction. *Microscopy Research and Technique* 2011;**74**:772–7.
13. Plachokova AS, Nikolidakis D, Mulder J, Jansen JA, Creugers NH. Effect of platelet-rich plasma on bone regeneration in dentistry: a systematic review. *Clinical Oral Implants Research* 2008;**19**:539–45.
14. Bornfeldt KE, Raines EW, Graves LM, Skinner MP, Krebs EG, Ross R. Platelet-derived growth factor. Distinct signal transduction pathways associated with migration versus proliferation. *Annals of the New York Academy of Sciences* 1995;**766**:416–30.
15. Pierce GF, Tarpley JE, Allman RM, Goode PS, Serdar CM, Morris B, et al. Tissue repair processes in healing chronic pressure ulcers treated with recombinant platelet-derived growth factor BB. *American Journal of Pathology* 1994;**145**:1399–410.
16. Bolander ME. Regulation of fracture repair by growth factors. *Proceedings of the Society for Experimental Biology and Medicine* 1992;**200**:165–70.
17. Cromack DT, Porras-Reyes B, Mustoe TA. Current concepts in wound healing: growth factor and macrophage interaction. *Journal of Trauma* 1990;**30**:S129–33.
18. Tsay RC, Vo J, Burke A, Eisig SB, Lu HH, Landesberg R. Differentiatial growth factor retention by platelet-rich plasma composites. *Journal of Oral and Maxillofacial Surgery* 2005;**63**:521–8.
19. Lundquist R, Dziegiel MH, Agren MS. Bioactivity and stability of endogenous fibrogenic factors in platelet-rich fibrin. *Wound Repair and Regeneration* 2008;**16**:356–63.
20. Tayaponsak P, O'Brien DA, Monterio CB, Arceo-Diaz LL. Autologous fibrin adhesive in mandibular reconstruction with particulate cancellous bone marrow. *Journal of Oral and Maxillofacial Surgery* 1994;**52**:161–5.
21. He L, Lin Y, Hu X, Zhang Y, Wu H. A comparative study of platelet-rich fibrin (PRF) and platelet-rich plasma (PRP) on the effect of proliferation and differentiation of rat osteoblasts in vitro. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontology* 2009;**108**:707–13.
22. He L, Lin Y, Hu X, Zhang Y, Wu H. A comparative study of platelet-rich fibrin (PRF) and platelet-rich plasma (PRP) on the effect of proliferation and differentiation of rat osteoblast in vitro. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontology* 2009;**108**(November (5)):707–13.
23. Sohn DS, Moon JW, Moon YS, Park JS, Jung HS. The use of concentrated growth factors (CGF) for sinus augmentation. *Journal of Oral Implant (Japan)* 2009;**38**:25–35.