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Effect of platelet-derived materials (PRF, CGF) on new bone formation after endodontic microsurgery

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신생골 형성에 미치는 영향

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치 의 학 과

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지도교수 유 재 식

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치 의 학 과

정 겨 운

정겨운의 박사학위 논문을 인준함

위원장 조선대학교 교수 김 홍 중 (인)

위 원 한양대학교 교수 황 경 균 (인)

위 원 조선대학교 교수 안 종 모 (인)

위 원 조선대학교 교수 오 지 수 (인)

위 원 조선대학교 교수 유 재 식 (인)

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조선대학교 대학원

Table of Contents

List of Tables

List of Figures

초록

치근단 절제술 후 혈소판 유래 물질(PRF, CGF)이 신생골 형성에 미치는 영향

정 겨 운
지도교수: 유재식, 치의학 박사
조선대학교 대학원 치의학과

연구 목적: 본 연구에서는 치근단 병소를 가진 환자 중 현미경을 이용한 치근단 절제술 및 낭종 적출술을 시행한 환자를 대상으로 한 전향적 연구를 통해 해당 골 결손부에 혈소판 유래 물질(혈소판 풍부 섬유소(PRF), 농축 성장인자(CGF))을 사용하여 신생골 형성에 미치는 효과를 평가하고자 하였다.

대상 및 방법: 상악 전치부 1치관 크기의 치근단 낭을 주소로 조선대학교 치과병원에 내원한 18명의 환자를 대상으로 하였다. 환자들은 대조군 (6명)과 PRF (6명)와 CGF (6명)를 넣은 실험군으로 나누어 무작위 대조 연구를 시행하였다. 현미경을 이용한 치근단 절제술을 시행하였고, 순측 치조골삭제의 크기는 평균 3-4mm, 근단부위 3mm는 MTA로 역충전하였다. 낭종 적출술 및 치근단 절제술 후 두 실험군 12명의 골 결손 부위에 혈소판 유래 물질 (PRF 6 명, CGF 6명)을 채우고, 대조군에는 아무것도 넣지 않았다. 수술 직후, 수술 후 3개월 및 6개월이 지났을 때, Cone-beam computed tomography (CBCT)을 촬영하여 신생골 형성 정도를 비교하였다. 골 결손부의 체적변화는 Mimics medical software를 이용하여 측정하였고, Kruskal-Wallis test와 Mann-Whitney U test를 사용하여 통계학적으로 분석하였다.

결과: 치근단 절제술 및 낭종 적출술 시행 3개월, 6개월 후 골 결손부의 재생은 PRF, CGF 를 넣은 그룹이 대조군보다 더 높게 나타났다. 그러나, 골 결손부 감소율은 3개월 후에 측정한 결과에서만 통계학적으로 유의성이 있었고 ($p < 0.05$), 6개월 후에 측정한 골 결손부 감소율은 세 그룹간 유의성이 없었다 ($p > 0.05$). PRF 와 CGF 그룹을 비교하였을 경우, CGF 를 넣은 그룹에서 골재생 비율이 약간 더 높게 나왔으나 이 역시 두 그룹 간에는 통계학적으로 유의미한 차이를 보이지는 않았다 ($p > 0.05$).

결론: 이 연구에서 대조군과 비교하여 PRF와 CGF를 넣은 실험군에서 현미경을 이용한 치근단 절제술 및 낭종 적출술을 시행한 골 결손부에서 뚜렷한 치근단 부위 골 재생이 관찰되었다. 이는 혈소판 유래 물질 (PRF, CGF)을 처리한 치료방법이 초기 신생골 형성에 유효한 효과를 보여준 것으로 치근단 낭의 치료 기간 단축을 기대해볼 수 있다. 그러나 외과적 근관치료에서 이러한 혈소판 유래 물질 (PRF, CGF)의 장점을 정확히 평가하기 위해서는 더 많은 환자들을 대상으로 추가적인 임상 연구가 필요할 것으로 생각된다.

주제어: 골 재생, 농축 성장 인자, 치근단 낭, 치근단 절제술, 혈소판 유래 물질, 혈소판 풍부 피브린

I. Introduction

Recently, substances that promote bone formation have been widely used in the field of dentistry for the repair of bone defects. These substances include transforming growth factor (TGF), platelet-derived growth factor (PDGF), and bone morphogenetic protein (BMP), which are known to be abundant in blood[1]. Platelet derived materials are produced by the centrifugation of blood, which is an effective and easy way to obtain growth factors. Platelet-derived materials, including platelet-rich fibrin (PRF) and concentrated growth factor (CGF), contain growth factors such as fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), transforming growth factor β (TGF-β), and epidermal growth factor (EGF). PRF and CGF are either used alone or as an adjunct for promoting soft and hard tissue regeneration in dentoalveolar and maxillofacial surgery[2].

Root canal treatment is usually successful, and symptoms may persist or recur in approximately 10 to 15% of the cases[3]. Such cases that remain unresolved with conventional endodontic therapy or retreatment are indicated for endodontic microsurgery to treat persistent apical periodontitis[4]. In addition, the success rate associated with surgical endodontics has increased with the introduction of microscope, ultrasonic instruments, micro-instruments, and the development of filling materials that are commonly used[5]. Recently, the use of platelet-derived materials during endodontic microsurgery has gained increasing interest[6,7]. Bone defect heals by spontaneous bone regeneration after cyst enucleation; however, in a large bone defect, a bone graft or platelet-derived materials are used to promote bone regeneration. PRF and CGF are platelet concentrates collected from a single fibrin membrane, which contains the components of blood that are beneficial for immunity and healing. These substances release growth factors and cytokines that stimulate bone and soft tissue healing. In addition, the fibrin matrix is responsible for immune regulation and angiogenesis[6,8].

Although many studies have been reported[9,10], there are few clinical studies on the efficacy of PRF and CGF in the field of surgical endodontics. The purpose of this study was to evaluate the effect of these substances on bone regeneration by applying these materials to bone defects after cyst enucleation and endodontic microsurgery.

Ⅱ. Subjects and methods

Subjects

This study was approved by the Institutional Review Board (IRB) of the Chosun University Dental Hospital (CUDHIRB 1902 010 R01).

This randomized controlled trial was conducted on patients who visited the Chosun University Dental Hospital from October 2019 to September 2021 with a periapical lesion. Diagnosis of periapical cysts was based on the clinical and radiographic findings of patients (age range; 18 to 68 years). Patients with systemic diseases, such as diabetes and osteoporosis that could have affected the bone metabolism and healing were excluded. Patients with cystic lesions in the maxillary anterior region and cysts of less than 10 mm (mesiodistal diameter) were included. A total of 18 patients (9 males and 9 females) participated in this clinical trial, and they were randomly assigned to a control group (untreated group, n=6) and an experimental group (PRF group, n = 6 and CGF group, n=6).

Surgical procedure and volumetric measurement

For each patient in the experimental group prior to surgery, PRF or CGF was prepared with 10 cc of venous blood collected from the patient's forearm according to the manufacturer's protocol (Fig. 1). Cyst enucleation and endodontic microsurgery were performed under local anesthesia in all patients (Fig. 2). The mean size of labial bone osteotomy was 3-4 mm, and retrograde filling up to 3 mm was done with mineral trioxide aggregate (MTA). After the surgical procedure, the apical bone defects in the patients of the two experimental groups were filled with each of the materials (6 by PRF and 6 by CGF). The unfilled cavities of six patients were used for control group. The surgical procedures were

performed by a single surgeon.

Cone beam computed tomography (CBCT) was performed immediately, 3 months, and 6 months after surgery using CS9300 3D CBCT (Carestream Health Inc., Rochester, NY, USA) with a setting value of 4-10 mA and 85-120 kV, with a voxel size of 0.2-0.3 mm. To measure the volume of bone defects, image files in digital imaging and communications in medicine (DICOM) were transferred to Mimics Medical 21.0, software (Materialize, Leuven, Belgium). The threshold was appropriately adjusted to mark the bone defects. After image segmentation of the bone defect in the axial, coronal, and sagittal views, the volume was calculated by converting it into a 3D image (Fig. 3).

In this study, the standard threshold range was set at -326 to 670, and a fixed threshold was applied equally to all the patients. The volume of bone defects was measured immediately, 3 months, and 6 months after surgery (Fig. 4). All volumetric measurements were performed by a single observer. The volume of bone defects was measured three times at fortnightly intervals. Bone regeneration in each group was compared based on the volume reduction rate of bone defects. Since the size of the initial lesion varied for each patient, the “reduction rate of the bone defect” was calculated. The value was divided by the volume measurement taken immediately after surgery.

Statistical analysis

The reduction rate of bone defects was statistically compared among groups using the Kruskal–Wallis test, and the post-hoc Mann–Whitney U test with Bonferroni correction. Intraclass correlation coefficients (ICC) were used to estimate the reliability of volumetric measurements using Mimics Mdical software version 21.0. IBM SPSS software (SPSS 25.0, IBM Corp., NY, USA) was used to perform statistical analysis. Statistical significance was set at $p < 0.05$.

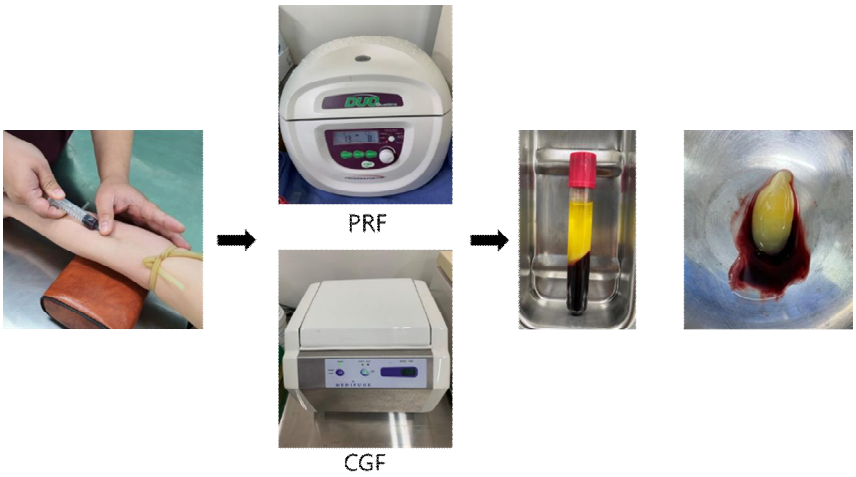

Fig. 1. The preparation of platelet-rich fibrin (PRF) and concentrated growth factor (CGF) from blood. Blood was collected from the patient and CGF or PRF was prepared according to the manufacturer's protocol.

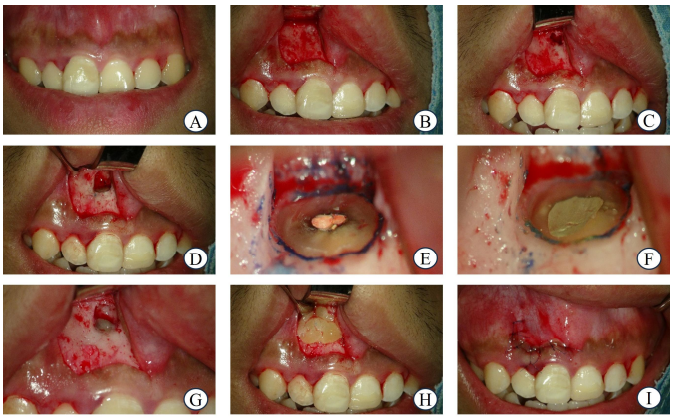

Fig. 2. Surgical procedure - cyst enucleation and endodontic microsurgery.
A-B: semilunar incision and full thickness periosteal flap, C-D: labial bone osteotomy and cyst enucleation, E: root-end resection, F-G: root-end filling with mineral trioxide aggregate, H: platelet-rich fibrin (PRF) or concentrated growth factor (CGF) insertion, I: suture

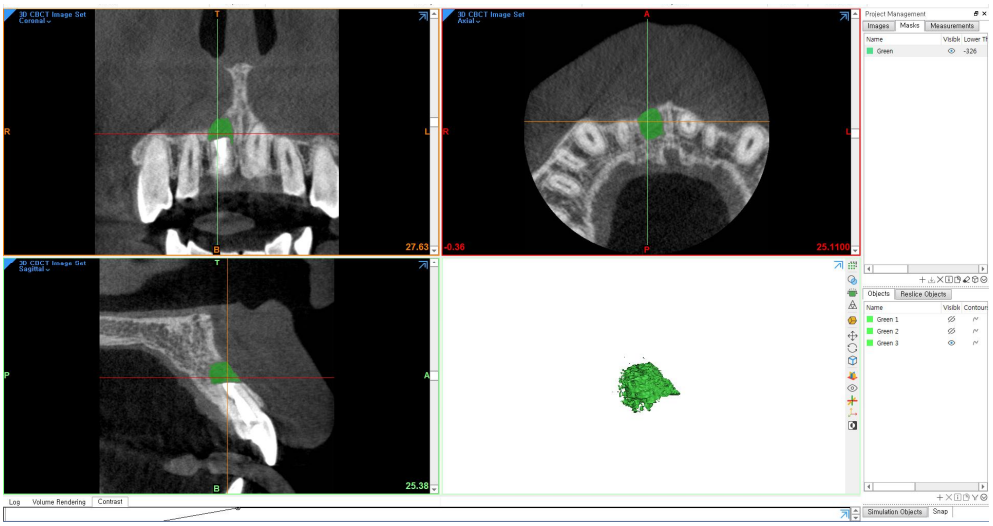

Fig. 3. Measurement of the volume of bone defects using Mimics medical software.

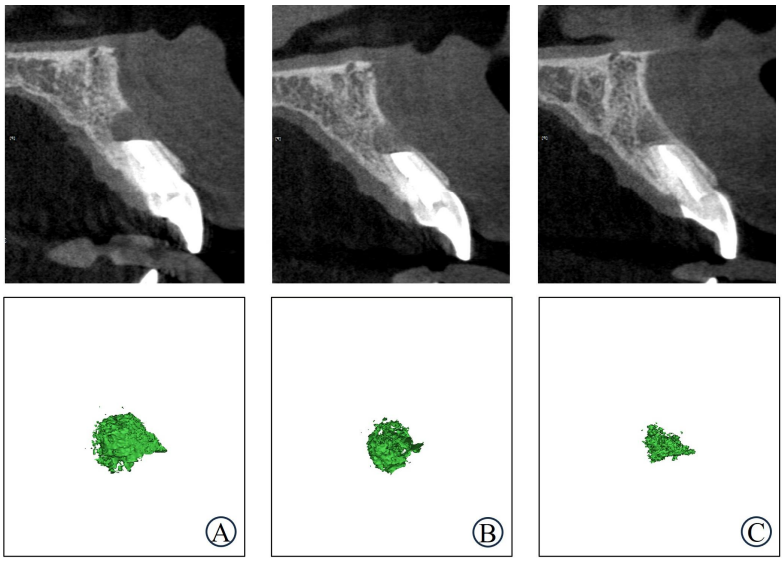

Fig. 4. Cone beam computed tomography (CBCT) sagittal view and the bone defect 3D image. A: immediately after surgery, B: 3 months after surgery, C: 6 months after surgery

Ⅲ. Result

The reliability of values for measured volumes was confirmed using intraclass correlation coefficients (ICC). The ICC was 0.997, indicating high intra-observer consistency.

There were no signs of infection or wound dehiscence in the patients, and soft tissue healing was achieved without complications.

The volume of the bone defect in the patients immediately after surgery varied. The bone defect volume immediately after surgery and the reduction rate of the bone defect after 3 months were compared in each group. In the control group, patient with the smallest bone defect volume was 30.72 mm^3 in volume. And the reduction rate of the bone defect was 49.38%, which showed the largest decrease in the area of bone defect (Table 1). In the PRF group, patient with the largest bone defect volume was 504.84 mm^3 in volume. And the reduction rate of the bone defect was 28.35%, which showed the largest decrease in the area of bone defect (Table 2). In the CGF group, the patient whose bone defect volume decreased the most after 3 months was 199.71 mm^3 in volume, and the rate of reduction in bone defect was 32.12 % (Table 3). Comparing the bone defect volume immediately after surgery to bone regeneration rate after 3 months, there was no direct proportion or correlation, which meant the smallest bone defect was not related to the faster bone regeneration rate.

After 3 and 6 months, the average reduction rate of bone defects in each group was compared (Table 4). After 3 months, the average reduction rate of bone defects was 55.74 ± 2.38 % in the control group, 41.19 ± 2.22 % in the PRF group, and 39.79 ± 1.68 % in the CGF group compared to immediately after surgery. After 6 months, the average reduction rate of bone defects was 26.85 ± 3.86 % in the control group, 21.76 ± 1.82 % in the PRF group, and 16.52 ± 1.52 % in the CGF group compared to immediately after surgery. Bone regeneration rate was the highest in the CGF group, followed by the PRF and

control groups.

For comparison among the three groups, the Kruskal–Wallis test were performed (Table 4). However, this was statistically significant only 3 months after endodontic microsurgery ($p < 0.05$) (Fig. 5). After 6 months, there were no statistically significant differences among the three groups ($p > 0.05$) (Fig. 6). The post-hoc Mann-Whitney U test with Bonferroni correction was performed for multiple comparisons of the reduction rate of bone defects 3 months after endodontic microsurgery (Table 4). Bone regeneration was higher in the PRF and CGF group than in the control group, which has statistical significance ($p < 0.05$). However, there was no significant difference between the PRF group and CGF group ($p > 0.05$). These results showed that platelet-derived materials (PRF, CGF) accelerate the early regeneration of new bone at the bone defect site.

Table 1. Measurement of the volume of bone defects in control group

patient	volume of the bone defect (mm^3)			reduction rate of the bone defect (%)	
	V_0	V_{3m}	V_{6m}	V_{3m} / V_0	V_{6m} / V_0
1	77.14	45.79	8.02	56.52	10.03
2	211.04	134.59	57.32	69.39	22.93
3	158.03	91.45	67.22	56.66	42.04
4	169.50	101.59	89.21	60.68	52.10
5	69.30	40.58	11.42	63.92	26.85
6	30.72	10.65	3.63	49.38	14.17

V_0: volume of immediately after surgery, V_{3m}: volume of 3 months after surgery, V_{6m}: volume of 6 months after surgery

Table 2. Measurement of the volume of bone defects in platelet-rich fibrin (PRF) group

patient	volume of the bone defect (mm^3)			reduction rate of the bone defect (%)	
	V_0	V_{3m}	V_{6m}	V_{3m} / V_0	V_{6m} / V_0
1	497.48	239.70	126.16	47.77	24.92
2	103.13	47.63	31.41	45.19	26.85
3	58.69	30.48	17.08	57.23	33.24
4	45.69	15.65	7.16	33.06	13.39
5	504.84	131.70	63.27	28.35	12.09
6	270.24	108.72	47.21	36.20	16.95

V_0: volume of immediately after surgery, V_{3m}: volume of 3 months after surgery, V_{6m}: volume of 6 months after surgery

Table 3. Measurement of the volume of bone defects in concentrated growth factor (CGF) group

patient	volume of the bone defect (mm^3)			reduction rate of the bone defect (%)	
	V_0	V_{3m}	V_{6m}	V_{3m} / V_0	V_{6m} / V_0
1	321.44	159.65	87.37	50.57	28.67
2	86.58	39.53	14.26	46.21	16.11
3	524.53	170.48	36.14	33.04	6.40
4	253.13	108.45	45.10	41.91	18.81
5	86.20	31.50	13.68	35.05	14.70
6	199.71	62.92	29.72	32.12	12.78

V_0: volume of immediately after surgery, V_{3m}: volume of 3 months after surgery, V_{6m}: volume of 6 months after surgery

Table 4. Comparison among three groups on the reduction rate of bone defects

	Mean ± SD (%)			*p-value	†Post-hoc		
	control	PRF	CGF		control-PRF	control-CGF	PRF-CGF
3 months	55.74 ± 2.38	41.19 ± 2.22	39.79 ± 1.68	0.000**	0.000**	0.000**	0.548
6 months	26.85 ± 3.86	21.76 ± 1.82	16.52 ± 1.52	0.173			

SD: standard deviation, PRF: platelet-rich fibrin, CGF: concentrated growth factor

*p-value was calculated by Kruskal-Wallis test

†Post- hoc p-values were calculated by Mann-Whitney U test using Bonferroni correction

**Statistical significance, $p < 0.05$

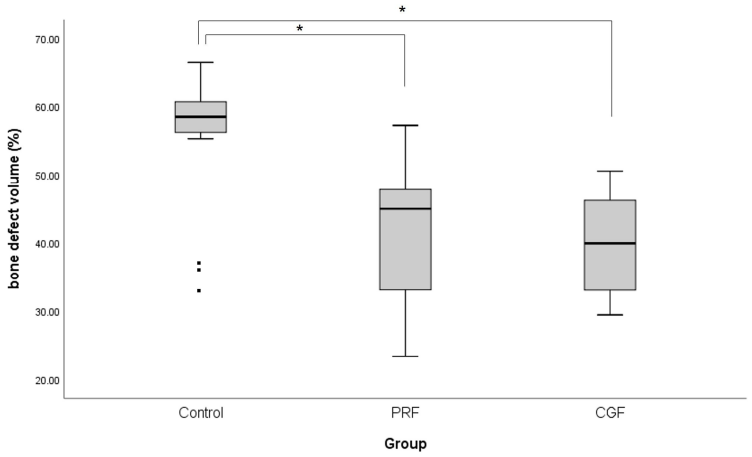

Fig. 5. Comparison among three groups after 3 months. Bone regeneration was higher in the PRF group and CGF group than in the control group. This has statistical significance (p = 0.000). But, there was no significant difference between PRF group and CGF group (p = 0.548). PRF: platelet-rich fibrin, CGF: concentrated growth factor, *p < 0.05

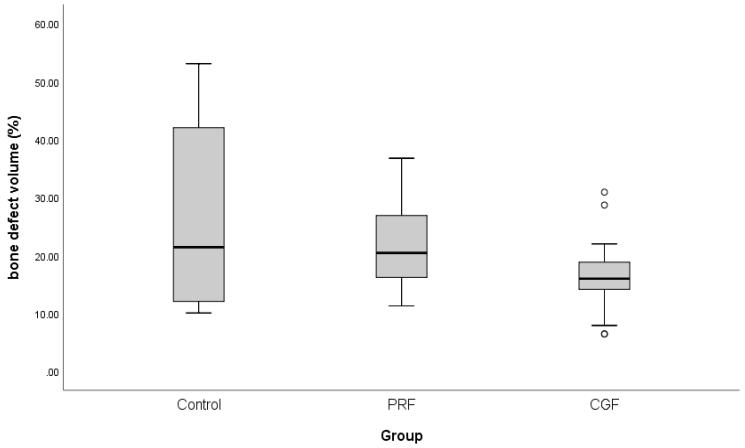

Fig. 6. Comparison among three groups after 6 months. After 6 months, bone generation was similar among the three groups, and there was no statistically significant difference (p = 0.173). PRF: platelet-rich fibrin, CGF: concentrated growth factor

Ⅳ. Discussion

Growth factors, including IGF, PDGF, TGF, VEGF, and TGF-β, are associated with cell migration, proliferation, differentiation, and angiogenesis, and promote tissue regeneration process[11]. IGF promotes new bone formation and regenerates damaged cells by acting on osteoblasts in the endosteum[12]. PDGF exists in α granules of platelets or giant cells. These growth factors promote angiogenesis and osteoblast proliferation. VEGF plays an important role in increasing plasma protein penetration in capillaries, maintaining the survival of new blood vessels, and inducing cell proliferation and differentiation[13]. TGF-β affects osteoblasts at an early stage of development and fibroblast to stimulate collagen synthesis, thus promoting bone and cartilage regeneration[14].

Blood consists of a liquid component, plasma, and cellular components, including white blood cells, red blood cells, and platelets. Platelets play an important role in hemostasis and contain growth factors that are involved in angiogenesis and tissue healing. Therefore, blood components are used to promote healing in dentistry. With the advanced techniques of procuring blood concentrates, substances including platelets, growth factors, and complex fibrin matrices of various concentrations have been developed[15].

Platelet-rich plasma (PRP) is a first-generation platelet-derived material with wound healing effects[16]. However, a double centrifugation process is required for PRPs and the addition of heterothrombin and anticoagulants may cause immune and infectious reactions[17]. Therefore, PRP is rarely used at present. In this study, other platelet-derived materials, such as PRF and CGF, were used to confirm the effects on bone regeneration.

The PRF reported by Choukroun et al.[18] is a second-generation platelet-derived material that contains more growth factors than PRP. Unlike PRP, PRF is produced only by a single centrifugation of autologous blood without the addition of anticoagulants or thrombin[18]. PRF obtained through the gradual coagulation

process has a three-dimensional structure with higher strength than natural fibrin coagulation. Its organization forms a highly elastic matrix structure that allows continuous release of various growth factors for a longer time[19]. Fibrin acts as a biological adhesive that allows the stabilization of the initial platelet cluster during coagulation[2].

CGF was first discovered by Sacco in 2006[20]. Similar to PRF, CGF can be obtained without the addition of biochemical agents, thereby preventing immune response, toxicity, and cross-contamination. A centrifuge (Medifuge, Silfradent Srl, Italy) to obtain CGF is specially designed such that the centrifugation rate varies with time[20]. This centrifugation process produces a dense fibrin matrix with abundant growth factors[8,21]. The increased cohesion by fibrinogen, factor XIII, and thrombin protects fibrin against plasmin degradation, which increases the tensile strength and stability of fibrin[9]. CGF has similar structural properties to PRF, but has a more complex three-dimensional fiber structure and contains more number of growth factors[8,22].

Many studies have reported the effects of PRF and CGF on bone regeneration. Kim et al.[9] showed that bone mineral density and bone volume were high when PRP, PRF, and CGF were applied to the cranial bone defects rabbits. However, there were no significant differences between the experimental groups. Rochira et al.[23] demonstrated that CGF has a direct effect on the osteogenic differentiation of human bone marrow stem cells. In addition, CGF induces the differentiation of periodontal ligament stem cells into osteoblasts *in vitro* and promotes bone formation during differentiation[10]. PRF and CGF promote osteogenic differentiation and proliferation of gingival-derived mesenchymal stem cells by regulating the expression of BMP 2.

However, PRF and CGF do not always have a positive effect on bone formation. In a study by Knapen et al.[24], PRF did not seem to have any additional effect on the quality, quantity, and kinetics of bone in guided bone regeneration. In addition, Faot et al.[25] reported that L-PRF temporarily affected

the expression of RUNX2 and VEGFA during early bone healing, and that there was no effect of promoting bone regeneration in the bone defect. Many studies on PRF and CGF have been conducted both *in vivo* and *in vitro*, but the effectiveness of PRF and CGF remains controversial.

Studies related to bone regeneration by PRF and CGF have been reported in various clinical studies. PRF and CGF in implant surgery provide stabilization of the bone graft in the defect area and minimize bone loss during healing[26]. other clinical studies have reported the application of PRF and CGF to the extraction socket for the treatment of alveolar osteitis. PRF and CGF have been shown to reduce the healing time of soft tissue, postoperative pain, edema, and trismus after extraction of third molars[27,28]. Platelet-derived materials can be used alone or as adjuvant regenerative materials in the treatment of periodontal intrabony defects. PRF significantly reduced pocket depth and improved clinical attachment level[29]. The use of PRF promoted hard and soft tissue healing and alleviated postoperative discomfort. The study by Lei et al.[30] showed that A-PRF and CGF have the advantage of improving the outcome of guided tissue regeneration by stimulating the steady release of growth factors.

Several studies have clinically evaluated the effect of PRF on bone regeneration by applying PRF alone after periapical cyst enucleation[6,31]. It was found to promote faster bone regeneration within 3 months after surgery, and complete bone regeneration and bone density were observed at 6 months after surgery. When PRF was used for bone defects in the oral cavity after cyst excision, bone regeneration was promoted because growth factors from the fibrin matrix were gradually released[6]. This was the same as the result that bone regeneration was promoted after 3 months in the group to which PRF was applied in this study. Although the study by Sureshbabu et al.[32] have shown that the use of bone graft material and CGF promotes bone regeneration in periapical cyst, there are few clinical studies that prove the effect of CGF alone after endodontic microsurgery. In this study, it was confirmed that a single application

of CGF promoted bone regeneration.

Studies on the application of PRF and CGF in regenerative endodontic treatment are being conducted. Huang et al.[33] investigated the effect of PRF on pulp cells and reported that PRF increased pulp cell proliferation and promoted osteoprotegerin and alkaline phosphatase activity. Hiremath et al.[34] reported affirmative experimental results from pulpotomy using PRF and concluded that pulpotomy using PRF could be an alternative treatment for root-end filling materials such as MTA. In addition, several studies have suggested that PRF may be an excellent biomaterial for apical sealing and pulp-dentin complex[35,36]. The application of CGF in chronic periapical lesions can accelerate bone regeneration and healing of inflammation, greatly shortening the healing period[3].

In this study, the effectiveness of PRF and CGF after cyst enucleation and endodontic microsurgery were evaluated. In the experimental groups, after 3 months, new bone formation was higher than that in the control group, demonstrating that PRF and CGF promote bone regeneration. However, there was no statistically significant difference between the PRF and CGF groups in this study. This result demonstrates that platelet-derived materials are effective in early new bone formation. In the evaluation of new bone after 6 months, similar values were found in all the three groups. It is thought that there was no significant difference in bone regeneration after 6 months because the size of the lesion was small. In this study, no bone graft material was used to evaluate bone regeneration. This is because the use of a bone graft material becomes an obstacle in measuring the volume of new bone, which has been reported in a study by Clark et al.[37].

As mentioned above, the effects of PRF and CGF applied after endodontic microsurgery were evaluated using the Mimics program, and the results were found to be effective in bone regeneration. However, this study had certain limitations. First, the number of subjects who participated in the experiment was too small. Second, the age or gender of the patients was not taken into

consideration. These factors may affect the action of growth factors or the healing status. Third, the size of periapical cysts varied. And, in the case of small-sized cysts, the bone defect reduction rate in the control group was similar to that in the experimental group. It is thought that the size of cystic lesions did not show any effect on bone regeneration. Thus, additional studies are required in patients with larger and similarly sized lesions.

This study was successfully managed with endodontic treatment followed by surgical intervention. Bone regeneration in the control, PRF, and CGF groups was evaluated through the volume of the bone defect using CBCT images. This study clinically demonstrated that PRF and CGF have an affirmative effect on early new bone formation and is expected to further enhance the therapeutic effectiveness of endodontic microsurgery. Large-scale prospective clinical studies are needed to further evaluate the possible benefits of PRF and CGF in endodontic microsurgery.

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