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The identification of growth factors contained in platelet rich fibrin

조선대학교 대학원

치의학과

이 준 우

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혈소판 풍부 피브린에 함유된 성장인자의 확인

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조 선 대 학 교 대 학 원

치 의 학 과

이 준 우

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지도교수 김 병 옥

이 논문을 치의학 석사학위신청 논문으로 제출함.

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조 선 대 학 교 대 학 원

치 의 학 과

이 준 우

이준우의 석사학위 논문을 인준함

위원장　조선대학교　교 수　유 훈 인

위 원　조선대학교　교 수　정 문 진 인

위 원　조선대학교　교 수　김 병 옥 인

2010 년　11 월　일

조선대학교 대학원

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혈소판 풍부 피브린에 함유된 성장인자의 확인

이준우
지도교수 : 김병옥
조선대학교 대학원 치의학과

치과 수술에서 다양한 생체 재료들이 사용되고 있으며, 이식 생체 재료들은 구강악안면외과 수술, 치주 수술, 임프란트 수술 등 다양한 분야에서 적용되고 있다. 특히 해부학적 한계나 불량한 골양, 골질 때문에 임플란트를 심기 어려웠던 부위도 골이식을 통해 많이 극복되어 왔다. 그 중 합성골 이식에 대한 많은 연구가 행해지고 있는데, 우수한 골전도 효과에도 불구하고 골유도 효과가 없기 때문에 골유도성 물질인 성장 인자, 골형성 단백질 등을 혼합 사용함으로써 골 형성 속도와 형성량을 증가시키고, 손상된 조직의 재생 속도를 가속화 시키고자 하는 노력이 계속 되어왔다. 그 중 대표적인 방법이 자가혈액에서 골유도성 성장인자를 함유한 Platelet Rich Fibrin(PRF)로 발치와, GBR, 상악동 거상술 등 여러 구강외과, 치주과 및 임플란트 수술에 임상적으로 널리 이용되고 있고, 그에 대한 많은 증례가 발표되고 있다. 하지만 국내에서는 이러한 PRF의 성장인자에 대한 연구가 미미한 상태로, 본 연구에서는 PRF에 어떠한 성장인자가 어느정도의 양이 존재하는지에 대해 확인하고자 한다.

전신적으로 건강하며 흡연을 하지 않은 20대의 성인 10명을 대상으로 혈액 20cc를 채취한 후 원심분리를 시행하여 PRF를 얻는다. 이를 bowl에 두고 10분동안 두어 삼출물을 추출한다. 추출해 낸 삼출물을 -70도에 우선 보관한 후, 본 실험에서는 PDGF-BB, IGF-1, VEGF 등 3가지 성장인자의 정량적 평가를 위해 ELISA 분석을 시행하였다. 분석 결과 피실험자마다의 농도의 차이는 있었지만, PDGF-BB, IGF-1,

VEGF 등의 성장인자가 존재함을 관찰할 수 있었다. 이러한 성장인자는 치유반응을 촉진시키며, 보다 빠른 조직 재생의 효과에 기여하는 것으로 밝혀진 바 있다. 본 실험을 통해 PRF의 발치와, GBR, 상악동 거상술 등 여러 구강외과, 치주과 및 임플란트 수술 등의 임상적인 사용에 대한 기본적 근거를 확립할 수 있고, 3개의 성장인자의 역할을 통해 PRF의 역할 또한 확인할 수 있었다. 하지만 이번 실험을 바탕으로 더 많은 성장인자의 존재의 확인과 기본적 근거에 기초한 PRF의 제작, 적용방법 등에 대한 정확한 프로토콜의 확립 및 PRF에 성장인자의 양을 증가시켜 임상 결과를 향상시키는 방법 등을 모색하는 연구가 뒷받침되어야 할 것이다.

Ⅰ. Introduction

In dental surgeries, various bio-materials are being used and graft bio-materials are applied in various fields in dentistry such as oral, maxillofacial, periodontal and implant surgeries. Especially, bone graft enabled implant placement possible even in sites where it was considered to be difficult due to anatomical limits, poor bone quantity or quality.

Among many bone grafts, auto-graft with bone formation, osteo-induction, osteo-conduction and fast healing effects is the most ideal.[1)] Also, it shows no immunologic rejection. However, due to limited amount of harvested bone and disadvantages of causing inevitable resorption and secondary defects, allograft, heterograft and synthetic bones were developed and used in clinics. Especially, many researches about synthetic bone were performed.[2,3)]

Since synthetic bone has no osteo-induction effect despite excellent osteo-conduction effect, bone formation does not occur in synthetic bone. Therefore, efforts to increase bone formation rate and to accelerate regeneration rate of damaged tissues by using mixture of osteo-inductive growth factors, bone formation proteins and synthetic bone were actively made.[4)]

Among them, one of the representative method is using Platelet Rich Plasma (PRP) containing osteo-inductive growth factors extracted from autogenous blood with synthetic bone graft, which showed excellent bone formation effect.[5)] PRP played an important role in healing and regenerating process and showed excellent bone formation effect by containing proteins acting as matrix for movement of highly concentrated growth factors, bone, connective tissues and epithelium.[6-9)] However, according to many researches, bone formation effect of PRP was not excellent and process of production was complex. The procedure was technique sensitive and concerns about mad-cow disease due to the use of bovine thrombin as coagulation initiative were reported.[10.11)]

To produce highly concentrated abundant biologic factors in PRP more stably and simply, Choukroun and Dohan et al suggested Platel Rich Fibrin (PRF) as a new method of extracting growth factors from autogenous blood.[12-16] The production process of PRF was simple compared to that of PRP. After harvesting blood from veins of patients and centrifuging for 10 minutes in 400G, three layers were appeared. Among the three layers, the middle layer was PRF, which is a new generation of concentrated platelets acquired without any biochemical blood treatment. Also, it looked like autologous cicatricial matrix, a fibrin network similar to natural things. In this form, PRF played an important roles as natural guide of angiogenesis, constituents of a natural support to immunity, guides to coverage of injured tissues and traps of circulating stem cells.

The PRF is clinically used in various oral and maxillofacial, periodontal and implant surgeries such as extraction sockets, GBR and maxillary sinus lifting. Many cases about PRF were reported. However, studies about the growth factors in PRF are very little in Korea. In this study, we identified the presence of growth factors in PRF and quantified the amount of each growth factor. Samples were prepared as described in materials and method. This study is to confirm the kinds and amount of growth factors existing in PRF.

Ⅱ. Materials and method

1. Subjects

10 healthy adults in their 20s who do not smoke were selected as subjects.

2. Experimental design

Blood sampling of 20cc from the subjects were performed 2 hours after meals and two subjects a day. The blood samples were centrifuged with 400G for 10 minutes. Only the PRF layer which does not contain red blood cells in the lower layer of the centrifuged blood was seperated. After collecting nine exudate samples of 100ul per one subject from the seperated PRF which was in the bowl for 10 minutes, they were stored into Eppendorf collection tube. And then, triplication was performed with PDGF-BB (3 samples), IGF-1 (3 samples), VEGF (3 samples). Overall, 90 samples were collected from 10 subjects. The samples were preserved at temperature of -70℃. For quantative analysis of three growth factors, PDGF-BB, IGF-1 and VEGF, ELISA analysis were performed (10 samples) by using ELISA reader (Thermo Electron corporation ELISA reader. America) and Quantikine (R&D Systems, Minneapolis, Minn) kit. The ELISA analysis process was as follows. First, samples were thawed on ice and diluted (30x). After transferring wells to new cases and returning to 4 degree celsius, assay buffer was added to each sample in wells and well trips were sealed and kept for 2 hours at RT. Conjugate solution was added and samples were washed 4 times and 400ul of distilled water was used for one well in each wash. After adding substrate solution, samples were kept for 30 minutes at RT. And then reacion was completed. Finally, after adding stop solution, OD was measured.

This study protocol was approved by the Chosun University Dental Hospital Institutional Review Board (CDMDIRB-015-003)

Ⅲ. Results

The results of identified three growth factors, PDGF-BB, IGF-1, and VEGF are as follows.

1) PDGF-BB: As shown in the Table 1, various concentrations of growth factors were confirmed with the range from 303 (pg/ml) to 4377 (pg/ml). Also, it was shown that deviation of concentration values of the subjects was large (Fig. 1). The average concentration of PDGF-BB in 10 subjects was 1241 (pg/ml) and the standard deviation 1269 (pg/ml) which was not significantly different from the average concentration of 1000 (pg/ml) reported by Dohan in 2006 (Fig. 2).
2) IGF-1: IGF-1 values showed large difference in concentrations, depending on subjects, with the range from 3573 (pg/ml) to 700773 (pg/ml) (table 2). Even in IGF-1, concentration deviation was large according to the subjects and it was larger than that of PDGF-BB (Fig. 3). As the average concentration of about 200000 (pg/ml) reported by Dohan in the year of 2006, the average concentration of IGF-1 in this study was 131624 (pg/ml), and the standard deviation 194331 (pg/ml) was not very different (Fig. 4).
3) VEGF: As shown in the table 3, VEGF showed a wide range of concentrations from 21 (pg/ml) to 195 (pg/ml) depending on the subjects. Also, even in VEGF, concentration deviation was large according to the subjects (Fig. 5). The average concentration of VEGF in 10 subjects was about 92 (pg/ml), and the standard deviation 59 (pg/ml) which was not significantly different from the reported value 110 (pg/ml) by Dohan(Fig. 6).

Ⅳ. Discussion

Since growth factors are essential in normal cell cycle, they are important factors in lives of animals. Though they do not always exist in animal bodies, growth factors, which are formed from local sites of a body according to its necessity, have functions of accelerating growth, proliferation, differentiation and movement of cell itself or its adjacent cell. These growth factors contained in PRF accelerate healing effect and show tissue regeneration effect in periodontics, oral and maxillofacial surgeries and implant - related surgeries in dentistry.[17.18)]

Among these growth factors, PDGF-BB, IGF-1, and VEGF play important roles in wound healing and tissue regeneration. PDGF-BB originates from plaetelets, macrophages and monocytes. PDGF released from platelets enables migration of leukocytes and fibroblasts. PDGF originated from macrophages contributes to fibrosis process. PDGF originated from monocytes induces migration and proliferation of fibroblasts and the formation of new connective tissues.[19)] Also, PRF participates in wound healing process by inducing angiogenesis and collagen synthesis and bone regeneration.[20.21)] IGF, existing in platelets, has functions as precursor of osteoblasts and these actions enable formation of bone, muscles, nerves and blood vessel tissues and regeneration of damaged cells.[22)] Finally, VEGF, a cytokine which increases the penetration of plasma proteins in blood capillarym, accelerates differentiation and movement of cells. VEGF also has a function of inducing protease which causes the reconstruction of cells, maintains the survival of newly formed blood vessels through inhibition of apoptosis and induces growth and differentiation of cells. Accelerating migration of blood vessel cells, which sequently accelerates generation and differentiation of newly born cells, increases skin composition.[23.24)]

PRF acts as storage for these growth factors and helps fast healing and fast tissue and bone regeneration.[15] Clinically, grafting mixture of synthetic bone and PRF, which is most frequently used grafting method in case of bone defects, showed excellent bone formation in radiographic and histologic measurement.[25]

In this study, the existence of three growth factors, PDGF-BB, IGF-1, and VEGF was confirmed. There were some difference in concentrations of PRF depending on the subjects. Comparing with the results in other international papers, the average concentrations of growth factors were similar. Confirmation of the existence of growth factors in PRF established the basic evidence about the clinical use of PRF in various oral and maxillofacial, periodontal and implant surgeries such as extraction sockets, GBR, and maxillary sinus lifting. But confirming only three growth factors is insufficient. Identification of more growth factors in PRF, establishment of exact protocols about the PRF production and application method based on basic evidence like confirmation of existence of these growth factors will be necessary in future studies. Further research about methods of increasing the growth factors in PRF will be necessary.

Ⅴ. Conclusions

In this study, we confirmed the presence of growth factors PDGF-BB, IGF-1, and VEGF in PRF obtained from human blood sample. With it, basic evidence about the presence of growth factors in PRF obtained from human sample was established.

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ABSTRACT

The confirmation of growth factors contained in platelet rich fibrin

Jun-Woo Lee
Advisor: Byung-ock kim, D.D.S., M.S.D., Ph.D.
Department of Dentistry,
Graduate School of Chosun University

Various bio-materials are used in dental surgeries and graft biomaterials are applied in various fields such as oral maxillofacial, periodontal and implant surgeries. Especially, bone grafts made implant placement possible even in the sites where it was difficult due to anatomical limitation or poor bone quantity and quality. Many researches about the grafts of synthetic bone are going on. Since the synthetic bone does not have the effect of osteo-induction despite the great effect of osteo-conduction, efforts were made to increase the rate and quantity of bone formation and accelerate the regeneration rate of damaged tissues by using the mixture of synthetic bone and materials having bone inducing effect such as growth factors and bone formation proteins. The representative method among them is Platelet Rich Fibrin (PRF) which contains osteo-inducing growth factors from autogenous blood. This is used clinically in various oral maxillofacial, periodontal and implant surgeries such as extraction sockets, GBR, maxillary sinus lifting. However, in Korea, studies about the growth factors of PRF were rare. Therefore, in this study, the presence and the amounts of growth factors in PRF were investigated.

Ten adults in the 20s who do not smoke and are healthy without any systemic diseases were selected for the extraction of 20cc of blood. After centrifuging the blood, PRF was derived from it. Exudates were extracted by putting the blood in the bowl for 10 minutes. After preserving the extracted exudates at -70℃, ELISA analysis was performed for quantitative evaluation of three growth factors PDGF-BB, IGF-1, and VEGF.

In the result of analysis, though there was some difference in the concentrations depending on the subjects, presence of growth factors such as PDGF-BB, IGF-1, and VEGF was confirmed. These growth factors are known to accelerate healing process and contribute to fast tissue regeneration. By this study, basic evidence about the clinical use of PRF in various oral maxillofacial, periodontal and implant surgeries such as extraction sockets, GBR, and maxillary sinus lifting were established and the role of PRF was also found by looking into the roles of three growth factors. However, further research about the clinical efficacy of PRF is necessary to confirm the presence of other growth factors and establish exact protocols about the production and application method of PRF.

Figures

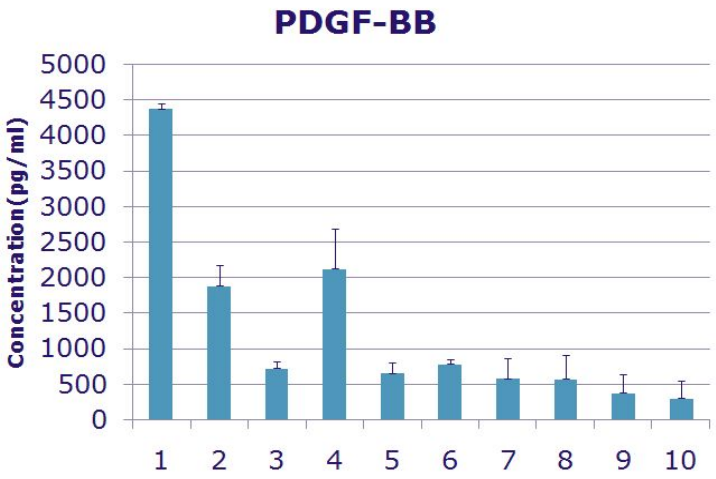

Fig. 1. The concentration of PDGF-BB in each 10 subjects.

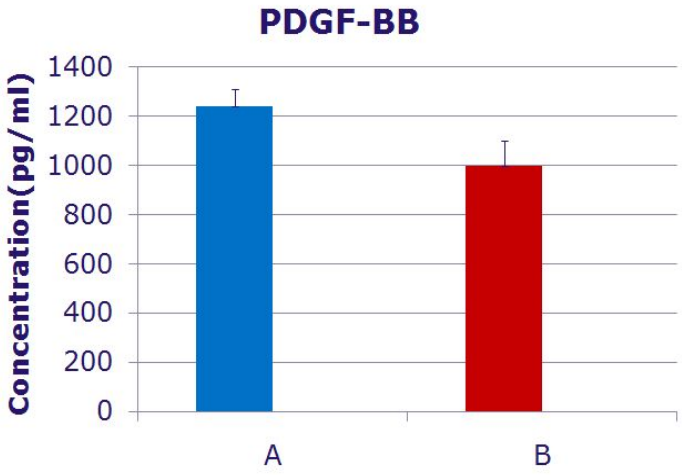

Fig. 2. Comparison of PDGF-BB concentrations of this study (A) with the one of Dohan et al (B).

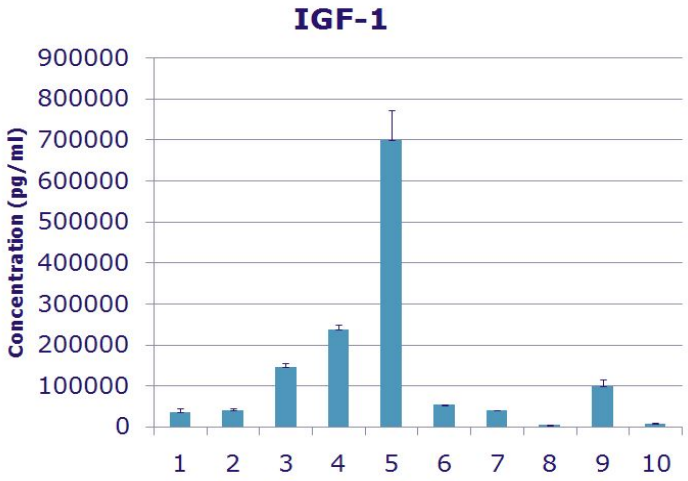

Fig. 3. The concentration of IGF-1 in each 10 subjects.

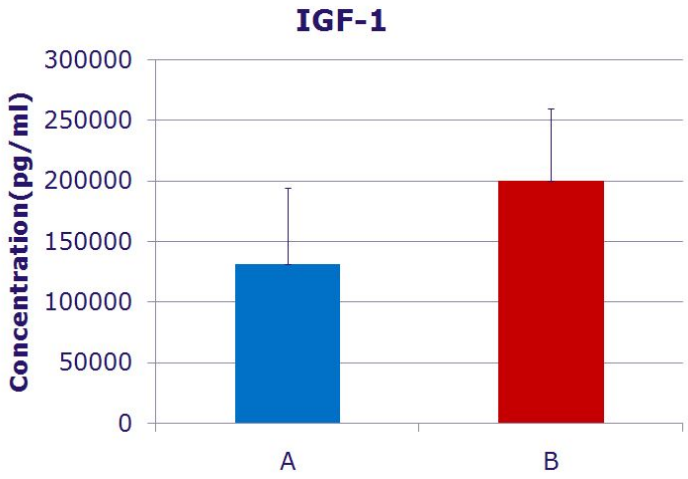

Fig. 4. Comparison of IGF-1 concentrations of this study (A) with the one of Dohan et al (B).

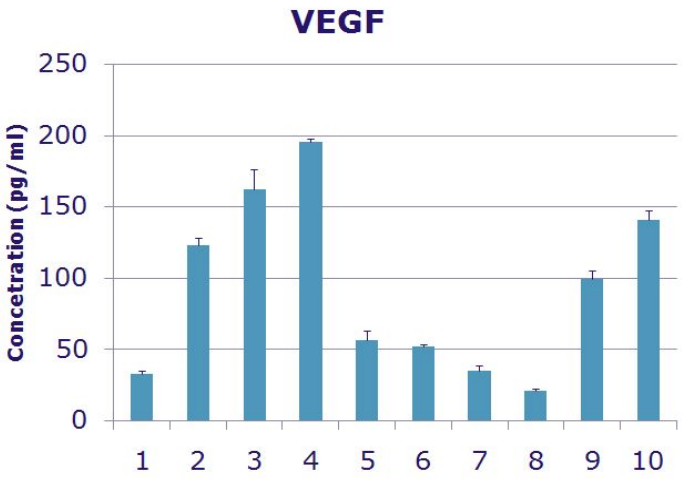

Fig. 5. The concentration of VEGF in each 10 subjects.

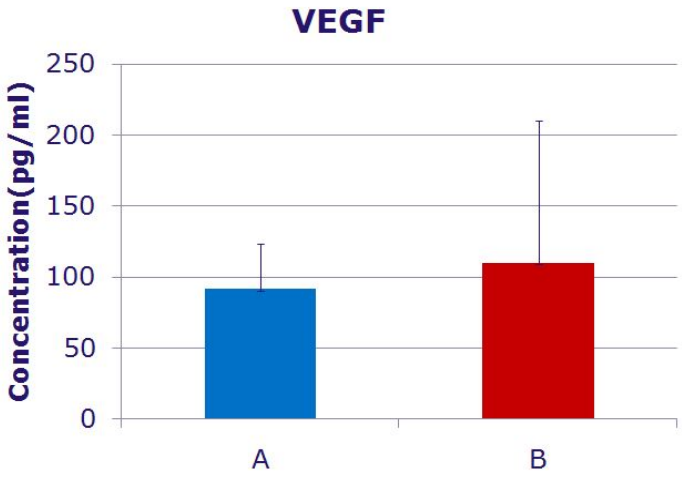

Fig. 6. Comparison of VEGF concentrations of this study (A) with the one of Dohan et al (B).

Table

Table1. PDGF-BB concentrations

Patient	Mean(pg/ml)	SD
1	4377.8	7.01
2	1887.8	29.34
3	727.8	9.14
4	2127.8	56.08
5	662.8	13.88
6	782.8	6.80
7	580.9	28.2
8	576.2	33.4
9	383.2	25.5
10	303.2	24.8
Average	1241.04	1269.86

Table 2. IGF-1 concentrations

Patient	Mean(pg/ml)	SD
1	35783	10134
2	39773	6022
3	144973	11085
4	238273	10806
5	700773	72310
6	52873	1905
7	39473	1587
8	3573	917
9	98673	17104
10	6973	2406
Average	131624.83	194331

Table 3. VEGF concentrations

Patient	Mean(pg/ml)	SD
1	32.4	2.4
2	122.8	5.0
3	162.2	13.6
4	195.1	2.4
5	56.5	6.2
6	51.7	1.7
7	34.7	3.8
8	20.5	1.6
9	99.1	5.7
10	140.5	7.1
Average	91.54	59.28

저작물 이용 허락서

학 과	치의학과	학 번	20097361	과 정	석사
성 명	한글 이준우	한문 李 浚 玗		영문 Lee Jun Woo	
주 소	500-103 광주 북구 두암 3동 868-14번지				
연 락 처	E-mail : aime-82@hanmail.net				
논문제목	한글 혈소판 풍부 피브린에 함유된 성장인자의 확인				
	영문 The identification of growth factors contained in Platelet Rich Fibrin				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건 아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함.
2. 위의 목적을 위하여 필요한 범위 내에서의 편집과 형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포·전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5년으로 하고, 기간종료 3개월 이내에 별도의 의사 표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물 이용의 허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음.
7. 소속 대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송·출력을 허락함.

동의여부 : 동의(○) 반대( )

2010년 10 월

저작자: 이 준 우 (인)

조선대학교 총장 귀하