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Effects of low intensity pulsed ultrasound and light emitting diode on the release of growth factors from platelet-rich fibrin in vitro

조선대학교 대학원

치의학과

석 상 동

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저강도파동형의 초음파와 발광다이오드가
혈소판풍부섬유소내 성장인자들의 유리에 끼치는 영향

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Contents

List of Figures ····· ii

List of Tables ····· iii

Abstract in Korean ····· iv

I. Introduction ····· 1

Ⅱ. Materials and methods ····· 3

Ⅲ. Results ····· 5

Ⅳ. Discussion ····· 9

Ⅴ. Conclusion ····· 12

References ····· 13

List of Figures

Figure 1. Schematic representation of the 3 centrifugation strata after PRF processing ····· 4

Figure 2. PRF exudate taken ····· 4

Figure 3. LIPUS used in this study ····· 4

Figure 4. LED used in this study ····· 4

List of Tables

Table 1. Values of PDGF-BB ····· 5

Table 2. Values of IGF-1 ····· 6

Table 3. Values of VEGF ····· 6

Table 4. Statistic values of PDGF-BB ····· 7

Table 5. Statistic values of IGF-1 ····· 7

Table 6. Statistic values of VEGF ····· 8

국문초록

저강도파동형의 초음파와 발광다이오드가 혈소판풍부섬유소내 성장인자들의 유리에 끼치는 영향

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골내임플란트를 임상적으로 사용했을 때 그 장기간의 성공률이 높다고 보고되어 상실된 치아를 대체할 수 있는 처치법으로 각광을 받고 있으나, 임플란트와 골융합 기간을 단축시키기 위해서 그리고 골질이 좋지 않은 부위나 골량이 부족한 부위에서 골재생을 향상시키기 위하여 임플란트 디자인과 표면의 형태수정, 골이식재, 성장인자, 골형성단백질, 혈소판풍부피부린 (platelet rich fibrin, PRF) 그리고 생체물리적인 자극(biophysical stimulation)요법이 연구되고 있다.

이 연구는 골재생술의 효능을 향상시키기 위하여 임상에서 사용되고 있는 PRF 젤에 생체물리적인 자극요법인 저강도파동형의 초음파 (low intensity pulsed ultrasound, LIPUS), 발광다이오드 (light emitting diode, LED)를 조사하여 gel내에 존재하는 platelet derived growth factor-BB (PDGF-BB), insulin-like growth facotr-1 (IGF-1), vascular endothelial growth factor (VEGF)의 농도 변화를 평가하고자 시행되었다.

이 연구를 위하여 전신적으로 건강하며 비흡연자인 20대 성인 10명을 대상으로, 오전식후 2시간 후에 상완정맥에서 혈액 20 ㏄를 채취한 후 원심분리(400g. 10분)를 시행하여 PRF 젤을 채득하였다. 채득된 젤을 상온에서 10분 동안 방치시켜 삼출물을 추출하였다. 실험은 3군 (대조군, LIPUS 조사군, 그리고 LED 조사군)으로 구분하여 시행되었다. PDGF-BB를 분석하기 위해서 2 ㎖ 에펜도르프 튜브에 100 ㎕, IGF-1과 VEGF를 분석하기 위하여 각각 400 ㎕씩 넣어서, 총 270개의 표본을 준비하여 수집용 박스에 넣은 후 -70℃에 보관하였다. 각각의 실험은 3번씩 시행되었다. 여기에서 사용된 LIPUS (Br-sonic® DENTOVE, Japan)는 제조회사의 지시대로 240 ㎽에서 15분 동안, 그리고 LED (Osseopulse®, BIOLUX, Canada)는 고정된 주파수에서 20분

동안 조사되었다.

조사된 샘플내에 존재하는 성장인자의 정량적 평가를 위하여 ELISA (Enzyme-linked immunosorbent assay) 분석을 시행하였다. 3가지 성장인자는 Quantikine (R&D Systems, Minneapolis, Minn, USA) kit를 이용하여 ELISA reader (Multiskan EX, Thermo Electron Co. USA)로 분석하였다.

분석결과, LIPUS와 LED를 조사한 군에서 PDGF-BB와 IGF-1은 대조군에 비해 유의성 있는 증가를 보인 반면, VEGF는 감소되는 경향을 나타냈다.

이 제한된 연구 결과를 살펴보면, LIPUS나 LED는 PRF 젤내에 있는 성장인자의 농도를 증가시킬 수 있음을 시사하고 있다. 따라서, 이 방법을 임상에서 적용한다면 환자의 불편감을 감소시킬 수 있으며 골재생을 향상시킬 수 있을 것이라고 생각된다.

I. Introduction

Long-term successful osseointegration of endosseous dental implants in regenerated edentulous defects and extraction sockets requires adequate wound healing and sufficient regeneration of bone in quality and quantity at their sites.

Several methods have been performed to enhance bone regeneration at defected areas: mechanical stimulation, bioactive materials, biological growth factors, biophysical stimulation such as pulsed electromagnetic fields and low intensity pulsed ultrasound (LIPUS), platelet concentrate technology and phototherapy using lighting emitting diode (LED).

PRF was developed in France by Choukroun et al in 2001. This technique required only centrifuged blood without additives. It offers simplified and optimized protocols for a new kind of fibrin adhesive, concentrated platelet-rich plasma, and looks like an autologous cicatricial matrix.[1)] Dohan et al.[2)] quantified PDGF-BB, TGF-β1, and IGF-1 with platelet poor plasma supernatant and PRF exudate serum. They reported that PRF would be able to progressively release cytokines during fibrin matrix remodeling, and might reduce the healing time. Aroca et al.[3)] concluded that the addition of a PRF membrane positioned under the modified coronally advanced flap provided an additional gain in gingival/mucosal thickness at 6 months compared to conventional therapy.

PRF acts as storage for these growth factors and helps fast healing and fast regeneration of tissue and bone.[4)] 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.[5)]

LIPUS is a form of mechanical energy that is transmitted through and into living tissue as acoustic pressure waves and its stimulatory effects are often

observed during the soft callus formation phase and not during the remodeling phase.[6] It is widely being used clinically to treat the fracture healing[7], to modify the mandible growth[8], and to improve distraction osteogenesis and osseointegration of dental implants.[9]

Phototherapy using LED become feasible due to the availability of high-efficiency monochromatic diodes at biologically active wavelengths. Some of the disadvantages of the low level laser therapy ares small optical footrprint, excessive hardware and bulk for treating large areas and intense energy density. Brawn and Kwong-Hing[10] reported that bone healing in the phototherapy treated HA socket graft might be accelerated.

Human and animal experimental research has verified that biophysics can accelerate implant stability and enhance bone formation. However, the combined effect of LIPUS or LED irradiation on the PRF clot exudate serum has not been previously evaluated. In this study, platelet-derived growth factors-BB (PDGF-BB), vascular endothelial growth factor (VEGF), and insulin-like growth factor-1 (IGF-1) were quantified in the PRF clot exudate serum irradiated with LIPUS or LED.

Ⅱ. Materials and methods

A. Blood collections and PRF preparation

Blood collection was carried out on 20 healthy volunteers. Non-smoker males from 22 to 27 years of age. The volunteers were given information beforehand on the nature and the objectives of our study.

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

Blood was collected 20 ㎖ from each volunteer. Blood samples were taken without anticoagulant in 10-㎖ glass-coated plastic tubes that were immediately centrifuged at 3,000 rpm (approximately 400g) for 10 minutes[2)](Fig. 1 and 2). All experiments were triplicated. The experimental results were tested by using paired t-test, with a 5% significance threshold.

B. Irradiation of LIPUS and LED

The experiments were subdivided into 3 groups; group 1: only PRF clot exudate serum as a control, group 2: PRF irradiated with LIPUS (PRF-LIPUS), and group 3: PRF irradiated with LED (PRF-LED).

After obtaining the PRF clot exudate serum, LIPUS (Br-sonic® DENTOVE, Japan)(Fig. 3) for 15 minutes, at 240 mW or LED (Osseopulse®. BIOLUX, Canada)(Fig. 4), was irradiated as instructed by a manufacturer for 20 minutes.

C. Enzyme-linked immunosorbent assay (ELISA) analysis

The concentration of PDGF-BB, IGF-1, and VEGF was quantified in these samples stimulated with LIPUS or LED by ELISA (Quantikine; R&D Systems, Minneapolis, Minn, USA).

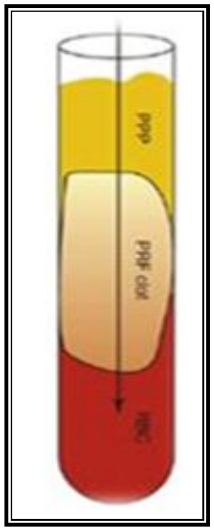


Fig. 1. Schematic representation of the 3 centrifugation strata after PRF processing.

Fig. 2. PRF exudate taken.

Fig. 3. LIPUS used in this study.

Fig. 4. LED used in this study.

Ⅲ. Results

In this study, 3 growth factors were quantified in the PRF clot exudate serum irradiated with LIPUS or LED. Statistical analyses of the results obtained were as follows.

The values of 3 growth factors in the PRF gel showed extremely individual variation (Table 1, 2, and 3). So, author analysed statistical difference of the each average (Table 4, 5, and 6).

1) Values of 3 growth factors irradiated with biophysics in each individual

Table 1 Values of PDGF-BB irradiated with biophysics in each individual (unit: pg/ml)

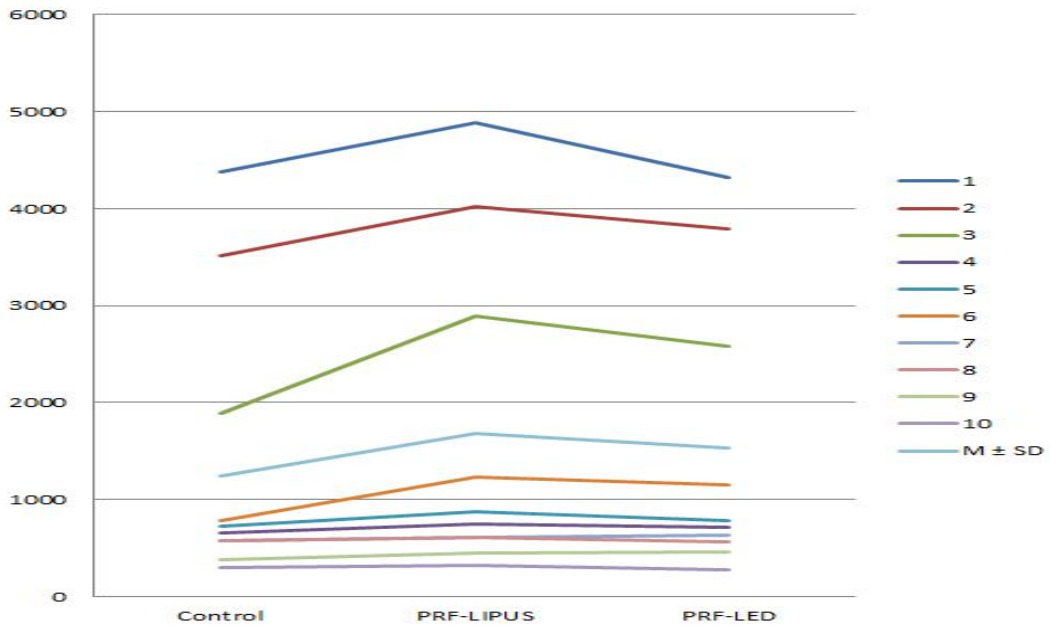


Table 2 Values of IGF-1 irradiated with biophysics in each individual (unit: pg/ml)

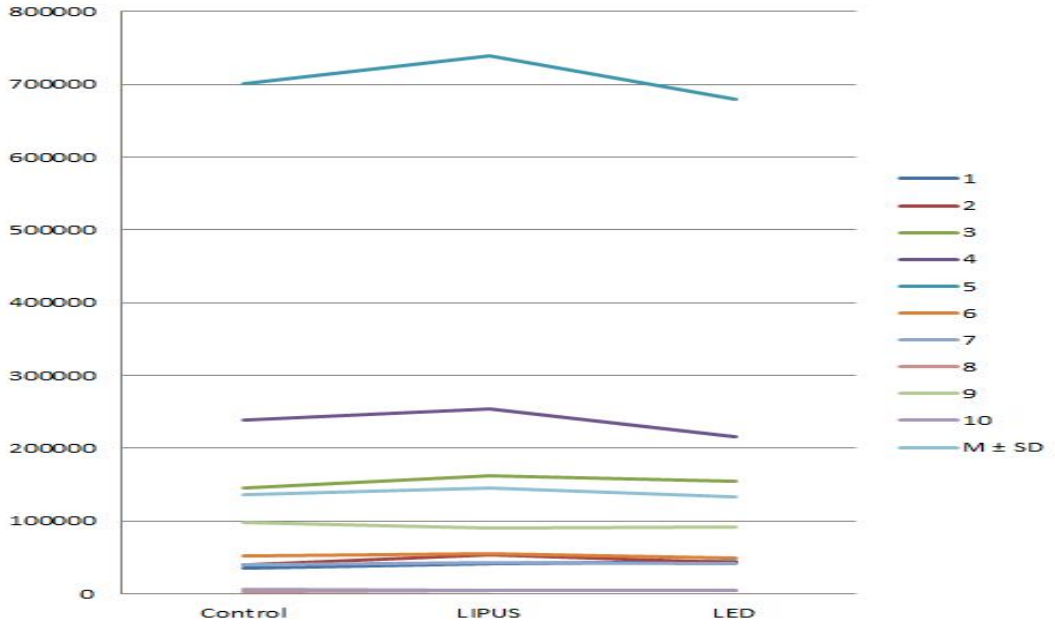
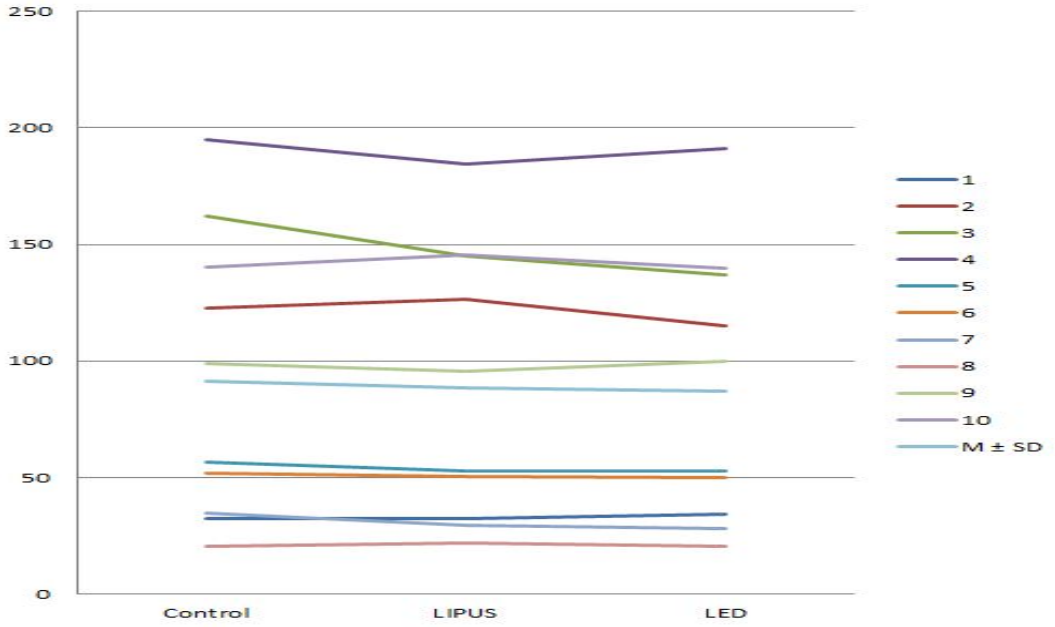


Table 3 Values of VEGF irradiated with biophysics in each individual (unit: pg/ml)



2) Values of 3 growth factors analysed statistically

(1) Values of PDGF-BB

There are significant differences between control group and PRF-LIPUS, and control and PRF-LED *(P < 0.05)*(Table 4).

Table 4 Statistic values of PDGF-BB

group	N	mean	S.D.	P value
C - B	30	-426.76000	664.12423	.001+
C - L	30	-286.67667	625.38322	.018+
B - L	30	140.08333	202.82243	.001+

S.D.: standard deviation, C; control, B: PRF-LIPUS group, L; PRF-LED group. N: number
+ : Significantly different between two groups (P<0.05)

(2) Values of IGF-1

There are significant differences between control group and PRF-LIPUS, and control and PRF-LED*(P < 0.05)* (Table 5).

Table 5 Statistic values of IGF-1

	N	mean	S.D.	P value
C - B	30	-13178.16667	31394.81508	.029+
C - L	30	4639.73333	19430.23063	.201
B - L	30	8538.43333	21545.91168	.038+

S.D.: standard deviation, C; control, B: PRF-LIPUS group, L; PRF-LED group . N: number
+ : Significantly different between two groups (P<0.05)

(3) Values of VEGF

There are no significant differences between each group*(P > 0.05)* (Table 6).

Table 6 Statistic values of VEGF

	N	mean	S.D.	P value
C - B	30	3.10567	8.92882	.067
C - L	30	-.08200	6.61623	.946
B - L	30	-3.02367	9.17641	.082

S.D.: standard deviation, C; control, B: PRF-LIPUS group, L; PRF-LED group. N: number

+ : Significantly different between two groups (P<0.05)

Ⅳ. Discussion

PRF membrane is a complex biomaterial with a specific biology, and Dohan et al.[11] showed that it releases high quantities of three growth factors (transforming growth factor-β1, PDGF-AB, VEGF) and a glycoprotein (thrombospondin-1) during 7 days.

Su et al.[12] proposed that the PRF membrane should be used immediately after formation to maximize release of growth factors to the surgical site, and the remaining fluid can be recovered as an additional source of growth factors for grafting. So author stimulated LIPUS or LED in the PRF close by chair-side.

In this study, author selected 3 growth factors, PDGF-BB, IGF-1 and VEGF, related to wound healing. PDGFs act as a chemoattractant and recruit mesenchymal cells into the wound[13], and have been shown to activate collagenase within the latter stages of woun healing.[14] PDGFs in bone remodeling and modeling are to increase the number of cells necessary for bone formation at the repair site, trigger capillary formation through its potent mitogenic activity, enhance site debridement, and provide a continued source of growth factors for bone repairs.[15]

IGF-1 has been shown to be chemotatic for cells derived from the periodontal ligament, and has strong effects on periodontal ligament fibroblast mitogenesis and protein synthesis in vitro.[16]

Some studies focused on a combination of PDGF and IGF-1. They found that this combination promoted new bone, cementum, and periodontal ligament in vivo[17,18] and promoted bone formation around press-fit and immediate extraction socket implant.[19]

Vascular endothelial growth factor (VEGF) is an important regulator that induces the microvascular permeability and angiogenesis during the stage of proliferation.[20,21] Johnson et al.[22] found that VEGF was significantly lower

within normal than within diseased gingiva, and may be a factor in initiation and progression of gingivitis to periodontitis. Shweiki et al.[23] suggested that expression of VEGF is hormonally regulated and excreted during every wound healing by keratinocytes.

Energy of ultrasound used in this study is absorbed at a rate proportional to the density of the tissues in which it passes through.[6]

Wijdicks et al.[24] domonstrated LIPUS enhances bone formation induced by rhBMP-2. Especially, Sena et al.[25] have demonstrated that LIPUS stimulates a transient increase in the expression of the early response genes (c-jun, c-myc, COX-2, Egr-1, and TSC-22), as well as the bone differentiation marker genes (ON and OPN), in bone marrow derived osteoblastic cells.

There are some theories about the mechanisms of LIPUS on bone healing, such as (1) mechanical strain and micromotion in the healing callus by the differential absorption of LIPUS[26,27] (2) increase in micromechanical blood pressure by way of the change in membrane permeability by LIPUS[28], and (3) temperature increase associated with energy absorption.[29,30] Even though the pathway of signals remains to be thoroughly understood, the current studies indicate that LIPUS has a favorable effect in PRF gel, especially PDGF-BB and IGF-1.

The LED array used in this study operates in the visible red spectrum at a continuous wavelength of 605-631 nm. Recently, in the effect of LED on implant stability, Uysal et al.[31] showed that significant increase was found in implant stability quotient values of LED photobiomodulation therapy applied titanium orthodontic miniscrews,

Lee[32] showed that mean value of each PDGF-BB, IGF-1, and VEGF was 1,241.0±1269.9, 136,123±204571, and 91.5±59.3, respectively. The values of growth factors in the PRF depended on the subjects. Comparing with the Dohan et al's result, the average values of growth factors were similar. In author's data, mean value of each PDGF-BB, IGF-1, and VEGF was 1677.8±1596.1, 1448031±215111, and 88.4±56.7, respectively, after PRF-LIPUS

stimulation and 1527.7±1433.0, 133063±196502, and 86.9±56.1, after PRF-LED, respectively.

As shown in the table, the values of 3 growth factors in the PRF gel showed extremely individual variation. So, author analysed statistical difference of the each average. Differences between the control and 2 each experimental group were statistically significant. The highest value in this study analysed were PRF-LIPUS, PRF-LED, and control for PDGF-BB and IGF-1. On the other hand, the value of VEGF showed decreasing trend.

There were significant differences between control group and biophysical stimulation in PDGF-BB and IGF-1. Thus, the therapy using biophysics near by chair can be very convenient for patients and dentists clinically because there might be no use irradiating the biophysics in the wound during the given period. But, we have to keep in mind that there might be a little difference in the effect of PRF because the values of growth factors in the PRF gel showed extremely individual variation.

In this study, PDGF-BB, VEGF, and IGF-1 were quantified in the PRF clot exudate serum irradiated with LIPUS or LED. Within the limited results, biophysics stimulation to improve wound healing by growth factors such as PDGF and IGF-1 may widen the clinical application of the PRF in periodontology and implant dentistry.

V. Conclusion

Within this limited study, the result suggests that LIPUS and LED can increase the concentration of platelet-derived growth factor-BB and insulin like growth factor-1 in the platelet-rich fibrin. But, in the future, it is necessary to make sure these results clinically through larger scale studies.

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저작물 이용 허락서

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논 문 제 목	한글 : 저강도파동형의 초음파와 발광다이오드가 혈소판풍부섬유소내 성장인자들의 유리에 끼치는 영향				
	영문 : Effects of low intensity pulsed ultrasound and light emitting diode on the release of growth factors from platelet-rich fibrin in vitro				

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( )

2011년 2월

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