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Effect of low intensity pulsed ultrasound on the gene expressions of bone differentiation markers in human periodontal ligament fibroblasts

조선대학교 대학원

치의학과

김 성 호

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사람 치주인대섬유모세포에서 파동형의 저출력 초음파가
골분화표지 유전자 발현에 미치는 효과

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치 의 학 과

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지도교수 김 병 옥

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치 의 학 과

김 성 호

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By Kim Sung-Ho

Advisor : Prof. Kim Byung-Ock, D.D.S., M.S.D., Ph.D.

Department of Dentistry
Graduate School of Chosun University

A thesis submitted of the faculty of the Chosun University in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in the Department of Dentistry

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Major Advisor

김성호의 박사학위논문을 인준함

위원장　　원광대학교　교 수　신 형 식　인

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ABSTRACT

Effect of low intensity pulsed ultrasound on the gene expressions of bone differentiation markers in human periodontal ligament fibroblasts

Kim Sung-Ho
Advisor: Kim Byung-Ock Ph.D.
Department of Dentistry
Graduate school of Chosun University

Low intensity pulsed ultrasound (LIPUS) has been demonstrated to accelerate bone growth during fracture healing and distraction osteogenesis. This study was to evaluate the effect of LIPUS on the alteration of the expressions of osteogenesis related genes in human periodontal ligament fibroblasts (hPDLFs) using the polymerase chain reaction (PCR) method.

HPDLFs were pre-cultured for eight days in the presence of differentiation media and then exposed to LIPUS for 7 days each 15-min. 6 passages of hPDLFs were used in this study.

RT-PCR was used to determine the effect of LIPUS on expression of several genes associated with bone differentiation markers in hPDLFs. The expression of the genes was increased compared to controls. Increases in both gene expressions and bone nodule formation were greatest at 7 days after last LIPUS exposure, implying that there may be a time dependence for the stimulus of bone differentiation markers in hPDLFs. LIPUS treatment could accelerate the differentiation of the hPDLFs into osteoblasts or cementoblasts. In the future, attempts to stimulate bone defect healing and periodontal tissue remodelling using LIPUS may focus on the use of other treatment protocols.

I. Introduction

Ultrasound is a propagating pressure wave that transfers mechanical energy into tissues, and low intensity ultrasound in pulse/echo mode is used commonly for imaging[1]. But, high-energy and intensity ultrasound is absorbed by tissues and has been used to heat tissues and kill malignant cells[2].

Ultrasound signal is delivered by a transducer that is coupled to the skin with water-based gel. The ultrasound waves are produced by vibrations in a piezoelectric material, such as lead zironate titante, as a result of a high-frequency electric field set up across the material[3].

Low intensity pulse ultrasound (LIPUS) is a biophysical form of intervention in the fracture-repair, process, which through several mechanical accelerates healing of fresh fractures and enhances callus formation in delayed unions and nonunions[4]. LIPUS is a form of mechanical energy that is transmitted through and into biological tissues as an acoustic pressure wave and has been widely used in medicine as a diagnostic and therapeutic tool[1].

In 1994, LIPUS was approved for the stimulation of fresh bone fracture healing in the United States by the Food and Drug Adminstration and, in 2000, approval was extended to the treatment of established nonunions[5]. But despite of the fact that the effect of LIPUS during fracture healing has a pronounced tendency, the underlying mechanism of action of it still remain unclear.

LIPUS has been demonstrated to accelerate bone growth during mandibular fracture healing[6], and distraction osteogenesis[7], enhance mandibular growth[8], and induce apoptosis[9]. Other cell culture studies using various treatment schedules have shown that LIPUS can stimulate expression of bone morphogenic proteins, bone-related genes, growth factors and its receptors, and bone markers[10,11] and cytokine release[12], transcriptional expression[13], in various cells.

In these days, almost all studies have reported the stimulatory effect of LIPUS on fracture wound healing, while there were a few studies that have not detected any positive effect to LIPUS. Schortinghuis et al.[14,15] reported that LIPUS does not stimulate bone defect healing.

Few studies about gene expression of collagen type-1 (COL-1), alkaline phosphatase (ALP), osteocalcin (OC), and osteopontin (OPN) associated with bone differentiation marker using LIPUS were performed in periodontal ligament fibroblasts (hPDLFs). This study was to evaluate the effect of LIPUS on the alteration of the expressions of these genes in hPDLFs using the polymerase chain reaction (PCR) method.

II. Materials and Methods

1. HPDLFs collection and culture

The hPDLFs were obtained and cultured from a healthy premolar extracted for orthodontic treatment or third molar. Before extraction, to minimize the infection of periodontal ligament plaque and calculus were removed with the use of Cavitron® (Dentsply, USA) and wash of the oral cavity was performed for 3 minutes using POTADINE SOLN® (Povidone-iodine solution, SAMIL PHARM. CO., Korea) and CHLOHEXIN GARGLE® (Chlorhexidine gluconate solution, DAEWOONG PHARM. CO., Korea) The extracted tooth was rinsed with Hanks' Balanced Salt Solution (HBSS, GIBCO BRL, USA) to remove the blood clot and other foreign bodies and the hPDLFs were harvested using a sterilized Gracey curette from the middle portion of root surface.

The hPDLFs were stored in the mixture of Hanks' Balanced Salt Solution (HBSS, GIBCO BRL, USA) and Antibiotic-Antimycotic (AA , GIBCO BRL, USA), washed several times with this solution and cut them 1-2 ㎟ pieces under dissecting microscope.

The incised tissue sections were placed on a slide glass to prevent the movement of tissues, and cultured in the mixture containing 10 % Fetal Bovine Serum (FBS, GIBCO BRL, USA), Dulbeco's Modified Eagles Medium (DMEM, GIBCO BRL, USA) and 1% Antibiotic-Antimycotic (AA, GIBCO BRL , USA) under the condition 37°C, 5% CO_2, and 100% humidity. Culture medium was changed every two days, and proliferating hPDLFs were cultured in series, and the sixth generation cells were used.

2. Ultrasound stimulation and proliferation assay

1) Ultrasound stimulation

LIPUS exposure (exposure time: 15 minute, per day, for 7 days) was applied to culture dishes. LIPUS was applied with the culture dishes in the tissue culture incubator (37℃, 5% CO_2, 95% air). Six groups were studied (Fig. 1).

(1) group 1: frequency: 3 ㎒, intensity: 320 ㎽/㎠
(2) group 2: frequency: 3 ㎒, intensity: 160 ㎽/㎠
(3) group 3: frequency: 5 ㎒, intensity: 320 ㎽/㎠
(4) group 4: frequency: 3 ㎒, intensity: 240 ㎽/㎠
(5) group 5: frequency: 1 ㎒, intensity: 160 ㎽/㎠
(6) control : no exposure

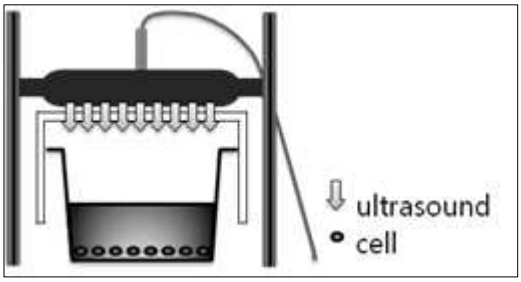

Fig. 1. Illustration of ultrasonic stimulation of cells. Ultrasound was transmitted through the upper of the wells between the ultrasonic transducer and the plate. The diameter of ultrasound transducers was the same as that of the walls (35 mm).

2) Proliferation assay

To observe the proliferation of hPDLFs to LIPUS stimulation, MTT assay was performed. For this study, three of 6 exposure modalities were selected based on the proliferation rate. The experiments were triplicated (Fig. 2).

3. Bone nodule

Alizarin-red staining was performed to observe the bone nodule at immediate after 7-day LIPUS-exposure, 7-day after 7-day LIPUS-exposure, and 14-day after 7-day LIPUS-exposure, respectively (Fig. 2).

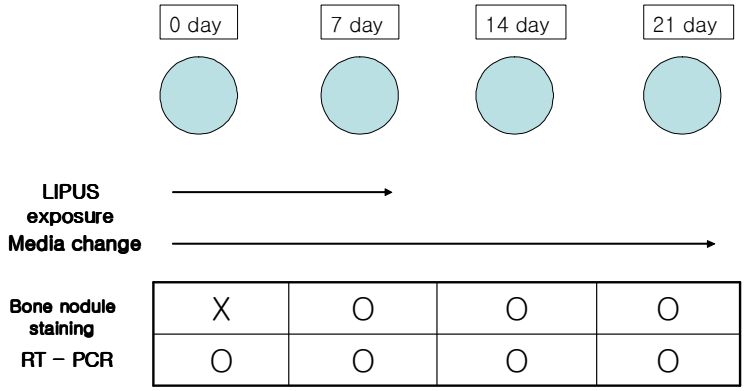

Fig. 2. Schematic diagram for experimental design

4. Isolation of total RNA and reverse transcription polymerase chain reaction (RT-PCR)

HPDLFs were exposed by LIPUS at immediate after LIPUS-exposure, immediate after 7-day LIPUS-exposure, 7-day after 7-day LIPUS-exposure, and 14-day after 7-day LIPUS-exposure, respectively. And then, total RNA was extracted from cells by homogenizing with Trizol Reagent on days 1-, 7-, 14- and 21-day of culture. cDNA was synthesized by reverse transcription of 5 ㎍ samples of RNA in 20 ㎕ of master mix containing 200 U/㎕ superscript™ II (Invitrogen), 5 mM $MgCl_2$, first strand buffer, 1 mM dNTP, 1 U/㎕ RNase inhibitor™, and 2.5 mM oligodT in DEPC-treated distilled water. The master mix was incubated in a PCR at 42℃ for 50-min. and 96℃ for 10-min. Synthesized cDNAs were subjected to 30 cycles of amplification under the following conditions: 94℃ denaturing for 5-min., 65℃ annealing for 1-min. and 72℃ extension for 1-min (Fig. 2).

5. Statistical analysis

Numerical values are expressed as the mean ±SD, n=3 per group. In all studies, three similar experiments were performed for each group. Statistical differences among the experimental groups were evaluated by analysis of variance followed by Kruscal-Wallis test; *, p values < 0.05, **, p values < 0.005 versus control were considered statistically significant.

Table 1. RT-PCR primers and conditions

Primer		*Sequences 5′-3′*	*Product size*	*NCBI Accession No.*
GAPDH	sense	5'-GGAGTCCACTGGCGTCTTCA-3'	182	NM_002046
	antisense	5'-AGCAGTTGGTGGTGCAGGAG-3'		
ALP	sense	5'-CGTGGTCACTGCGGACCATT-3'	219	NM_000478
	antisense	5'-GCAGACTGCGCCTGGTAGTT-3'		
COL-1	sense	5'-CTTCCTGCGCCTGATGTCCA-3'	192	NM_000088
	antisense	5'-CTCGTGCAGCCATCGACAGT-3'		
OPN	sense	5'-ACAGCCAGGACTCCATTGACTCGAACGACTCT-3'	198	NM_000582
	antisense	5'-CCACACTATCACCTCGGCCATCATATGTGTCT-3'		
OC	sense	5'-AGCGGTGCAGAGTCCAGCAA-3'	190	NM_199173
	antisense	5'-AGCCGATGTGGTCAGCCAAC-3'		

GAPDH; glyceraldehyde-3-phosphate dehydrogenase: ALP; alkaline phosphotase: COL-1; collagen type 1: OPN; osteopontin: OC; osteocalcin.

III. Results

1. Effect of LIPUS on hPDLFs proliferation

In the MTT assay for the proliferation of hPDLFs to LIPUS, the value of MTT was ordered to (1) group 4 (2) group 5 (3) group 2, 3 and control, and (4) group 1. So. author chose 3 of 6 exposure modalities, based on the value of MTT, the highest (condition 1: 3MHz, 240 mW), moderate (condition 2: 3MHz, 160 mW), and lowest (condition 3: 3MHz, 360 mW) (Fig. 3).

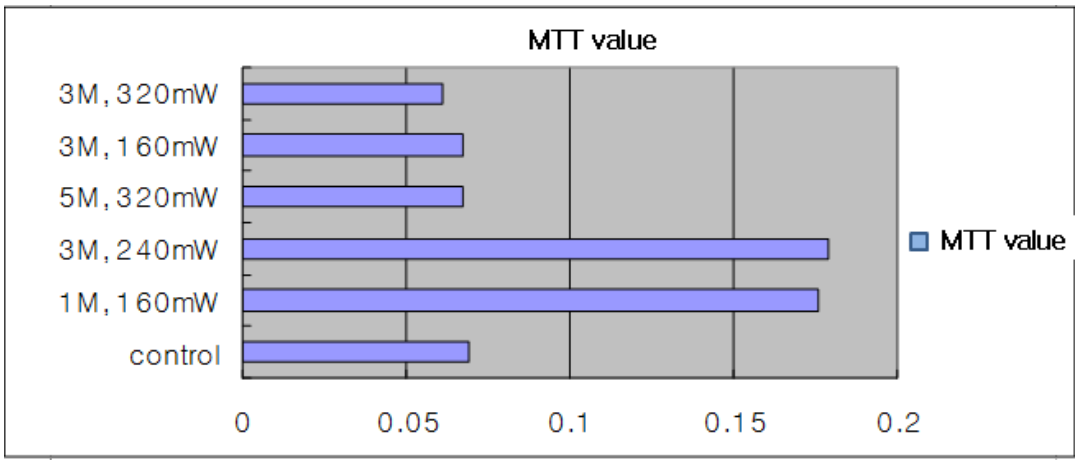

Fig. 3. MTT assay to evaluate the effect of LIPUS on proliferation of hPDLFs.

2. Effect of LIPUS on bone differentiation marker genes

1) ALP mRNA

The expression of ALP mRNA was shown increased tendency in condition 1 and 2 at 14 days after LIPUS exposure, and in condition 3 at 21 days after LIPUS exposure ($p>0.05$) (Fig. 4).

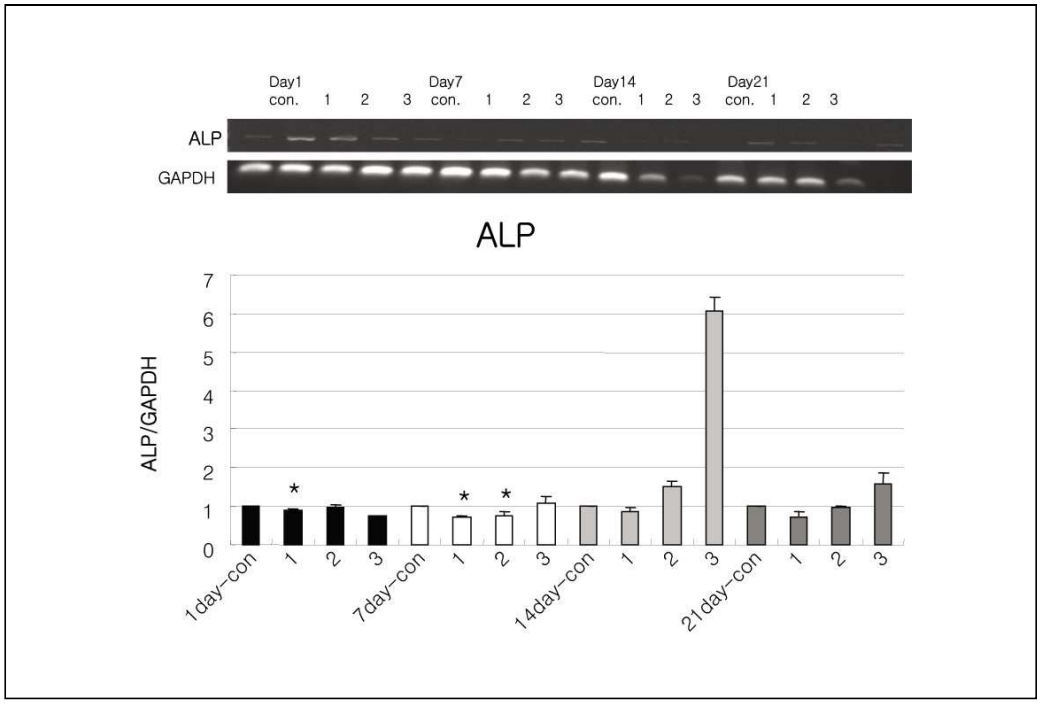

Fig 4. Effect of LIPIS on expression of ALP mRNA in hPDLFs.

2) COL-1 mRNA

The expression of COL-1 mRNA was shown significantly increased in condition 2 at 14 days after LIPUS exposure, and in condition 3 at 21 days after LIPUS exposure compared to control ((p<0.05, p<0.005) (Fig. 5).

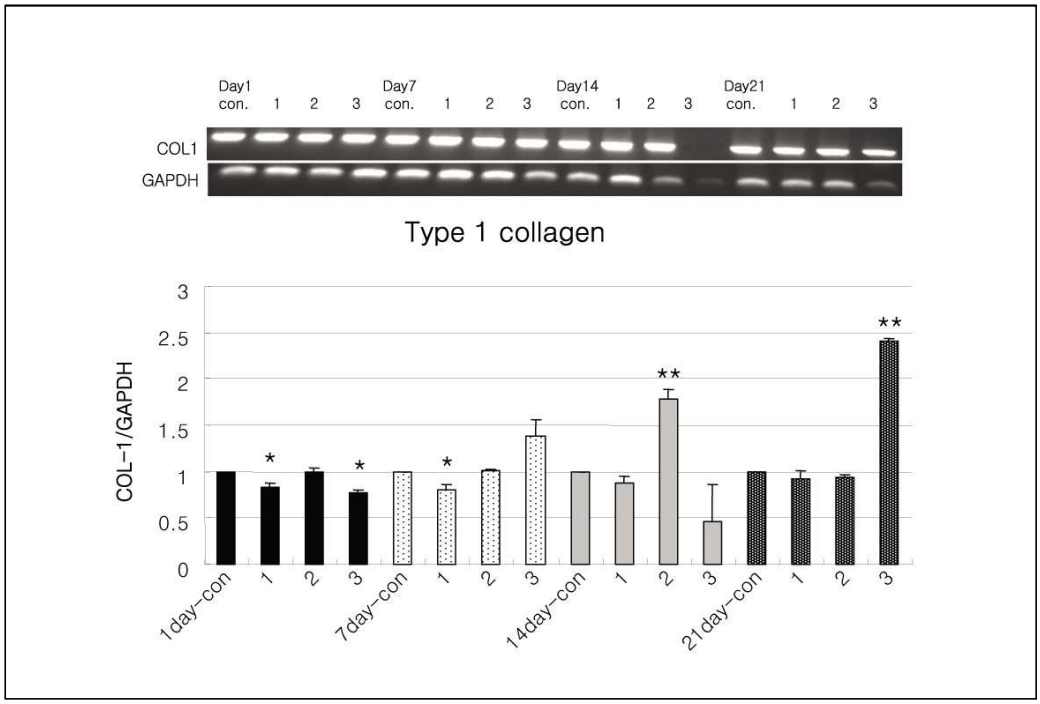

Fig 5. Effect of LIPIS on expression of COL-1 mRNA in hPDLFs.

3) OC mRNA

The expression of OC mRNA was shown significantly increased tendency in condition 1, and 2 at 14 days after LIPUS exposure compared to control ($p>0.05$) (Fig. 6).

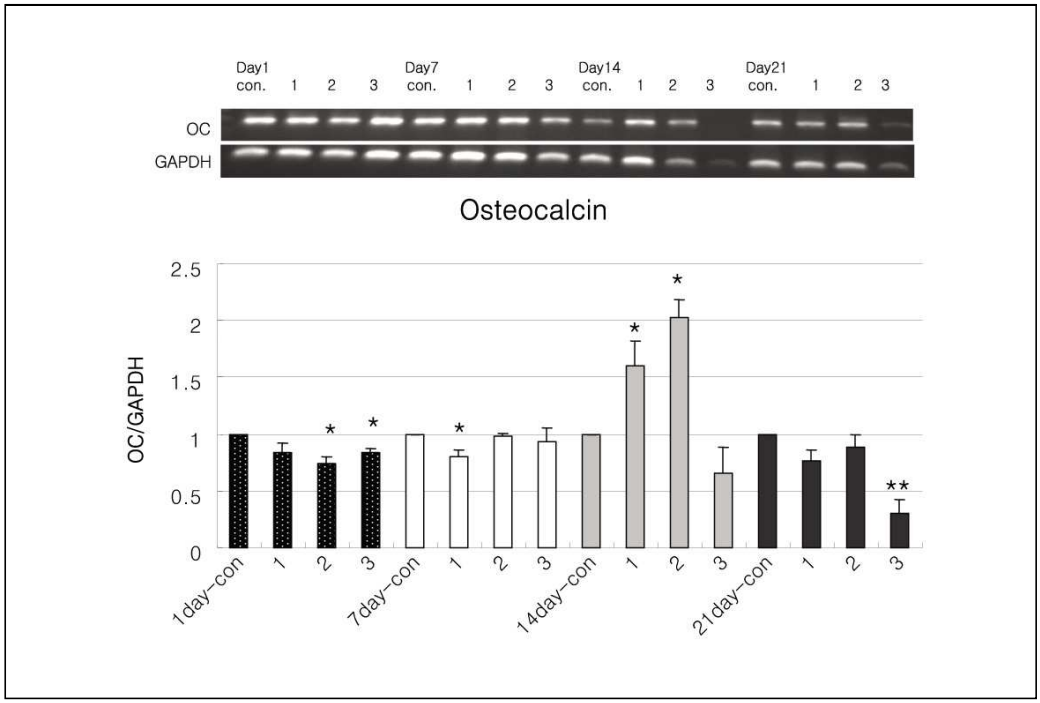

Fig. 6. Effect of LIPIS on expression of OC mRNA in hPDLFs.

4) OPN mRNA

The expression of OPN mRNA was shown significantly increased tendency in condition 1, 2 and 3 at 14 days after LIPUS exposure compared to control ($p<0.05$) (Fig. 7).

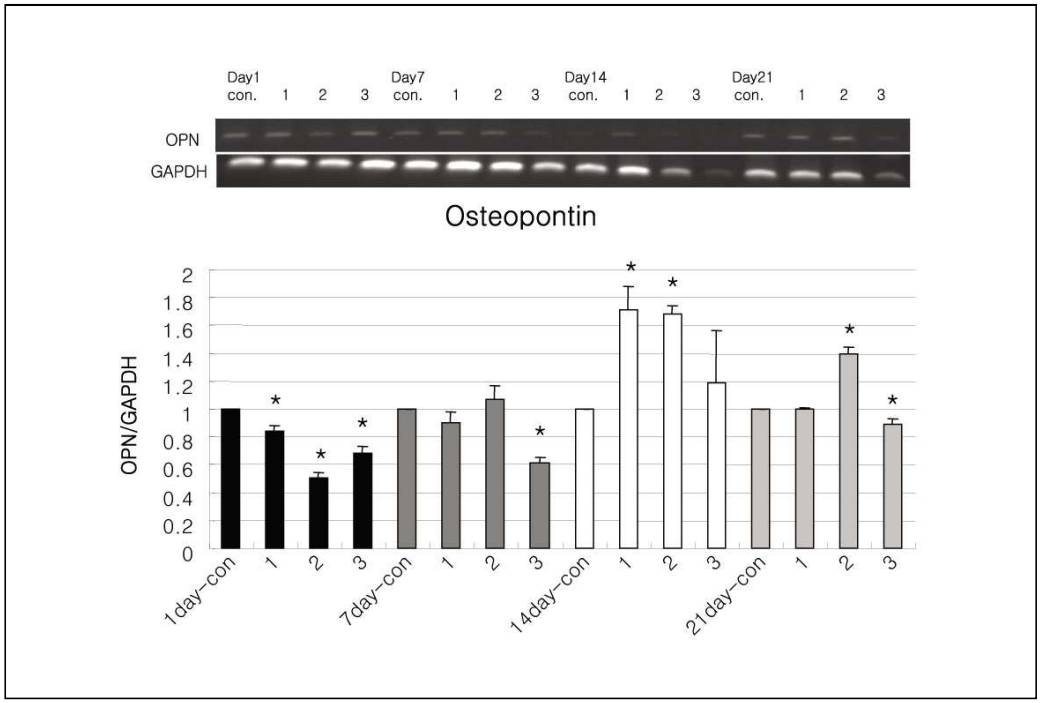

Fig. 7. Effect of LIPIS on expression of OPN mRNA in hPDLFs.

3. Effect of LIPUS on bone nodule formation

From 14 days after last LIPUS exposure, typical bone nodules began to be observed in control and all the experimental groups. Lots of bone nodules were observed at 14-day LIPUS exposure groups compared to 7 days after LIPUS exposure groups, and LIPUS groups compared to control ($p>0.05$) (Fig. 8,9).

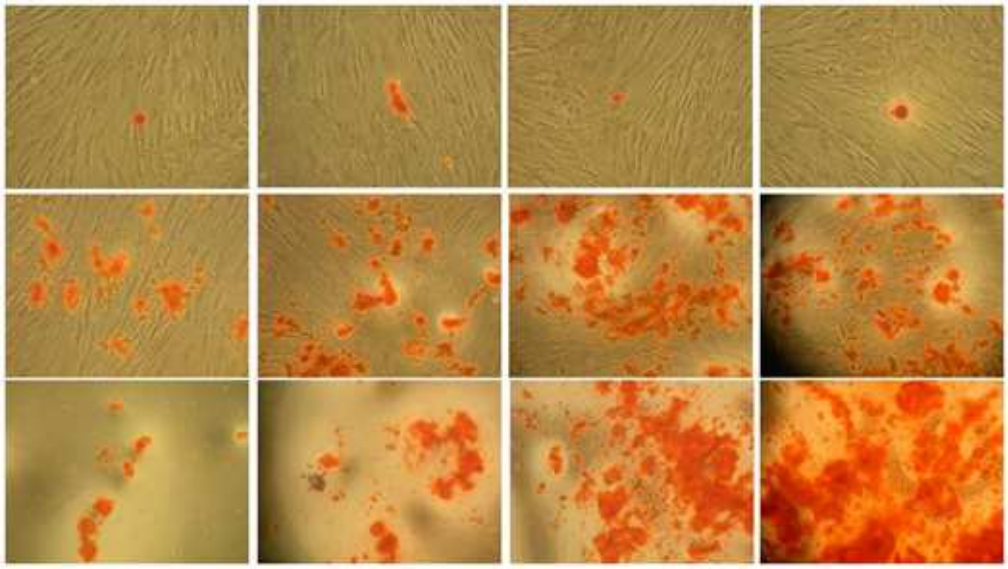

Fig. 8. Mineral nodule formation in hPDLF cell culture observed under microscope with 10x magnification. The row on the top is day 7 group, the middle is day 14 group and the bottom is day 21 group after LIPUS stimulation. The cells were cultured with DMEM supplemented with 10% FBS and mineralization ingredients.

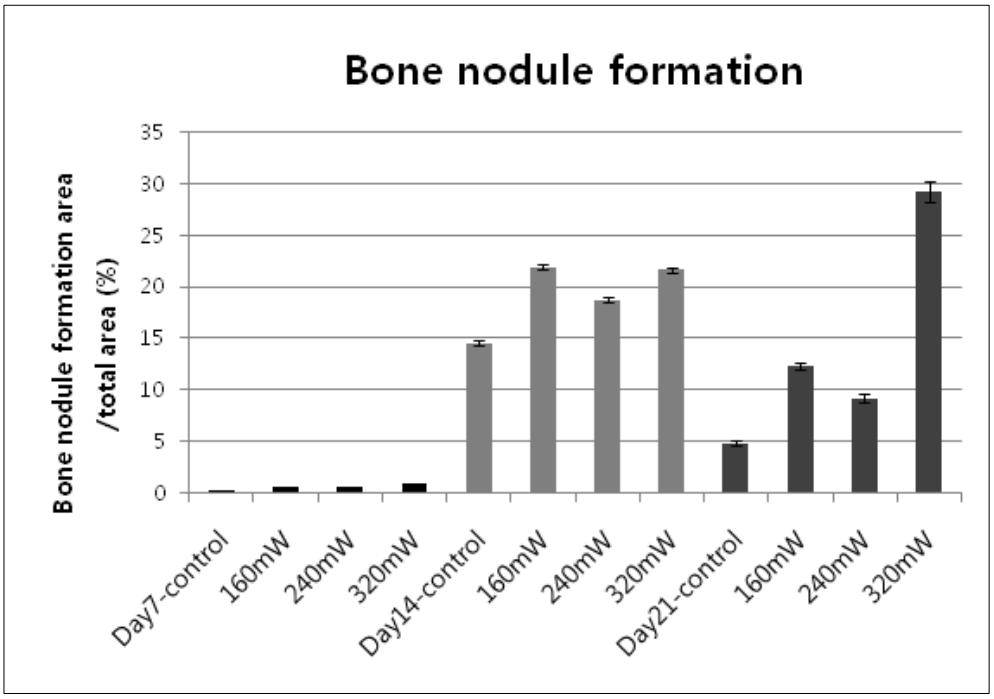

Fig. 9. Bone nodule formation measured in the divided four parts of each well after culture at 37℃ for 7-, 14-, and 21-days. Values represent mean±SD. 10 samples measured per group.

IV. Discussion

In recent years, various treatment modalities using bone morphogenic proteins, and growth factors are introduced to accelerate the bone healing to osseous defects. In the same manner, LIPUS is used for stimulation of bone healing. Application of LIPUS to fracture wounds has been shown to increase the mechanical strength of the callus, in animal models[16,17].

Bone remodelling is a process by which bone grows and turns over. Biochemical markers of bone turnover can be classified according to the process that underlie in markers of bone formation, products of the osteoblast activity [bone ALP, OC, procollagene I C- and N-terminal propeptides] and markers of bone resorption, products of the osteocalst activity [pyridinuim crosslinks, collagen I C- and N-terminal telopeptides (CTX-I and NTX-I), tartrate resistent acid phosphatase (TRACP) isoform 5b][18].

There are some studies that small temperature fluctuation (< 1℃) at the fracture site[19], or a temperature elevation of 0.15℃ in the presence of an intramedullary rod in bone with a 20-minute application of LIPUS[3]. But, it has been demonstrated that thermal increase is not a likely mechanism for LIPUS stimulated changes in cell behavior when used at low intensity[20]. So, we did not measure culture temperature.

ALP, Cbfa-1, ON, OPN are bone differentiation marker gene. Cbfa-1 is one of the transcription factors expressed by cells committed to the osteoblast lineage during bone formation[21]. The coordinate action of these transcription factors results in the expression of several genes that are characteristic of osteoblast differentiation, such as ALP, bone sialoprotein, COL-1, OC and OPN that are expressed sequentially during the process of osteogenesis[22].

In the present study, author was focused on changes in gene expression related to bone differentiation markers, such as ALP, COL-1, OC, and OPN, in hPDLFs.

Sant'Anna et al.[10] reported that increases in gene expressions were greatest after 3 days exposure to LIPUS, and the absence of continued elevation of gene expression for genes that were stimulated most with 3 days exposure to LIPUS may be the result of loss of sensitivity to the stimulus, which could be a result of the cells reaching a developmental stage at which they were no longer responsive to the stimuli, or becoming refractory to further stimulus LIPUS. In my results, increases in gene expression were greatest after 7 days exposure to LIPUS. These differences may be a result of the differences in cell type or time course of experiments.

For ALP, the gene was shown increased tendency in condition 1 and 2 at 7 days after LIPUS exposure, and in condition 3 at 14 days after LIPUS exposure. For COL-1, the gene was shown increased tendency in condition 2 at 14 days after LIPUS exposure, and in condition 3 at 21 days after LIPUS exposure compared to control. For OC, the gene expression was shown increased tendency in condition 1, and 2 at 14 days after LIPUS exposure compared to control. For OPN, the gene expression was useful to accelerate at 3 MHz of frequency in hPDLFs. For bone nodule, from 7 days from beginning of the experiment, typical bone nodules began to be observed in control and all the LIPUS groups. Lots of bone nodules were observed at 7 days after 7-day LIPUS exposure groups compared to immediate day after 7-day LIPUS exposure groups, and LIPUS groups compared to control.

Sena et al.[5] quantified the changes in gene expression in rat bone marrow derived stromal cells to LIPUS during early time points after the ultrasound application. LIPUS at 1.5 MHz, 30 ㎽/㎠ was applied to cells for a single 20 min treatment. They demonstrated that LIPUS treatment induced elevated transient expression of the bone differentiation marker genes, OC and OPN, at 3-hour. Syuhou et al.[23] suggested that according to the color image analysis, irradiation with 3.0 ㎒ LIPUS was almost three times as effective as non LIPUS for osseointegration, and LIPUS treatment was useful to reduce the period between

primary and secondary operation of dental implants. Sant'Anna et al.[10] determined that how LIPUS altered gene expression such as Cbfa-1/Runx2, IGF-receptor, Alk-3, ALP, OPN, TGF-beta1, BMP-7, in rat bone marrow stromal cells and to see if combining this stimulation with 100 *ng*/㎖ BMP-2. They concluded that Increases in gene expression were greatest after 3 days exposure to US, with similar results for BMP-2 treatment. Takayama et al.[24] investigated the effect of LIPUS on osteogenesis by examining the effect of LIPUS stimulation on cell proliferation, ALP activity, osteogenesis-related gene expression, and mineralized nodule formation in a rat osteosarcoma cell line. The mineralized nodule formation and the calcium content in mineralized nodules were markedly increased on day 14 of culture after LIPUS stimulation. Leung et al.[25] suggested that LIPUS stimulated human periosteal cell proliferation, differentiation, and showed increased bone forming effect in calcium nodule formation.

Taken altogether, Increases in gene expression were greatest 7 days after last exposure to LIPUS, implying that there may be a time dependence for the stimulus of bone differentiation markers in hPDLFs. For most genes the pattern of expression was similar, even though slight different according to the exposure modalities, and longer exposure of LIPUS could not obtain the additional positive effects in gene expression, in the culture environment. Our results indicated that LIPUS treatment could accelerate the differentiation of the hPDLFs into osteoblasts or cementoblasts. And we suggests that the condition of LIPUS exposure will be changed based on gene expression period, since the expression of several genes that are characteristic of osteoblast differentiation is expressed sequentially during the process of osteogenesis. too.

Although there is an extensive literature on the application of LIPUS on skeletal tissues, there is limited information on the molecular mechanisms in periodontal tissue remodelling. In the future, attempts to stimulate bone defect healing and periodontal tissue remodelling using LIPUS may focus on the use of other treatment protocols.

References

1. Ziskin MC. Applications of ultrasound in medicine-comparison with other modalities. In: Parvizi J and Vegari D. Pulsed low-intensity ultrasound for fracture healing. Foot Ankle Clin N Am. 2005;10:595-608.

2. Goss SA, Frizzell LA, Dunn F. Ultrasonic absorption and attenuation in mammalian tissues. Ultrasound Med Biol. 1979;5(2):181-186.

3. Parvizi J and Vegari D. Pulsed low-intensity ultrasound for fracture healing. Foot Ankle Clin N Am. 2005;10:595-608.

4. Malizos KN, Hantes ME, Protopappas V. Papachristos A. Low-intensity pulsed ultrasound for bone healing: An overview. Injury. 2006;S56-S62.

5. Sena K, Leven RM, Mazhar K, Sumner DR, Virdi AS. Early gene response to low intensity pulsed ultrasound in rat osteoblastic cells. Ultrasound Med Biol. 2005;31(5):703-708.

6. Erdogan O, Esen E, Ustün Y, Kürkçü M, Akova T, Gönlüşen G, Uysal H, Cevlik F. Effects of low-intensity pulsed ultrasound on healing of mandibular fractures: an experimental study in rabbits. J Oral Maxillofac Surg. 2006;64(2):180-188.

7. Uglow MG, Peat RA, Hile MS, Bilston LE, Smith EJ, Little DG. Low-intensity ultrasound stimulation in distraction osteogenesis in rabbits. Clin Orthop Relat Res. 2003;417:303-312.

8. El-Bialy T, Hassan A, Albaghdadi T, Fouad HA, Maimani AR. Growth modification of the mandible with ultrasound in baboons: A preliminary report. Am J Orthod Dentofacial Orthop. 2006;130:435.e7-435.e14.

9. Feril LB Jr, Kondo T, Cui ZG, Tabuchi Y, Zhao QL, Ando H, Misaki T, Yoshikawa H, Umemura S. Apoptosis induced by the sonomechanical effects of low intensity pulsed ultrasound in a human leukemia cell line. Cancer Lett. 2005;28(2):145-152.

10. Sant'Anna EF, Leven RM, Virdi AS, Sumner DR. Effect of low intensity pulsed ultrasound and BMP-2 on rat bone marrow stromal cell gene expression. J Orthop Res. 2005;23(3):646-52.

11. Unsworth J, Kaneez S, Harris S, Ridgway J, Fenwick S, Chenery D, Harrison A. Pulsed low intensity ultrasound enhances mineralisation in preosteoblast cells. Ultrasound Med Biol. 2007;33(9):1468-1474.

12. Li JK, Chang WH, Lin JC, Ruaan RC, Liu HC, Sun JS. Cytokine release from osteoblasts in response to ultrasound stimulation. Biomaterials. 2003;24(13): 2379-2385.

13. Gleizai A, Li S, Pialat JB, Beziat JL. Transcriptional expression of calvarial bone after treatment with low intensity ultrasound: an in vitro study. Ultrasound Med Biol. 2006;32(10):1569-1574.

14. Schortinghuis J, Ruben JL, Raghoebar GM, Stegenga B. Ultrasound to stimulate mandibular bone defect healing: a placebo-controlled single-blind study in rats. J Oral Maxillofac Surg. 2004;62(2):194-201.

15. Schortinghuis J, Ruben JL, Raghoebar GM, Stegenga B, de Bont LG. Does ultrasound stimulate osteoconduction? A placebo-controlled single-blind study using collagen membranes in the rat mandible. Int J Oral Maxillofac Implants. 2005;20(2):181-186.

16. Chan CW, Qin L, Lee KM, Cheung WH, Cheng JC, Leung KS. Dose-dependent effect of low-intensity pulsed ultrasound on callus formation during rapid distraction osteogenesis. J Orthop Res. 2006;24(11):2072-2079.

17. Chan CW, Qin L, Lee KM, Zhang M, Cheng JC, Leung KS. Low intensity pulsed ultrasound accelerated bone remodeling during consolidation stage of distraction osteogenesis. J Orthop Res. 2006;24(2):263-270.

18. Pagani F, Francucci CM, Moro L. Markers of bone turnover: biochemical and clinical perspectives. J Endocrinol Invest. 2005;28(10 Suppl):8-13.

19. Wu J, Du G. Temperature elevation in tissues generated by finite-amplitude tone bursts of ultrasound. J Acust Soc Am. 1990;88:1562-1577.

20. Chang WH, Sun JS, Chang SP, Lin JC. Study of thermal effects of ultrasound stimulation on fracture healing. Bioelectromagnetics. 2002;23:256-263.

21. Ducy P, Karsenty G. Genetic control of cell differentiation in the skeleton. Curr Opin Cell Biol. 1998;10(5):614-619.

22. Stein GS, Lian JB, Stein JL, Van Wijnen AJ, Montecino M. Transcriptional control of osteoblast growth and differentiation. Physiol Rev. 1996;76(2):593-629.

23. Syuhou F, Tadayasu K, Kuniteru N, Kohji Y. Usefulness of low-intensity pulsed ultrasound in reducing the healing period of dental implant therapy. J Jap Soc Oral Imp. 2004;17(2):183-195.

24. Takayama T, Suzuki N, Ikeda K, Shimada T, Suzuki A, Maeno M, Otsuka K, Ito K. Low-intensity pulsed ultrasound stimulates osteogenic differentiation in ROS 17/2.8 cells. Life Sci. 2007;80(10):965-971.

25. Leung KS, Cheung WH, Zhang C, Lee KM, MPhil JKL. Low intensity pulsed ultrasound stimulates osteogenic activity of human periosteal cells. Clin Orthop. 2004;418:253-259.

ABSTRACT IN KOREAN

사람 치주인대섬유모세포에서 파동형의 저출력 초음파가 골분화표지 유전자 발현에 미치는 효과

김 성 호
조선대학교 치의학과
(지도교수 : 김병옥)

파동형의 저출력 초음파치료는 파절부 창상와 골신연술후에 창상치유를 촉진한다고 보고된 바, 이 연구에서는 RT-PCR법을 이용하여 파동형의 저출력 초음파치료가 골분화유전자 표현을 변화시키는 지를 평가하였다.

이 연구를 수행하기 위해서 치주인대섬유모세포는 교정치료를 위해 발거된 치아의 치근에서 채취하였으며 6세대의 세포를 이용하였다. 실험은 다음과 같이 6군으로 구별하여 초음파를 15분 동안 조사하여 MTT법으로 증식률을 측정하였다. (조사 1군; 주파수: 3 ㎒, 강도: 320 ㎽/㎠, 조사 2군: 주파수; 3 ㎒, 강도: 160 ㎽/㎠, 조사 3군; 주파수: 5 ㎒, 강도: 320 ㎽/㎠, 조사 4군; 주파수: 3 ㎒, 강도: 240 ㎽/㎠, 조사 5군; 주파수: 1 ㎒, 강도: 160 ㎽/㎠, 대조군: 초음파조사를 하지 않은 군.) 증식률을 토대로, 증식률이 가장 높은 군을 실험 1군 (주파수: 3 ㎒, 강도: 240 ㎽/㎠) 으로, 증식률이 중간인 군을 실험 2군 (주파수: 3 ㎒, 강도: 160 ㎽/㎠), 그리고 가장 낮은 군을 실험 3군 (주파수: 3 ㎒, 강도: 320 ㎽/㎠)으로 구분하였다.

치주인대섬유모세포에서 초음파치료의 유용성을 평가하기 위해서 RT-PCR법을 이용하여 골분화유전자인 alkaline phosphatase, collagen type 1, osteocalcin, 그리고 osteopontin과 bone nodule의 생성을 평가하였다. 전체적으로, 초음파를 7일 동안 조사하고 7일 후에 골분화유전자의 표현이 대조군에 비해 증가되었으며, 또한 bone nodule의 생성도 많았다. 즉, 치주인대섬유모세포가 골모세포나 백악모세포로의 분화가 촉진됨을 알 수 있었다. 골분화유전자의 발현양상이 시간적으로 차이가 있으므로 향후에는 초음파 조사방법을 달리하여 경조직 뿐만아니라 연조직개조에 관한 연구도 필요하리라 생각된다.

(별 지)

저작물 이용 허락서

학 과	치의학과	학 번	20057470	과 정	박 사
성 명	한 글: 김 성 호 한 문 : 金 聖 鎬 영 문 : Kim Sung-Ho				
주 소	광주 광역시 북구 일곡동 현대아파트 304-602				
연 락 처	E-MAIL : deutlovel@hanmail.net				
논문제목	한글 : 인간의 치주인대섬유아세포에서 파동형의 저출력 초음파가 alkaline phosphotase, collagen type-1, osteocalcin, osteopontin 유전자 발현에 끼치는 효과				
	영문 :Effect of low intensity pulsed ultrasound on collagen type-1, osteocalcin, alkaline phosphotase, osteopontin in human periodontal ligament fibroblasts				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 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 편집·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 또는 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송·출력을 허락함.

동의여부 : 동의(○) **반대**( )

2008년 2월 일

저작자: 김 성 호 (서명 또는 인)

조선대학교 총장 귀하