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Low-intensity pulsed ultrasound accelerates periodontal wound healing after flap surgery

H. Ikai[1], T. Tamura[1], T. Watanabe[2], M. Itou[1], A. Sugaya[1], S. Iwabuchi[4], Y. Mikuni-Takagaki[3], S. Deguchi[1]

Departments of [1]Periodontology, [2]Prosthodontics and [3]Functional Biology, Kanagawa Dental College, Yokosuka, Japan and [4]Research Associate Bio-medical Engineering Laboratories, Teijin Pharma, Tokyo, Japan

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Background and Objective: A study was conducted to evaluate the effects of low-intensity pulsed ultrasound on wound healing in periodontal tissues after mucoperiosteal flap surgery.

Material and Methods: Bony defects were surgically produced bilaterally at the mesial roots of the mandibular fourth premolars in four beagle dogs. The flaps were repositioned to cover the defects and sutured after scaling and planing of the root surface to remove cementum. The affected area in the experimental group was exposed to low-intensity pulsed ultrasound, daily for 20 min, for a period of 4 wk from postoperative day 1 using a probe, 13 mm in diameter. On the control side, no ultrasound was emitted from the probe placed contralaterally. After the experiment, tissue samples were dissected out and fixed in 10% formalin for histological and immunohistochemical analyses.

Results: The experimental group showed that the processes in regeneration of both cementum and mandibular bone were accelerated by low-intensity pulsed ultrasound compared with the control group. In addition, the expression level of heat shock protein 70 was higher in the gingival epithelial cells of the low-intensity pulsed ultrasound-treated tooth.

Conclusion: Our results suggest that osteoblasts, as well as cells in periodontal ligament and gingival epithelium, respond to mechanical stress loaded by low-intensity pulsed ultrasound, and that ultrasound accelerates periodontal wound healing and bone repair.

Shinji Deguchi, Department of Periodontology, Kanagawa Dental College, 82 Inaoka-cho, Yokosuka, Kanagawa 238–8580, Japan
Tel: +81 46 8228855
Fax: +81 46 8228855
e-mail: deguchis@kdcnet.ac.jp

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Low-intensity pulsed ultrasound has been used in orthopedic surgery to accelerate the fracture healing process (1–3). Among the responses of live animals exposed to ultrasound, increased tissue temperature as a result of the continuous ultrasound waves, or mechanical stress induced by the pulsed waves, has been reported. Acceleration of fracture healing by low-intensity pulsed ultrasound is to be considered attributable to the production of pressure waves, which induce biochemical and molecular events at the cellular level. However, the mechanotransduction pathways through which low-intensity pulsed ultrasound stimulates living tissues are still not completely understood. Yang *et al.* have reported that exposure to low-intensity pulsed ultrasound increased aggrecan gene expression in a rat femoral fracture model (4). Parvizi *et al.* have reported that low-intensity pulsed ultrasound stimulation increased the intracellular concentration of calcium in isolated rat chondrocytes (5,6), while Naruse *et al.* have reported Ca-independent mechanisms that involve activation of the phosphatidylinositol 3-kinase pathways (7,8). Wang *et al.* have reported that low-intensity pulsed ultrasound accelerated endochondral ossification along with the enhanced mechanical properties of healing bone callus (9).

The reported reactions at the cellular level, especially those in fracture healing processes, are likely to be similar to the cellular responses in periodontal wound healing after flap surgery. Therefore, we examined the effects of low-intensity pulsed ultrasound in a canine model system of periodontal wound healing.

Material and methods

Experimental animals

Four Beagle dogs (2–8 years in age; 10–15 kg in weight) were used in this study. For the surgical procedure and low-intensity pulsed ultrasound treatment, the dogs were anesthetized with an intramuscular injection of 12.5 mg/kg of Kethalar®50 (Sankyo Co., Tokyo, Japan). After making intracrevicular incisions, buccal mucoperiosteal flaps were reflected. After removal of the 5 × 5-mm buccal alveolar bone on the mesial roots of the mandibular fourth premolars, the periodontal ligament and cementum of these areas were completely removed by means of hand instruments. To serve as a reference point for histological measurement, a notch was made on the root surface located at the bottom of the bony defects. The flaps were then adapted to the original position and sutured. To control plaque, daily brushing and spraying with 0.2% chlorhexidine digluconate solution (Hibitane®; Sumitomo Chemical Industries, Tokyo, Japan) were continued until the dogs were killed.

The experimental procedures of this study were reviewed and approved by the committee of ethics on animal experiments of Kanagawa Dental College. Experiments were carried out under the guidelines for animal experimentation of Kanagawa Dental College.

Low-intensity pulsed ultrasound treatment

The Sonic Accelerated Fracture Healing System (SAFHS®; Smith & Nephew, Memphis, TN, USA) generated the ultrasound signals composed of a 200-μs burst sine wave of 1.5 MHz repeated at a frequency of 1.0 kHz. The intensity was 30 mW/cm^2. To ensure that the wound was exposed to the ultrasound, the original adaptor was prepared with heavy body putty (EXAFINE putty type; GC Dental Products Corp., Tokyo, Japan) and a rapid-setting self-curing resin (Shofu Co., Kyoto, Japan) before the experiment according to a dental impression taken for each dog. The affected area of the experimental side of the mandible was exposed to low-intensity pulsed ultrasound using a probe, 13 mm in diameter, for 20 min each day for a period of 4 wk from postoperative day 1 (Fig. 1). No ultrasound was emitted from the probe placed contralaterally for the control side.

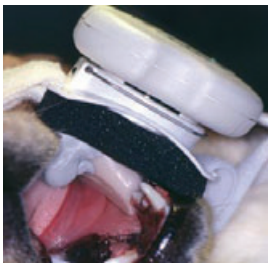

Fig. 1. A Sonic Accelerated Fracture Healing System (SAFHS®) probe was applied to generate the ultrasound signals composed of a 200-μs burst sine wave of 1.5 MHz repeated at a frequency of 1.0 kHz. The intensity was 30 mW/cm^2.

Histological measurement

Block sections of the experimental tooth and adjacent tissue were dissected out immediately after the dogs were killed. After fixation in 10% buffered formalin solution for 1 wk, the sections were demineralized in 10% formic acid for 3 mo. After embedding in paraffin, the tissue was cut into 5-μm-thick serial buccolingual sections, which were either stained with hematoxylin and eosin or processed for immunohistochemical observation.

For histological measurements, 15 sections, corresponding to the center of the mesiodistal bone defect, were selected at an interval of 100 μm from each group. At a 40-fold magnification, the distance between the apical border of the notch and the cemento–enamel junction was determined. Relative to this distance (100%), the following distances were determined and expressed as percentages (Fig. 2).

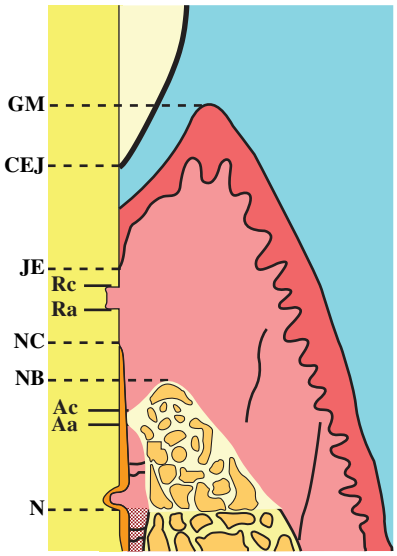

Fig. 2. Reference points for the evaluation of histometric parameters. Aa, the apical border of the ankylosis; Ac, the coronal border of the ankylosis; CEJ, cemento–enamel junction; GM, gingival margin; JE, the most apical level of the junctional epithelium; N, apical border of the notch; NC, the most coronal level of newly formed cementum; NB, the most coronal level of newly formed bone; Ra, the apical border of the root resorption; Rc, the coronal border of the root resorption.

(a) Gingival recession restraint: the distance from the apical border of the notch to the gingival margin.
(b) Epithelial downgrowth restraint: the distance from the apical border of the notch to the most apical level of the junctional epithelium.
(c) New cementum formation: the distance from the apical border of the notch to the most coronal level of newly formed cementum.
(d) New bone formation: the distance from the apical border of the notch to the most coronal level of newly formed bone.
(e) Root resorption: the total distance from the apical border to the coronal border of the root resorption.
(f) Ankylosis: the total distance from the apical border to the coronal border of the ankylosis.

Immunohistochemical analysis

Immunohistochemical staining was performed with mouse monoclonal antibody to human heat shock protein 70 (1 : 500 dilution) as the primary antibody. Diaminobenzidine substrate was used for signal detection. Any positive background staining was blocked by excess endogenous peroxidase.

Statistical analysis

The statistical significance of intergroup differences between these parameters was determined with the Mann–Whitney *U*-test.

Results

Histological analyisis

In the control group, epithelial downgrowth occupied half of the root surface where the dentin had been exposed by root planing. New cementum formation was observed in the area between the apical border of the notch and the apical one-third of the root surface. The new bone formation was limited to the bottom of the bone defect (Fig. 3). In the experimental group, the apical migration of epithelium was limited to the coronal quarter of the root surface. The new cementum formation was observed between the apical border of the notch and the most apical end of the epithelium. The new bone formation was observed in the apical quarter of the root surface (Fig. 4). Although slight resorption was observed in the root in both groups, no dental ankylosis was found. The histometric results are shown in Table 1. No significant differences were found between the two groups with regard to gingival recession and root resorption. Epithelial downgrowth restraint, new cementum formation and new bone formation were more significantly progressed in the experimental group compared with the controls.

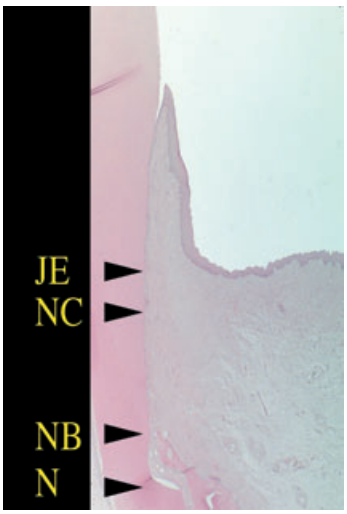

Fig. 3. Buccolingual section of the central portion of a mesial root representing the control result. JE, junctional epithelium; N, notch; NB, newly formed bone; NC, newly formed cementum. Hematoxylin and eosin staining with original magnification ×5.

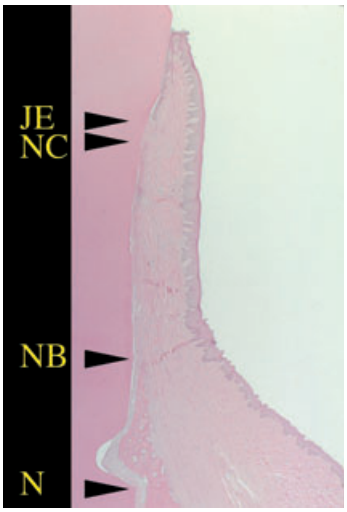

Fig. 4. Buccolingual section of the central portion of a mesial root representing the experimental result. JE, junctional epithelium; N, notch; NB, newly formed bone; NC, newly formed cementum. Hematoxylin and eosin staining with original magnification ×5.

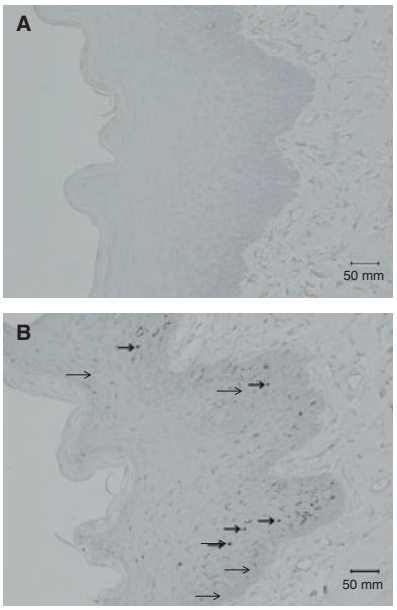

Fig. 5. Expression of heat shock protein 70 in gingival tissues of the control group (A) and of the low-intensity pulsed ultrasound-treated group (B). Bars represent 50 μm at the original magnification of ×40. Arrows indicate areas where a positive reaction occured.

Immunohistochemical analysis

In the present study, heat shock protein 70-positive cells were seen around the basal and spinous layers of the gingival epithelium in the experimental groups (Fig. 5). However, there was no positive reaction in the control group.

Discussion

The therapeutic ultrasound creates mechanical stress in cells involved in fracture healing, which occurs via processes involving various cellular reactions, some of which are accelerated by the low-intensity pulsed ultra-

Table 1. The difference in histometric parameters between control and experimental groups

	Control	Experiment
Gingival recession restraint	107.5 ± 3.9	110.3 ± 2.8
Epithelial downgrowth restraint	54.0 ± 2.5	79.4 ± 1.7[a]
New cementum formation	42.8 ± 2.5	67.5 ± 1.6[a]
New bone formation	11.8 ± 1.1	23.4 ± 0.8[a]
Root resorption	3.6 ± 0.1	2.3 ± 0.1

Data are expressed as mean ± standard deviation.
Significance in difference ([a] $p < 0.05$) between the two groups was analyzed with the Mann–Whitney *U*-test.

sound. Many cell types, such as fibroblasts, chondrocytes and osteoblasts, all of which respond to mechanical stress, play important roles in the fracture healing process (10). Naruse *et al.* reported that low-intensity pulsed ultrasound acts on osteoblastic cells at a specific stage of differentiation and induces direct anabolic reactions, which results in bone matrix formation (7,8). In an oral environment, similar cell-specific responses to low-intensity pulsed ultrasound are expected from such *in vitro* studies with periodontal ligament cells or gingival fibroblasts (11,12). Certain applications were studied and results of positive (13) and negative (14) efficacy have been reported.

In untreated periodontal disease, teeth are lost through destruction of the attachment apparatus and tooth-supporting structures. In addition to conventional approaches (e.g. flap debridement) to access root surfaces, reduce periodontal pockets and attain improved periodontal architecture, new surgical procedures such as guided tissue regeneration, which aim at complete regeneration of affected periodontal tissues. have also been developed (15). Currently, the anticipated therapeutic approaches will be towards noninvasive treatment, such as low-intensity pulsed ultrasound or low-intensity laser therapy (16), which is much less stressful and more suitable for patients at the earlier disease phases.

We therefore examined the effects of low-intensity pulsed ultrasound in a canine model of periodontal wound healing. In our study, immunohistochemical detection localized heat shock protein 70-positive cells only in the low-intensity pulsed ultrasound-exposed gingival epithelium around the basal and spinous layers. In a previous report, high-level expression of heat shock protein 70 has been well correlated with the healing process (17). Moreover, Morton reported that the vastus lateralis muscle biopsies of individuals, after running on a treadmill for 45 min, showed induction of heat shock proteins 70 and 60, and heat shock cognate 70, typically occurring 48 h postexercise, to 210, 170 and 139% of pre-exercise levels, respectively. In contrast, neither heat shock protein 27, alpha B-crystallin, superoxide dismutase-2 (SOD-2) (manganese-SOD) protein content, nor the activity of SOD or catalase, was induced by the exercise (18). Our findings of elevated heat shock protein 70 expression level in the low-intensity pulsed ultrasound-exposed gingival epithelium at 4 wk suggest certain anabolic mechanical induction mechanisms shared by such moderately demanding and nondamaging running exercise. Elevated expression of heat shock protein 70 may have contributed to the proliferation of epithelial cells and prevented their downgrowth. In addition to heat shock protein 70 expression, activation of early response genes, such as Egr-1, an early growth response gene, was reported in mechanically stretched fetal lung epithelial cells (19). Interestingly, it was also reported that nuclear factor-κB was responsible for the sustained and progressive increase in expression of heat shock protein 70. Variable transient or progressive increases in gene expression of the early response genes reported may also occur in our system after mucoperiosteal flap surgery. Moreover, we showed that new bone and cementum formation is significantly elevated in the experimental group compared with the controls. These results imply that periodontal ligament cells, mandibular osteoblasts and gingival epithelial cells all respond to the mechanical stress of low-intensity pulsed ultrasound in an anabolic manner in the processes of wound healing, although individual cellular responses in growth factor production, for example, is yet to be investigated. Studies in cells derived from a more readily available rodent model, at earlier time points, is currently underway. Other areas of *in vivo* research (13,20–25) support our contention that low-intensity pulsed ultrasound provides a safe and effective method applicable to oral ailments.

Acknowledgements

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References

1. Heckman JD, Ryaby JP, McCabe J, Frey JJ, Kilcoyne RF. Acceleration of tibial fracture-healing by non-invasive, low-intensity pulsed ultrasound. *J Bone Joint Surg Am* 1994;**76:**26–34.
2. Kristiansen TK, Ryaby JP, McCabe J, Frey JJ, Roe LR. Accelerated healing of distal radial fractures with the use of specific, low-intensity ultrasound. A multicenter, prospective, randomized, double-blind, placebo-controlled study. *J Bone Joint Surg Am* 1997;**79:**961–973.
3. Leung KS, Lee WS, Tsui HF, Liu PP, Cheung WH. Complex tibial fracture outcomes following treatment with low-intensity pulsed ultrasound. *Ultrasound Med Biol* 2004;**30:**389–395.
4. Yang KH, Parvizi J, Wang SJ *et al.* Exposure to low-intensity ultrasound increases aggrecan gene expression in a rat femur fracture model. *J Orthop Res* 1996;**14:**802–809.
5. Parvizi J, Parpura V, Greenleaf JF, Bolander ME. Calcium signaling is required for ultrasound-stimulated aggrecan synthesis by rat chondrocytes. *J Orthop Res* 2002;**20:**51–57.
6. Parvizi J, Parpura V, Kinnick RR, Greenleaf JF, Bolander ME. Low intensity ultrasound increases intracellular concentration of calcium in chondrocytes. *Trans Orthop Res Soc* 1997;**22:**465.
7. Naruse K, Mikuni-Takagaki Y, Azuma Y *et al.* Anabolic response of mouse bone-marrow-derived stromal cell clone ST2 cells to low-intensity pulsed ultrasound. *Biochem Biophys Res Commun* 2000;**268:** 216–220.
8. Naruse K, Miyauchi A, Itoman M, Mikuni-Takagaki Y. Distinct anabolic response of osteoblast to low-intensity pulsed ultrasound. *J Bone Miner Res* 2003;**18:**360–369.
9. Wang SJ, Lewallen DG, Bolander ME, Chao EY, Ilstrup DM, Greenleaf JF. Low intensity ultrasound treatment increases strength in a rat femoral fracture model. *J Orthop Res* 1994;**12:**40–47.
10. Azuma Y, Ito M, Harada Y, Takagi H, Ohta T, Jingushi S. Low-intensity pulsed ultrasound accelerates rat femoral fracture healing by acting on the various cellular reactions in the fracture callus. *J Bone Miner Res* 2001;**16:**671–680.
11. Doan N, Reher P, Meghji S, Harris M. In vitro effects of therapeutic ultrasound on cell proliferation, protein synthesis, and cytokine production by human fibro-

blasts, osteoblasts, and monocytes. *J Oral Maxillofac Surg* 1999;**57:**409–419.
12. Harle J, Salih V, Mayia F, Knowles LC, Olsen I. Effects of ultrasound on the growth and function of bone and periodontal ligament cells in vitro. *Ultrasound Med Biol* 2001;**27:**579–586.
13. El-Bialy T, El-Shamy I, Graber TM. Repair of orthodontically induced root resorption by ultrasound in humans. *Am J Orthod Dentofacial Orthop* 2004;**126:**186–193.
14. Schortinghuis J, Bronckers AL, Stegenga B, Raghoebar GM, de Bont LG. Ultrasound to stimulate early bone formation in a distraction gap: a double blind randomised clinical pilot trial in the edentulous mandible. *Arch Oral Biol* 2005;**50:**411–420.
15. Wang Y, Nakashima N, Sekiguchi T, Nishimoto T. Saccharomyces cerevisiae GTPase complex: *Gtr*1p-*Gtr*2p regulates cell-proliferation through Saccharomyces cerevisiae Ran-binding protein, Yrb2p. *Biochem Biophys Res Commun* 2005; **336:**639–645.
16. Azevedo LH, de Paula Eduardo F, Moreira MS, de Paula Eduardo C, Marques MM. Influence of different power densities of LILT on cultured human fibroblast growth: a pilot study. *Lasers Med Sci* 2006;**21:**86–89.
17. Oberringer M, Baum HP, Jung V *et al*. Differential expression of heat shock protein 70 in well healing and chronic human wound tissue. *Biochem Biophys Res Commun* 1995;**214:**1009–1014.
18. Morton JP, MacLaren DP, Cable NT *et al*. Time course and differential responses of the major heat shock protein families in human skeletal muscle following acute nondamaging treadmill exercise. *J Appl Physiol* 2006;**101:**176–182.
19. Copland IB, Post M. Stretch-activated signaling pathways responsible for early response gene expression in fetal lung epithelial cells. *J Cell Physiol* 2007;**210:** 133–143.
20. Efanov O. Effect of low-intensity ultrasound on the microcirculation in periodontosis. *Vopr Kurortol Fizioter Lech Fiz Kult* 1981;**3:**5–8.
21. ElHag M, Coghlan K, Christmas P, Harvey W, Harris M. The anti-inflammatory effects of dexamethasone and therapeutic ultrasound in oral surgery. *Br J Oral Maxillofac Surg* 1985;**23:**17–23.
22. Harris M. The conservative management of osteoradionecrosis of the mandible with ultrasound therapy. *Br J Oral Maxillofac Surg* 1992;**30:**313–318.
23. El-Bialy TH, Royston TJ, Magin RL, Evans CA, Zaki AE, Frizzel LA. The effect of pulsed ultrasound on mandibular distraction. *Ann Biomed Eng* 2002;**30:** 1251–1261.
24. El-Bialy TH, Zaki AE, Evans CA. Effect of ultrasound on rabbit mandibular incisor formation and eruption after mandibular osteodistraction. *Am J Orthod Dentofacial Orthop* 2003;**124:**427–434.
25. Erdogan O, Esen E, Ustün Y *et al*. Effects of low-intensity pulsed ultrasound on healing of mandibular fractures: an experimental study in rabbits. *J Oral Maxillofac Surg* 2006;**64:**180–188.