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THE EFFECT OF ULTRASONİC WAVES (LOW İNTENSİTY ULTRASOUND) DURİNG PERİODONTAL SURGERY ON BONE CELL ACTİVİTY OF MANDİBLE İN DOG

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ABSTRACT

Background: One of the main goals of treatment of periodontal disease is preservation of alveolar bone in order to survive the teeth. It is believed that stimulating osteoblast cells activity and preventing osteoclastic activity of bone cells during the treatment process, rebuild the bone structure. The positive effect of low intensity ultrasound on the bone cells activity has been approved. Therefore, the use of ultrasound devices is an effective method to accelerate the treatment process of bone deterioration diseases and fractures.
Material and method: present study was conducted as pilot study on two dogs. Orthodontic o rings were used to create periodontitis in cervical of molars on the light and left sides of mandible for 4 weeks. Debridement and Root planning were performed on the right side of mandible (control group) with manual tip and on the left side of it (target group) with Piezo tip (mectron-20$^{mW}/_{cm^2}$). After debridement on the left side, Piezo tip was moved on the bone to stimulate bone cells activity for 10 minutes. After 14 days, the bone of the right and left sides of mandible was sampled from sample1. Sampling was conducted on sample 2 after 21 days.
Results: the results of present study showed that low intensity ultrasound cannot statistically have positive or negative effects on regeneration of bone, including the number of osteoblasts and osteoclasts, vascularity, formation of collagen fibers, inflammatory cell infiltration, callus formation, evidences of bone remodeling and presence of mature cartilage (p>0.05).
Conclusion: according to the results, it seems that in order to achieve more accurate and more confident results about the priority of each of these methods, designing and performing complementary studies with larger sample sizes are necessary.

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Introduction

Periodontal tissues have 4 main components of periodontal ligament, gums, cementum and alveolar bone that provide the support necessary to keep the teeth in function. These components are in a form of evolutionary and functional unit that each of them, including alveolar bone, may be damaged due to periodontal diseases. Alveolar bone is the bone that supports the teeth and dwindles due to the process of periodontal diseases or increased activity of osteoclasts and affects the treatment prognosis. Pocket surgical treatment can be used to ensure the removal of all triggers from the tooth surface and reduction in periodontal pocket depth or remove it. Periodontal treatment efficiency depends on the complete elimination of tartar, plaques

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and infected cementum from the tooth surfaces. The presence of serration on the root surface often make the surgery more difficult. With deeper pocket, the greater surface should be debrided, root surface seems rougher and access to root becomes weak. All these problems can be reduced by cutting or moving the wall of soft tissue of pocket, because it increases the view and access to root surfaces. With the properly selected disease, both reduction and enhancement techniques are effective in achieving this goal. Pocket creates the areas that keeping them clean is impossible for the patient and it results in increased depth of pocket [1]. Given that the most of periodontal diseases are associated with bone loss in the jaw bone. It is believed that stimulating osteoblast cells activity and preventing osteoclastic activity of bone cells during the treatment process, rebuild the bone structure and restore the survival of the teeth. One of the ways discussed to activate bone is to use ultrasound waves. Ultrasound waves have been routinely used in medicine to treat neuromuscular and musculoskeletal chronic diseases since Wood and Loomis published their article entitled "The physical and chemical effects of high-frequency sound waves" in 1927 [2]. The use of ultrasound has been firstly raised as an ultrasonic scalar in the treatment of periodontal diseases in 1955 by Zinner [3]. In fact, ultrasound waves refers to the waves that their frequencies are higher than human hearing range and they are produced by putting the crystals of material against intermittent electricity. At the clinic, piezoelectric transducer or magnetostriction converts electrical energy into ultrasonic waves [4]. Ultrasound waves has led to great advances in the devices used in non-invasive periodontal treatments and they can provide momentary information on the clinical aspects of periodontium such as pocket depth, attachment level, tissue thickness, histological changes, crime, and calculus, bone morphology and tooth structure for dentists. Spranger [4,5] tested the ultrasonography in periodontology for the first time to measure the surface of the alveolar crest. Palou et al. [6] have also tried to get the picture of alveolar crest by placing ultrasonic transducer parallel to the long axis of the teeth. Eger [7] succeeded to measure gum thickness using 5 MHz ultrasound [8].

Recently, bone cutting surgery using ultrasound waves, has been introduced as a possible alternative to traditional means used for craniofacial surgery due to their accurate and safe technical features. Piezo surgery is a new and innovative method that piezoelectric ultrasonic vibration is used for accurate and safe osteotomy. The method was firstly invented to overcome the limitations of traditional devices in orthopedic surgery in the mouth by Tomaso Vercelotti. In piezoelectric surgery, certain surgical devices are used that their power 3 times more than normal ultrasound devices. The used device has a special feature: when the device is used on mineralized tissue, its shear activity begins and when it is used on soft tissue, its cutting activity stops, so, the nerves, vessels and soft tissue adjacent to the targeted hard tissue aren't damaged by micro-vibration. The device has different frequency fluctuations and due to this, operator can apply specific vibration so that cutting area is free of bone chips. Leaf sinus surgery and raising the membrane from the bottom of sinus are performed using piezoelectric elevator and the force of physiological solution. The results of piezo surgery have shown the better reconstruction and remodeling of bone compared to diamond burs and carbide. The force required for cutting is less compared to rotary burs. Also, patients are more comfortable during surgery because of emitted sound of the turbines [5].

Given that in periodontal disease treatment, one of the structures may be injures is the bone supporting the teeth or alveolar bone, so, one of the main goals of treatment of periodontal disease is preservation of alveolar bone in order to survive the teeth. Given that the most of periodontal diseases are associated with bone loss in the jaw bone. It is believed that stimulating osteoblast cells activity and preventing osteoclastic activity of bone cells during the treatment process, rebuild the bone structure and restore the survival of the teeth. Medical science researchers have proven the positive impact of ultrasound waves on stimulating activity of bone cells. So, today, using ultrasound devices is an effective method to accelerate the treatment of wastes and bone fractures. It is clear that if ultrasound waves have similar effects on bone structure of the jaws and alveolar bone, they can be used to stimulate bone cells in periodontal diseases treatment during surgery and however, the use of ultrasound in periodontal diseases treatment is limited to SRP and removal of inflammatory granulation tissue r during surgeries. Therefore, present study aimed to evaluate the effect of low intensity ultrasound during periodontal surgery on bone cell activity of mandible in dog.

Material and Method

Present study is interventional animal research performed in animal research branch in the school of dentistry in Mashhad. Sampling method was non-probability and easy. This study was performed as pilot on two dogs aged about 6 months. Both dogs were adult and systematically healthy. The dogs were of Iranian mixed breed. They were quarantined for two weeks with the veterinary supervision and necessary drugs and Berirab rabies and Levamisol anti-parasitic and Paraziquantel anti-fungal vaccines were prescribed. After the quarantine period, the animals were cared in the Maintenance of Animal department in the school of Dentistry in Mashhad. During rearing, the animals were fed with standard and health food for dogs and proper supplements and all of these steps were recorded in birth certificate of each dog. All the steps of present study were the creation of periodontitis, periodontal surgery and sampling. Sampling in both dogs are quite similar to each other. After being sure that the animal was unconscious, orthodontic o rings were used to create periodontitis in cervical of molars on the light and left sides of mandible for 4 weeks. O ring were fixed in place by light-curing glass. After 4 weeks, animals were anesthetized and the creation of periodontitis in the areas of molars for periodontal surgery was confirmed by X-ray and probe. After preparation of the animal, block anesthesia and infiltration injections in the mandibular molar were performed using lidocaine 2% and adrenaline 1/100000. Sulcular incision was performed using the blade No. 15 so that margins and papilla were placed in the flap. Flap had been eliminated as full thickness Envelop so that the studied area would be fully in sight and reach. Then, debridement and Root planing were carefully performed on the right side of mandible (control group) with manual tips and on

the left side of mandible (experimental group) with Piezo tip (mectron-20$^{mW}/_{cm^2}$). After debridement on the left side, Piezo tip was moved on the bone to stimulate bone cells activity for 10 minutes. No osteoctomy and osteoplasty were performed on the bone. Then the flaps were closed by sutures(figure 8). After 14 days, sample 1 was anesthetized by doing vital perfusion and then, the bone of the right and left sides of mandible was sampled. Sampling was conducted on sample 2 after 21 days. The mandibles of dogs were separated from the posterior of last molar. The bone of surgical areas was separated as block section. After removing excess soft tissue, samples were placed in formalin 10% for ten days. Then, they were stored in normal saline for one day. Then they were placed in a solution containing 780 ml of tribasic sodium citrate 10% and 220 ml of formic acid 85%. After the decalcification, the samples were sent to the pathology laboratory for histological stages. In present study, longitudinal incisions are required. The specimens were placed under re-fixation and then, they were dehydrated. Transparency practice of samples was performed by replacing alcohol with Xylenol. Then, they were incubated in paraffin so that xylenol was replaced by paraffin and penetration of paraffin in tissue became complete, then, the samples were placed in paraffin frame in order to be prepared for cutting. After these stages, longitudinal incisions with 4 micron thickness were created by microtome device and placed on the slide. Hematoxylin and eosin method was used to stain. A pathologist performed histological evaluation in a quite blind form by binocular optical microscope LEICA BME (Made in America). Statistical tables and charts were used to describe the data and Mann-Whitney U test and Independent Samples Test were used to analyze the data by SPSS16 software and p-value was considered less than 0.05.

Results

In present study, two samples of both half jaw of two white and yellow dogs were studied that in yellow dog, the left and right sides of the jaw were treated by manual (control group) and Piezo (experimental group) tips, respectively and in white dog, the right and left sides of the jaw were treated by manual (control group) and Piezo (experimental group) tips, respectively. In this section, formation or absence of collagen fibers, acute or chronic inflammatory cell infiltration based on the number of cells: 1- None (0-2 inflammatory cells) 2. Small (2-5 inflammatory cells) 3. Average (5-10 inflammatory cells) 4. Great, callus formation or absence, presence or absence of evidences of bone remodeling, presence or absence of mature cartilage, counting the number of osteoblasts in 5 microscopic fields, counting the number of osteoclasts in 5 microscopic fields and counting the number of blood vessels in 5 microscopic fields were analyzed using statistical tests.

Table1. Comparison of formation or absence of collagen fibers in the two groups

Group	No.	Formation of collagen fibers	Absence of collagen fibers	Result
Control	3	3	0	P-value=1.000
Experimental	4	4	0	
	No.	callus formation	Callus absence	
Control	3	0	3	P-value=1.000
Experimental	4	0	4	
	No.	Presence of bone remodeling	Absence of bone remodeling	
Control	3	3	0	P-value=1.000
Experimental	4	4	0	
	No.	Presence of mature cartilage	Absence of mature cartilage	
Control	3	0	3	P-value=0.386
Experimental	4	1	3	

In above table, the two groups were compared in formation or absence of collagen fibers and formation of collagen fibers was observed in both of them and also, the results of Mann-Whitney U test showed no significant difference between the two groups in formation of collagen fibers (P-value=1.000). Also, no callus formation was observed in the both groups and the results of Mann-Whitney U test showed no significant difference between the two groups in callus formation (P-value=1.000) and finally, it was observed that there was no mature cartilage in control group but in experimental group, mature cartilage was observed only in one case and the results of Mann-Whitney U test showed no significant difference between the two groups in presence or absence of mature cartilage (P-value=0.386).

Table2. Comparison of inflammatory cell infiltration in the two groups

inflammatory cell infiltration	Control	Experimental
None (0-2 inflammatory cells)	3	3
Small (2-5 inflammatory cells)	0	1
Average (5-10 inflammatory cells)	0	0
Great	0	0
Result	P-value=0.386	

In above table, the two groups were compared in inflammatory cell infiltration and it was observed that no inflammatory cell infiltration was found in control group but in experimental group, inflammatory cell infiltration (small number of cells (2-5 cells)) was observed only in one case and the results of Mann-Whitney U test showed no significant difference between the two groups in inflammatory cell infiltration (P-value=0.386).

Table3. Comparison of the number of osteoblasts, osteoclasts and blood vessels in the two groups

	Group	Frequency	Mean	Standard deviation	Test result
Osteoblasts	Control	3	25	13.22	P-value=0.167
	Experimental	4	41.25	13.14	
Osteoclasts	Control	3	2.33	2.08	P-value=0.881
	Experimental	4	2.5	0.577	
Blood vessels	Control	3	28.33	12.58	P-value=0.096
	Experimental	4	43.75	7.5	

In above table, the two groups were compared in the number of osteoblasts and it was observed that the mean number of osteoblasts was 25±13.22 in control group and it was 41.25±13.14 in experimental group and the results of Independent Samples Test showed no significant difference between the two groups in the mean number of osteoblasts (P-value=0.167). Also, the mean number of osteoclasts was 2.33±2.08 in control group and it was 2.5±0.577 in experimental group and the results of Independent Samples Test showed no significant difference between the two groups in the mean number of osteoclasts (P-value=0.881). Finally, it was observed that the mean number of blood vessels was 18.33±12.58 in control group and it was 43.75±7.5 in experimental group and the results of Independent Samples Test showed no significant difference between the two groups in the mean number of osteoclasts (P-value=0.096).

Discussion

Low-intensity pulsed ultrasound (LIPUS) is a form of mechanical energy that is transmitted high-frequency sound pressure waves through the skin [9]. LIPUS intensity (30 mW / cm2) is within the intensity of ultrasound (1-50 Mw/cm2) used for therapy and is considered as non-thermal and non-destructive waves [10]. In present study, the effect of low intensity ultrasound during periodontal surgery on bone cell activity of mandible in two yellow and white dogs was investigated that in yellow dog, the left and right sides of the jaw were treated by manual and Piezo tips, respectively and in white dog, the right and left sides of the jaw were treated by manual and Piezo tips, respectively. Bone cells are sensitive to the stresses caused by physical load [11, 12]. Mechanoreceptors convert biophysical stimulus to biochemical responses that alter gene expression and cell adaptation [13]. Mechanical adjustment model increases bone tissue by a call proliferator or direct anabolic effect on bone cells [14]. Micromechanical stress caused by LIPUS, provides a substitute for the forces that applied naturally by the physical load and according to the Wolff law [15, 16]. However, the pressure caused at the tissue level by LIPUS is several times less than the maximum pressure caused by physical load bearing [15], the pressures with low intensity and high frequency can produce regulatory signals on the bone tissue [19-17]. The first successful use of LIPUS in treatment of nonunion and delayed union fractures in clinical field was described in 1983 by Xavier and Duarte [20]. LIPUS is a safe and easy treatment method and it requires only 20 minutes to be used in daily treatment of patients on an outpatient basis [21]. Randomized clinical trials conducted in the field of new fractures healing represent 38% acceleration in bone fracture healing as the result of treatment using LIPUS [22, 23]. Food and Drug Administration approval for the use of LIPUS as a way to accelerate new fractures with protective treatment program was received in 1994. In some studies, it has been shown that ultrasound waves play an effective role in the recovery and restoration of bone tissue. Claes, in a study, stated that using LIPUS during the treatment of bone fractures reduces recovery time and it is effective in stimulating all the cells involved in bone repair of mesenchymal cells, chondrocytes, osteoblasts and osteoclasts and their proliferation [24]. Also, Azuma et al. concluded that using LIPUS can positively impact on treatment of femoral fractures and all cell processes of bone healing [25]. In a study by Sun et al., it was found that LIPUS significantly increases the number of osteoblasts and reduces the number of osteoclasts [26]. However, in present study, no significant difference was observed between control (25±13.22) and experimental (25.41±13.14) groups in mean number of osteoblasts (p=0.167). Also, no significant difference was observed between control (2.08±2.33) and experimental (2.5±0.577) groups in mean number of osteoblasts (P=0.881). Nolte et al. showed that LIPUS can change the failure in repair of fractures in non-union cases and heal the fractures [27]. This evidence, along with some other clinical observations based on LIPUS regenerative capacity on impaired bone repair led to receiving FDA approval for use of LIPUS in non-union fractures LIPUS in 2000. In some studies, the ways in which ultrasound waves affect bone tissue cells have been studied in terms of molecule. Ultrasound waves increase the production of prostaglandin E2 through the induction of cyclooxygenase-2 in MC3T3-E1 osteoblast cells in-vitro [28]. In a study by Saini et al. [29] in 2011, the effect of LFSS long with LIPUS on the regulation of synthesis of prostaglandins H2 (PGHS-2) and prostaglandin E2 (PGE2) was studied and it was found that PGE2 levels after exposure of shear stress significantly increased after 3 and 24 hours. Additionally, applying ultrasound waves for 20 minutes before shear stress significantly increases the levels of PGE2 and PGHS-2. These results show that some positive anabolic effects on bone tissue after exposure of ultrasound waves are due to

the change in prostaglandin expression. PGE2 is a potential inflammatory mediators that contribute to the migration and proliferation of mesenchymal stem cells [28]. In a study by Takayama et al., it was found that LIPUS had no effect on the division of rat osteosarcoma cells and the activity of alkaline phosphatase activity increased 7 days after exposure. LIPUS increased the expression of Runx2, Msx2, Dlx5 and Osterix genes and sialoprotein and decreased the expression of AJ18. Also, the mineralized nodule formation and calcium products in the mineralized nodules specifically increased on 14th day [30]. The results of treatment with LIPUS in the metatarsal of mouse embryos in in-vitro conditions showed the direct impact of LIPUS on osteoblasts and ossification of cartilage by stimulating cell activity or differentiation (not cell proliferation) [31,32]. Also, in some studies [33, 34], it has been found that ultrasound waves participate not only in the proliferation of osteoblasts, but also in their differentiation towards osteocytes in the bone healing and repair process. However, Korstjens et al., due to the lack of effect of ultrasound on cell proliferation, knew the stimulation of cell differentiation and calcified matrix production and not the change in cell division as the reason for stimulation effect of LIPUS on endochondral ossification [21]. In in vivo animal studies on the effects of ultrasound waves on fracture healing, it was found that healing callus mechanical properties has been enhanced; also, the waves resulted in greater bone bridging at the fracture site [9,17,35]. As a result of stimulation of ultrasound waves in an ulnar osteotomy fracture model in dogs, vascularity surrounding the fracture site increased [36]. The positive effect of ultrasound waves on fracture healing may be due to stimulation of various cellular processes involved in fracture healing and bone formation, including the processes of angiogenesis, chondrogenesis, endochondral and intramembranous ossification [9]. It has been observed that ultrasound waves and specifically LIPUS increase and or accelerate clinical fracture healing, but its exact mechanism is not known. In our study, the mean number of blood vessels in the control and experimental groups was 18.33±12.58 and 43.75±7.5, respectively that despite the increased vascularity in experimental group compared to the control group, there was no significant difference between the two groups ($P = 0.096$). In addition to bone tissue, in some studies, the effect of ultrasound waves on oral and periodontal soft tissues has been discussed. Shiraishi et al. [37], in their study in 2011, showed that LIPUS, in addition to the impact on ossification, accelerates soft tissue repair by increasing CCN2 / CTGF. Also, Ikai et al. [38], in their study in 2008, showed that LIPUS accelerates cement and mandible reconstruction after mucoperiosteal flap. Harle et al. [39], in their study on the effects of ultrasound waves on human osteoblast-like cells and periodontal ligament cells, expressed that ultrasound waves potentially have significant effects on functional activity of connective tissue cells that also may affect tissue regeneration and reconstruction process in vivo. However, in present study, no significant difference was observed between the two groups in terms of the formation of collagen fibers, inflammatory cell infiltration, callus formation, evidences of bone remodeling and presence of mature cartilage ($p>0.05$). As observed in present study, LIPUS could have no positive effect on bone healing process. Such results are consistent with the results of other studies. For example, in Griffin et al.'s review study of 12 randomized clinical trial articles with a total of 622 participants with 648 fractures, it was found that LIPUS had no significant impact during the treatment of acute fractures in adult humans. On the other hand, Leskinen et al. [41] showed that with the increased expression of some genes in osteoblasts, ultrasound waves don't significantly influence the genes involved in the osteoblast differentiation and their expression remains unchanged. In present study, it was found that despite some differences between the two groups, ultrasound waves cannot statistically have negative or positive effects on bone healing process, including the number of osteoblasts and osteoclasts, vascularity, the formation of collagen fibers, inflammatory cell infiltration, callus formation, evidences of bone remodeling and presence of mature cartilage.

According to the results, it is suggested to perform future studies on the effects of ultrasound waves both in-vivo and in-vitro and to compare the results. Also, it is suggested to perform the study on different exposure duration of waves to find the possible effects of this variable on bone and periodontal tissues. It is suggested to conduct future studies on the factors such as shear stress that may influence the effectiveness of ultrasound waves on bone tissue repair.

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