ORIGINAL RESEARCH

Effect of Novel Low-intensity Pulsed Ultrasound Stimulation on Accelerated Implant Osteointegration in Canine

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Abstract

Aim: To evaluate the effect of low-intensity pulsed ultrasound (LIPU) application on dental implant accelerated osseointegration in the two-stage implant protocol.

Materials and methods: A total of 20 implants were placed in 10 mature mongrel dogs, two implants for each dog replacing the lower 3rd premolars bilaterally. After 3 months of extraction, implants were placed. After 24 hours of implantation, the right sides received LIPU for 20 mins/day, continuously for 20 days. The left sides didn't receive LIPU (control). Standardized radiographs were taken before LIPU and after 10 and 20 days for both sides. After 20 days of LIPU application, second-stage surgery was performed and provisional crowns were placed on each implant. Implants were subjected to functional occlusal loading for 4 weeks. Standardized radiographs were taken after 2 and 4 weeks of loading and analyzed to assess the peri-implant bone density changes. All data were collected, tabulated, and statistically analyzed.

Results: All tissues appeared clinically normal, with the absence of inflammation and peri-implant radiolucency. The survival rate was 100%. The LIPU group showed a statistically significantly higher percentage increase in mean bone density after 10 LIPU sessions, 20 LIPU sessions, and 2 and 4 weeks of loading, than a control group.

Conclusion: The LIPU stimulation radiographically increased the bone density around implant area and accelerated osseointegration in the two-stage implant protocol.

Clinical significance: Low-intensity pulsed ultrasound stimulation could be beneficial in accelerating osseointegration and thus shortening the waiting period for final prosthetic delivery.

Keywords: Accelerated-osteointegration, Dental implant, Low-intensity pulsed ultrasound, Two-stage implant protocol.

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Introduction

Implant osseointegration is an essential metabolic and remodeling process involving bone tissues surrounding implant surfaces. Achieving stable osseointegration during the healing period is a prerequisite for successful dental implantation.[1]

The prosthetic part after placing an implant depends greatly on the healing capacity of the individual patient. The healing period following implantation was generally estimated to be approximately 5 months in the maxilla and 4 months in the mandible.[2] Current implant healing times before occlusal loading are derived from the original work carried out by Brånemark and associates. It was reported that loads placed upon a healing implant would result in fibrous encapsulation of that implant inhibiting osseointegration.[1,2] If there were a method that could shorten the period of osseointegration, patients could receive their prosthesis earlier. Till now, achieving firm, reliable, and accelerated osseointegration after placing a dental implant is still a clinical challenge.

Numerous studies have been published trying to improve the quality and accelerate osseointegration. For this purpose, altering the surface and/or shape of the implant, dual acid etching of titanium, tissue-engineering dental pulp cells on various implant surfaces and biomimetic implant coatings containing bone morphogenetic protein has been frequently investigated.[3] In addition, numerous trials have been published trying to enhance endogenous bone healing around biomaterials and use different forms of biophysical stimulations such as pulsed electromagnetic fields and low-intensity pulsed ultrasounds (LIPU) treatment. All previous trials aimed to accelerate bone fracture healing.[3,4]

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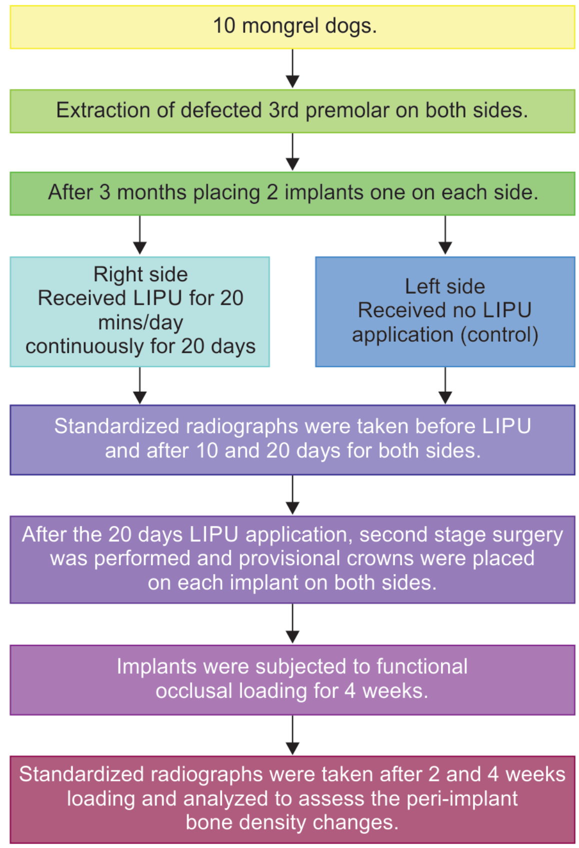
Ultrasound is a form of energy that is transmitted through biological tissues as high-frequency acoustic waves, which is widely used in medicine as a diagnostic, therapeutic, and operative tool.[5] One form of ultrasound signal has been hypothesized that, the micromechanical strains produced in biological tissues may result in biochemical events that could stimulate fracture healing and other osseous defects. This ultrasound signal consisted of a 1.5 or 3 MHz ultrasound wave pulsed at an intensity of ≤ 50 mW/cm^2 SATA (Spatial Average Temporal Average) applied for 20 minutes per day. This combination of parameters will be referred to as LIPU.[6] Low-intensity pulsed ultrasounds effects have been studied in various clinical studies for the acceleration of tibial fracture healing and adjunct for the treatment of closed or Grade I open tibial shaft fractures, nonunion fractures, and humorous fractures.[7–9] Jiang et al. have studied the osseointegration of dental implants in the maxillary first molars extraction sockets in mice and proved better osseointegration of the implant with LIPU.[10]

Low-intensity pulsed ultrasound treatment has been reported to be effective in liberating preformed fibroblast growth factors from a macrophage-like cell line (U937), stimulates angiogenesis during wound healing, stimulates anti-inflammatory process, enhances dental tissue formation, and enhances healing of orthodontically induced tooth root resorption.[3,6] Also, recent research shows that therapeutic ultrasound stimulates the expression of bone proteins (osteonectin, osteopontin, bone sialoprotein), which enhances bone healing after fractures and mandibular osteodistraction.[11]

Although numerous studies attempted to simulate shortening dental implant osseointegration time and to increase the osseointegration quality utilizing different methods, these studies evaluated implant osseointegration using the rabbit tibiae model or rat tibial model.[11,12] No publication up to the authors knowledge is available concerning LIPU treatment and dental implant osseointegration in maxillofacial bone involving functional occlusal loading in dogs. The effectiveness of LIPU in this hypothesis requires verifications firstly by animal experiments in which the optimal duration and frequency of the LIPU will be studied. The dogs as an experimental animal were selected because they have been extensively utilized in studies for the similarity of bone physiology with the human beings.[13,14] The present study was performed to evaluate the effects of LIPU treatment on bone osseointegration after implant placement and early occlusal loading into the dog mandible *via* clinical and radiographic evaluation (Flowchart 1). The Null hypothesis was the LIPU would not affect bone osseointegration.

Flowchart 1: Flowchart of study steps



Materials and Methods

Study Setting

This study was carried out in the experimental surgical laboratory at the Department of Veterinary Surgery at the Faculty of Veterinary Medicine, Cairo University, Giza, Egypt. The design of the study was recognized by the Ethics Committee for experimental studies at the Faculty of Pharmacy, Sinai University, Kantara, Egypt. With approval number: SUK.REC.2023/6-1. The study was conducted at the end of 2022 for 9 months duration. Implant containers was labeled; therefore, the surgeons were blinded to the implant side being chosen to do LIPU application.

Study Design

This study was designed according to the CONSORT 2010 clinical trial guideline. This is a randomized, controlled, single-blind (surgeon), split-mouth, experimental, clinical trial.

Sample Size Calculation

The software used for sample size calculation was PASS 15 (NCSS LLC, TX, USA), and the formula was a one-sample *t*-test. The calculated sample size (alpha was set to 0.05, target power was set to 80%). Therefore, 20 implants were included in the current study, 10 for each subgroup.

Care of the Experimental Animals

Ten healthy mature mongrel dogs were selected according to the following criteria: Inclusion criteria: (A) Males (B) 9–12 months old (C) Weighing between 12 and 15 kg (D) Healthy, active, and normally feeding (E) Free from any disease or worms (F) Had no previous operations (G) Needs to change his bilateral 3rd premolars. Exclusion criteria: (A) Aggressive or dominating dogs (B) Having fur insects, fungal infection, or skin disease (C) Any sign of animal abuse.

After primary selection, they were examined to rule out the presence of any disease and were kept under clinical observation for 3 weeks preoperatively and fed cooked meat, bread, milk, and water. The dogs were housed in separate cages, supplied with food and water, and allowed to live in optimal conditions in the Department of Veterinary Surgery at the Faculty of Veterinary Medicine, Cairo University. The cages were sprayed with 6/1000 neocidal diazinon. In addition, the dogs were injected with Ivermectin 0.1 mg/kg of body weight subcutaneously to guard against ecto-endoparasitic infestations repeated doses were injected periodically every 45 days.

Treatment Protocol

A total of twenty implants were placed in 10 dogs, two implants were placed for each dog replacing the lower third premolars bilaterally. The implant on the right side will be subjected to ultrasound treatment. While the implant on the left side will be left as a control. Single temporary crowns were placed above each implant. The dogs were allowed to eat for 1 month. Radiographs for each implant taken throughout the study (pre-ultrasound, after 10 times of ultrasound applications, after 20 times of ultrasound application, 2 weeks after loading, and 4 weeks after loading).

Preoperative Preparations

The night before the operation, food and water were kept away from the animals to prepare them for anesthesia. Animals were weighed to calculate the dose of the drugs to be used and to get a preliminary record of the general health condition of each dog. Atropine sulfate (The Egyptian Company for Chemicals and Pharmaceuticals, Egypt) was injected subcutaneously 10–30 minutes before the surgery in a dose of 0.05 mg/kg weight.

Induction of General Anesthesia

The anesthetic agent used was a mixture of Xylazine-HCL; 1 mg/kg body weight and ketamine HCL (5 mg/kg body weight). Using a 23 g intravenous cannula through the cephalic vein. Anesthesia was maintained through the operative time by venous drip of 500 mg thiopental sodium/500 mL dextrose 5% with drip rate (28–40 drops/minute).

Preoperative Impression Taking

Vinyl poly-siloxane impression material was used for taking upper and lower impressions, using custom-fabricated acrylic resin trays. Each impression was poured using Type IV extra hard stone three times; The first cast was for provisional crown construction, the second was for surgical stent, while the last was kept for reference.

Defected 3rd Premolars Extraction

Atraumatic extraction was performed to preserve the buccal, lingual, and lateral walls of alveolar sockets. Each extraction socket was irrigated thoroughly with sterile saline. The wound was closed using interrupted 3/0 chromic catgut.

Postsurgical Medications and Care

After extraction, ketorolac 1 mg/kg, tramadol 1.7 mg/kg, and buprenorphine 0.01 mg/kg were used to reduce pain. To prevent postoperative infection, a combination of penicillin strep and gentamicin sulfate 10% was injected every 12 hours for 7 days. Dogs were observed carefully until recovery. Each dog was fed a liquid diet for 2 weeks followed by a soft diet for 2 weeks. The extraction sites were allowed to heal for 3 months.

Preparation of Radiographic and Surgical Stents

Radiographic Stent

A vacuum-press stent was constructed on a waxed-up study model and filled with radiopaque material. A 4 mm stainless-steel ball of was used as a radio-opaque reference point and fixed with resin material. The occlusal film was used to allow a better view of the implant area. The height of bone at the prospective implant sites were estimated according to the following equation:

$$\text{Actual bone height} = \frac{\text{Actual ball diameter} \times \text{Radiographic bone height}}{\text{Radiographic ball diameter}}$$

Surgical Stent

The radiographic stent was used as a surgical stent to guide the pilot drill. In the proposed implant site, a hole 3 mm in diameter was made to accommodate the first drill.

Implant Insertion

After 3 months of healing, implants were placed to replace lower third premolars. Animals were prepared and anesthetized following the standard procedure with the same anesthesia as previously described.[15] Preoperative intraoral radiographs were taken. Using a Bard-Parker blade no. 11, a mucosal crestal incision was made from the distal of the second premolar to the mesial of the fourth premolar, and a mucoperiosteal flap was raised to expose the bone surface. The surgical template was placed on its position intraorally to allow the determination of proper implant positions.

Pilot Drill Preparation

Starting with a surgical round bur no. 2, a small indentation was done on the bone to allow stable drilling. A sharp pilot drill of 2.3 mm diameter (Surgical Drills: Spectra- System Implants, Implant direct LLC, USA.), was used in combination with 16:1 reduction contra angle hand-piece mounted on a micro-dispenser (Micro-despenser 3000, Nouvag). The drill was inserted through the stent to the center of the alveolar crest, using a speed of 14000 rpm; attention was taken to assure parallelism to the long axis of the teeth. Constant irrigation was imperative during the procedure.

The surgical stent was removed after marking the implant insertion site and the pilot drill was deepened gradually with the bur being brought in and out of the bone until reaching the required depth (8 mm). A paralleling pin was inserted to ensure parallelism with the long axis of adjacent teeth.

Enlargement of Pilot Drill

A twist drill 2.8 mm twist drill (Surgical Drills: Screwplant of Spectra-system, Implant Direct LLC, USA) was used to enlarge the pilot channels with a speed of 14,000 rpm. as maximum under sufficient irrigation. The implant a depth was checked using depth gauge and parallelism was rechecked with the paralleling pins.

The final drill (3.4 mm twist drill) was used to enlarge the channel with a low speed of 14,000 rpm. and copious irrigation. The depth and parallelism were finally rechecked.

The implant (Mini-threads textured by blasting with Soluble Blast Media of Hydroxyapatite), (Screwplant of Spectra- system, Implant Direct LLC, USA) length was 8 mm and 3.4 mm diameter was inserted in the prepared bone site under a low speed of 12000 rpm, insertion was completed with a 2.5 mm diameter hex tool.

Implant Level Impression

Double mix single step was employed using vinyl poly-siloxane impression material. Transfer implant-level impression techniques were selected due to a limited number of implants and for ease of the procedure. The implant well screws were cleaned, irrigated, and tightly sealed with the cover screw.

Postsurgical Care

The surgical wound was carefully debrided, copiously irrigated with saline, and was closed using interrupted 3/0 chromic catgut. Postoperative pain control was performed as described above. To prevent postoperative infection, dogs received a combination of 2 antibiotics (gentamicin and penicillin strep) for 7 successive days postoperatively. Dogs were kept on a soft diet (cooked meat, bread, and milk) for the first postoperative week. On the second postoperative week, they were able to eat the regular food.

Radiographic Exposure

Radiographs were taken for each side after implant placement. A specially fabricated and designed pure aluminum step wedge constructed to 15 steps of thicknesses 0.5, 1, 1.5, 2, 2.5 till 7 mm was attached and fixed on the film using double-faced stickers to act as a reference image on the radiographs for standardization of any processing variations during the developing of the films. Radiographs were taken while dogs were sedated and lying on their sides. The X-ray images were processed and interpreted.

LIPU Application

It started 24 hours after implant placement, on the right side of the mandibles of the dogs, while the left side was left as a control; A commercially available therapeutic ultrasound device was used for the ultrasound treatment (Prosound ULS-1000, Med Serve, UK). The device was set to transmit pulsed rate 1:3 ultrasound signals with 3 MHz operation frequency consisting of a burst width of 2 minutes with a repeating rate of 125 Hz, which produces 40 mW/cm^2 intensity.[16] Applied for 20 minutes periods/day (40, 51). Dogs were prepared and anesthetized with the same procedures described before. A specific coupling gel (Home Care Gel, Conductive Gel, China) was applied to the transducer head. The ultrasound transducer was placed touching the buccal surface of the mandible intraorally facing the implant site, with a circular movement, for 20 minute period. The 20 minutes sessions were repeated on a daily basis for 20 days. X-ray were taken at five times intervals; First X-ray: Before ultrasound application, (preoperative), (Immediate after implant placement). Second X-ray: After 10 ultrasound sessions. Third X-ray: After 20 ultrasound sessions. Fourth X-ray: After 2 weeks of implant loading. Fifth X-ray: After 4 weeks of implant loading.

Occlusal Loading

Construction of Provisional Crown

Soft tissue replica (Soft tissue mimic duplication material, Zhermack- Italy) material was injected around the implant analog in the impression; this is to reproduce gingival tissues which facilitate removal of the impression coping from the stone cast, the placement of subsequent abutment without breaking the stone and maintaining the references point of the soft tissue. All maxillary and mandibular impressions were poured using type IV extra-hard dental stone. The transfer was removed from the abutment by a disc. The comfort cap (Comfort Cap, ScrewPlant of Spectra- System, Implant Direct LLC, USA) was placed upon the abutment on the cast. This comfort cap was specially designed for each abutment by the manufacturer. It is made from a special plastic inert material, used as a provisional coverage for the abutment. A provisional crown was completed using a composite material Filtek Supreme, 3M ESPE, USA. The Composite material was filled in the impress of the lower 3rd premolar area in the Vacu-press stent previously formed. The filled stent was replaced upon the comfort cap on the abutment and light cured (Heliolux DLX, Ivoclar, Schaan, FL; 500 mW/mm^2 light intensity). The crowns were finished and polished.

Implant Loading

The day after implementation of the ultrasound therapy, anesthesia was administrated and the mucoperiosteum was punched and removed; using a 4 mm tissue puncher until the implant was exposed. The implant cover screw was removed, and the abutment was screwed to each implant. Composite crowns were cemented using glass ionomer and excess was removed before setting. (Fig. 1). The dogs were allowed to eat for 1 month their regular food. Other X-rays were taken for each implant after 2 and 4 weeks from the implant's loading (Fig. 2).

Fig. 1: Photograph of the cemented composite crown

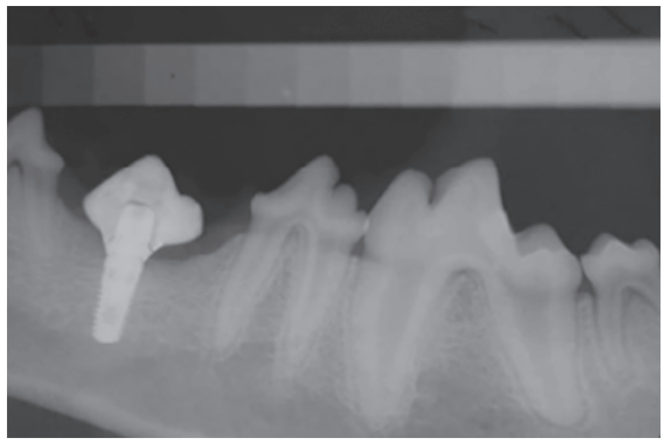


Fig. 2: Radiograph for the implant after 2 weeks occlusal loading

Methods of Evaluation

Clinical Evaluation

The follow-up period was every 2 weeks from implant placement till "two months" at the end of the study. The dogs were treated with a plaque control regimen that included implant cleaning three times per week using a toothbrush and dentifrice. Clinical evaluation included the general health condition of the dogs and intraoral examination to detect mobility of the implants and observation of any signs of inflammation or infections that may interrupt the normal wound healing.

Radiographic Evaluation

All radiographs for each implant taken throughout the study (pre-ultrasound, after 10 times of ultrasound applications, after 20 times of ultrasound application, 2 weeks after loading, and 4 weeks after loading) were digitized using a full-page scanner. After the images were digited, they were stored and processed by a computer. The stored images were projected onto a monitor.

Image Analysis

Digitized images were manipulated using specially designed software of the Digora system; by this software radiometric measurements were performed as follows:

Radiometric (Densitometric) Measurements (of the Region of Interest)

As an attempt to assess the peri-implant bone density changes around each of the studied implants, before and after therapy, a region of interest (ROI) was chosen just tangential to the bone-implant interface on the mesial and distal sides. This ROI was assessed radiometrically twice, once as a rectangle 2 × 2 in diameter, and another time in the form of three lines drawn parallel to each other and 1 mm apart starting just tangential to the bone-implant interface, the mean of the three lines was calculated. The means of the area and line measurements were pooled and included in further statistical analysis in an attempt to eliminate any localization measurement errors.

Radiometric Analysis of the Step Wedge

The procedure was repeated to record the mean gray levels of the standardized 2 × 2 mm area of each step of the used step wedge on each radiograph of every animal. Analysis and measurements were performed by the same researcher two times at two different sessions to eliminate the intraobserver errors and the means were then calculated.

Calculation of Bone Density

Using the calibration equation of the step wedge and the mean gray value of ROI, a corresponding gray value was calculated, to present the density of the defect using the following equation, all collected data were tabulated, statistically analyzed, and compared with that of the control group.

Statistical Analysis

Data were presented as mean and standard deviation values. Because this is a split-mouth study, paired *t*-test was used to compare the two sides (groups). Paired *t*-test was also used to study the changes by time within each group. Pearson's correlation coefficient (PCC) was used to determine the significant correlation between bone density. The significance level was set at $p \leq 0.05$. Statistical analysis was performed with statistical package for scientific studies (SPSS) 16.0 for Windows; Microsoft (version 365).

Results

Results of Clinical Evaluation

At the end of the study period, all tissues appeared clinically normal, with the absence of inflammation or suppuration. The gingival, which was generally pink in color, was not edematous and did not appear swollen.

The radiographic examination did not indicate any peri-implant radiolucency and the bone appeared to be in direct contact with all implants. All implants appeared to be integrated and were clinically stable (i.e., no mobility detected) after the 1 month loading period. The preimplant area was spectacularly good for both sides and all treated dogs. The clinical survival rate for implants placed was therefore 100%.

Results of Radiographic Evaluation

Comparison between Total Mean Bone Density for the Two Studied Groups

There was no statistically significant difference between mean bone densities of the preoperative and after 10 sessions. While, after 20 sessions of LIPU, 2 and 4 weeks of loading, the mean bone density of the LIPU group showed statistically significantly higher values than the control group (Table 1) (Fig. 3).

Table 1: Mean ± standard deviation (SD) values and results of paired *t*-test for total bone densities of the two tested groups

	LIPU	*Control*	
Period/Group	*Mean ± SD*	*Mean ± SD*	*p-value*
Preoperative	5.62 ± 1.3	5.14 ± 0.1	0.457
10 LIPU sessions	6.28 ± 1.2	4.61 ± 0.3	0.062
20 LIPU sessions	6.38 ± 0.6	5.00 ± 0.1	0.013*
2 weeks of loading	6.62 ± 0.4	5.23 ± 0.1	0.001*
4 weeks of loading (post-sacrifice)	6.90 ± 0.5	5.31 ± 0.1	0.002*

*Significant at $p \leq 0.05$

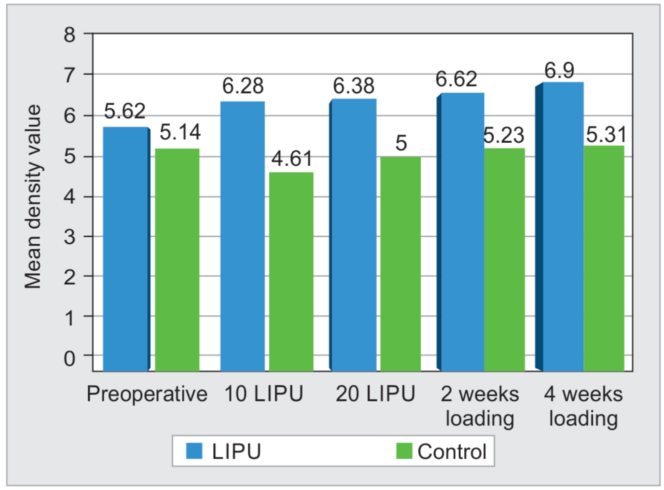


Fig. 3: Bar chart representing mean bone density of the two studied groups

Comparison between Percentage Changes in Mean Bone Density

Percentage changes in the total bone density of the two studied groups: The LIPU group showed a statistically significantly higher percentage increase in mean bone density than the control group during the following periods: After 10 LIPU sessions, 20 LIPU sessions, 2 and 4 weeks of loading, while through the other periods, there was no statistically significant difference between percentage changes in mean bone density of the two groups (Table 2) (Fig. 4).

Discussion

In the current study, certain procedural steps were taken to best fulfill the aim of the study. The dogs as experimental animals, were selected because they have been extensively utilized in studies for the similarity of bone physiology with human beings.[13,14] Another cause for selecting dogs was that the wide mouth opening for accessibility for the intervention and enabled the large head of the ultrasound device to reach the bone freely and allow some circular movement of the head during LIPU application following the manufacturer instructions.

The lower arch was selected for better bone quality while lower third premolars were selected for accessibility of surgery and ultrasound application. Lower third premolars were extracted bilaterally and allowed 3 months for complete healing before implant placement.[13,14] The implants were selected to be of 3.7 mm diameter for mesiodistal space suitability, and of 8 mm length according to the height of bone above the inferior alveolar canal by the aid of the used radiographic stent. The implants were placed bilaterally for comparison. The ultrasound parameters used in the study were, 3 MHz operation frequency which produces 40 mW/cm^2 intensity, pulsed 1:3 ultrasound signals (25% duty cycles).[17] The most common frequencies used in bone healing are either 3 or 1.5 MHz.[18] In the current study 3 MHz frequency was selected rather than 1.5 MHz due to its greater effect on superficial areas (absorbed more rapidly in the superficial tissues (2.5 cm depth) whilst the 1.5 MHz energy is absorbed less rapidly with progression through the tissues, and can therefore be more effective for deepest areas (5 cm depth).[19] That penetration of ultrasound is inversely proportional to its frequency. The low-intensity ultrasound signals used in this study were reported to stimulate bone repair and reduce healing times experimentally and clinically.[5,6] While high intensities (≥100 mW/cm^2) ultrasound has been reported to delay bone healing.[20] Since the pulsed signals of the ultrasound beam, decrease the average intensity of the output and reduce the thermal effects thus allowing for the non-thermal effects to occur.[21] Furthermore, it was previously found that high-intensity continuous-wave ultrasound was deleterious to fracture healing in animal studies, while low intensity (30 mW/cm^2) pulsed mode ultrasound signal did accelerate fracture healing.[22] In the present study, LIPU was applied for 20 minutes for 20 sessions daily applied starting on the second post-operative day after implant following the recommendation of Tanzer[17] who reported that the LIPU effect is optimum during the first 2–3 weeks of treatment.[23–25] Radiographs were taken after 10 and 20 sessions (in the current study). This protocol was chosen to throw some light on the efficiency of the exact number of LIPU applications for the acceleration of bone formation.

Table 2: Mean percentage change (%) ± standard deviation (SD) values and results of paired *t*-test for total percentage changes in bone density of the two tested groups

	LIPU	*Control*	
Period/Group	*Mean ± SD*	*Mean ± SD*	*p-value*
Preoperative – 10 LIPU sessions	14.2 ± 5.1	–10.3 ± 6.1	0.001*
Preoperative – 20 LIPU sessions	20.3 ± 8.8	–2.6 ± 1.3	<0.001*
Preoperative – 2 weeks of loading	24.6 ± 10.2	1.8 ± 0.9	<0.001*
Preoperative – 4 weeks of loading	29.8 ± 10.2	3.3 ± 1.9	<0.001*
10 LIPU sessions – 20 LIPU sessions	4.1 ± 1.5	7.8 ± 2.7	0.646
10 LIPU sessions – 2 weeks of loading	8.2 ± 2.7	11.7 ± 7.5	0.630
10 LIPU sessions – 4 weeks of loading	12.5 ± 7.7	15.5 ± 7.3	0.787
20 LIPU sessions – 2 weeks of loading	4.4 ± 1.8	4.5 ± 2.2	0.982
20 LIPU sessions – 4 weeks of loading	8.6 ± 2.4	6.1 ± 1.8	0.601
2 weeks of loading – 4 weeks of loading	4.2 ± 2.6	1.5 ± 0.6	0.136

*Significant at $p \leq 0.05$

For estimating the bone density around the implants, indirect digital radiography was used, where an aluminum step wedge was utilized as a reference image. The step wedge standard compensates for any processing variations. It could be considered an indispensable tool for compensating for any contrast density variations because its radiation absorption characteristics were similar to bone and it also provided a unit for quantitating the density of bone in the form of standardized aluminum equivalents.

Moreover, they stressed that bone scatters radiation to the same extent as aluminum. If only bone is to be investigated, aluminum would be the ideal material for constructing the step wedge Kribbs et al.[26]

The end of the follow-up period was chosen to be "2 months" post-implant placement. Interpreting the results from this study depends on the relationship between the dogs and human bone physiology. Differences in the mean value as well as the rate of bone turnover, have been observed between the two species. This value had been determined to be lower for dogs than for humans, meaning that bone healing response starts during the first week after implant insertion, peaks around 3–4 weeks, and arrives at a relatively steady state at 6–8 weeks after implant insertion.[27] For this reason, it was decided to stop comparing the X-ray at 2

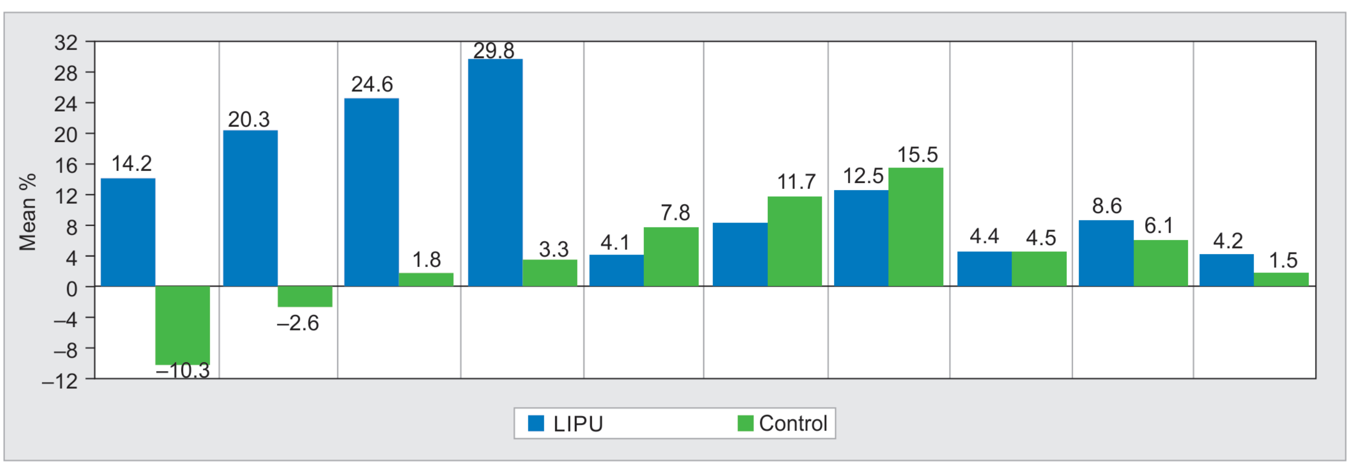


Fig. 4: Bar chart representing mean percentage change in bone density of the two studied groups

months. If X-ray measurement period extended up to 3 months, any differences in the implant-bone interface would have been masked by the greater rate of bone turnover in the animals. This is in accordance with the recommendation by Shigino et al. who found that the bone contact ratio of 1 month loaded implant after 18 times of capacity coupled electrical field (CCEF) application was not significantly different from the control group of 3 months loaded implant.[13]

All implants in all groups were immobile, with healthy peri-implant tissue, and no preimplant tissue infection was observed. These results go in accordance with many earlier studies.[27,28]

The results of the present study revealed a statistically significant increase in mean percentage change of bone density in the LIPU group than the control one throughout all the periods of the study, this result is in accordance with Erdogan et al.,[24] Tanzer et al.[17] and Ustun et al. in 2008.[11] Also, the results of the current study are consistent with the work of Tanzer et al.[17] who found an increase of about 18 % in bone ingrowth into fully porous titanium implants in the LIPU-treated group compared with non-treated, LIPU treatment had a greater effect in the first 2–3 weeks of stimulation. Bone density around the implant reflects the degree of compatibility and integration of the inserted implant under certain defined conditions, as reported by Ustun[11] who found that implants treated with LIPU treatment showed more resistance to torque removal forces on the 2nd, 4th, and 6th weeks than the control implants at the same time. They demonstrated that the higher resistance to removal torque forces indicates a better osseointegration. Azuma et al.[28] have shown that LIPU increases the rate of fracture healing at each stage of the process, with the biggest impact occurring when used throughout all the stages. The authors concluded that these data suggested that LIPU acts on cellular reactions involved in each phase of the healing process such as inflammatory reaction, angiogenesis, chondrogenesis, intramembranous ossification, endochondral ossification, and bone remodeling.[18]

However, the current results are in contradiction with Schortinghuis et al.[29,30] who demonstrated that LIPU has no stimulatory effects on bone healing in mandibular defects covered by osteoconductive membranes such as Expanded Polytetrafluoroethylene (E-PTFE). They attributed these results to two reasons, first enough ultrasound pressure did not reach the tissue behind the E-PTFE membrane. The EPTFE membranes used in this study are porous in nature. Due to this porosity, air may be trapped inside the membrane which, in turn, blocks ultrasound transmission. However, the second reason is that no significant ultrasound signals denoting therapy were detected. This may be due to the fact that the mandibles of rats were not responsive to the ultrasound signal, not even in the case of using the osteoconductive membrane which would not partially block the signal. This is an important finding because ultrasound does not necessarily stimulate bone healing in all circumstances. The main underlying mechanisms of osseointegration processes around implanted biomaterials are very similar to those occurring during bone fracture repair.[31]

Despite multiple studies in the field of biologic effects of the therapeutic ultrasound, the physical process through which low level ultrasound interacts with living tissue remains unknown. The difficulty in resolving this issue lies in the complex response of living tissue to these high frequency acoustic stimuli. On passing through the tissue, the ultrasonic energy is absorbed at a rate proportional to the density of the tissue. This differential absorption may play a critical role in targeting the ultrasound to the cells present inside and around the hard tissue (e.g., bone and teeth).[32] The mechanisms by which ultrasound may accelerate bone regeneration are still unclear and under research. Different mechanisms have been proposed in the literature: (A) LIPU is able to generate acoustic streaming and cavitation which have been shown to affect diffusion rates and membrane permeability. Changes in membrane permeability may result in an increase in micromechanical blood pressure which can accelerate fracture healing.[33] (B) LIPU possesses the ability to stimulate changes in tissues and cells may be due to the temperature increase, associated with energy absorption. Minimal heating effects (<10) may affect some enzymes which are very sensitive to small variations in temperature.[34] (C) LIPU uses a mechanical force to stimulate mechanical receptors, producing a biological response: During the use of LIPU, mechanical pressure waves transmit through the skin and soft tissue. When the signal reaches the injury site, these waves have a direct effect on the cells. Ultrasound causes movement in the extracellular matrix and the signal is detected by mechanical cell surface receptors which are called integrins. Integrins are found on a wide range of cells which are crucial to the healing process of fractures. Under normal static conditions, mechanosensitive integrins are in an inactive state. When stimulated by a mechanical force such as the low-intensity pulsed ultrasound signal, integrins are activated. Integrin-associated molecules such as Paxillin and F-actin cluster. This integrin-related signaling then initiates an intracellular cascade during which molecules that regulate gene expression are stimulated and move into the nucleus to perform their function. All the while, normal intracellular signaling, protein expression, and cellular behavior are enhanced.[35,36]

There is different biological effect of ultrasound many effects on living cells and tissues are known. These effects provide insights into the biological effects of ultrasound. It seems that the biological effect of ultrasound on bone is the result of a combination of physical and piezo-electric effects leading to cellular responses in which the cell membrane plays an important role.[30] The fracture healing ultimately depends on the activity of bone cells, especially osteoblasts. Some researchers have demonstrated that ultrasound stimulation increased the surface expression of integrins in osteoblasts and that long-term stimulation also enhanced osteoblastic differentiation and inhibited osteoclastogenesis. One study indicates that the cell population increased significantly when osteoblasts were treated with ultrasound.[37] It has been demonstrated that LIPU exposure increased (cyclooxygenase-2) mRNA expression which leads to an increase in PGE2 (prostaglandin E2) and plays an essential role in the osseointegration of dental implants.[38] Another research showed that LIPU stimulation led to significantly increased VEGF-A (Vascular Endothelial Growth Factor-A) mRNA and protein levels in human osteoblast cells.[39]

Low-intensity pulsed ultrasound was approved by the US Food and Drug Administration (FDA) as early as in 1994 and 2000 for accelerating fresh fracture healing and reconstitution of bone nonunion.[5]

At present, the clinical application of LIPUS in dental implantation is still in its infancy. However, according to the existing *in vivo* experimental studies and cell biology studies, authors speculate that LIPUS may have good application value in promoting the osseointegration of implants in future clinical practice.

In the present study, the ultrasound-stimulated implants demonstrated a 26.5% increase in bone density compared with their contralateral controls. Non-invasive low-intensity ultrasound had its greatest effect in the first 10 days. These results suggest that LIPU treatment promoted bone formation and accelerated the healing process at the bone-implant interface in a canine model. The null hypothesis was rejected.

However, this study has some limitations, first; lack of histological analysis. Second; additional clinical testing is advised.

The experimental results of the current study recommend the use of the noninvasive daily application of LIPU stimulation in conjunction with dental implant surgery. Especially in the initial post-implant placement period for the double-stage techniques.

A further recommendation is to the dental manufacturers who are invited to design and industrially produce appropriate LIPU apparatus with conveniently shaped small transducers to facilitate access and/or application intraorally to popularize the benefits of the technique to all patients due to receive dental implants.

Conclusion

Within the limitations of the present study, the following conclusions can be withdrawn:

- The LIPU stimulation radiographically increased the bone density of the peri-implant area.
- The greatest effect of the LIPU stimulation occurred in the initial ten-days period.
- The LIPU treatment shortened osseointegration waiting time in the two-stage operation.

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