Resonance frequency analysis of orthodontic miniscrews subjected to light-emitting diode photobiomodulation therapy

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SUMMARY The aim of this prospective experimental study was to evaluate the effect of light-emitting diode (LED) photobiomodulation therapy (LPT) on the stability of immediately loaded miniscrews under different force levels, as assessed by resonance frequency analysis (RFA). Sixty titanium orthodontic miniscrews with a length of 8 mm and a diameter of 1.4 mm were implanted into cortical bone by closed flap technique in each proximal tibia of 15 New Zealand white adult male rabbits (n = 30). The animals were randomly divided into irradiated and control groups under different force levels (0, 150, and 300 cN). OsseoPulse® LED device (Biolux Research Ltd.) 618 nm wavelength and 20 mW/cm² output power irradiation (20 minutes/day) was applied to the miniscrews for 10 days. The RFA records were performed at miniscrew insertion session (T1) and 21 days after surgery (T2). Wilcoxon and Mann–Whitney *U*-tests were used for statistical evaluation at P < 0.005 level.

It was found that initial primer stability of all miniscrews was similar in all groups at the start of the experimental procedure. Statistically significant differences were found for changes in implant stability quotient (ISQ) values between LED-photobiomodulated group and the control (0 cN, P = 0.001; 150 cN, P < 0.001; and 300 cN, P < 0.001). Significant increase was found in ISQ values of LPT applied miniscrews under 0 cN (+11.63 ISQ), 150 cN (+10.50 ISQ), and 300 cN (+7.00 ISQ) force during observation period. By the increase of force levels, it was determined that ISQ values decreased in non-irradiated control miniscrews. Within the limits of this *in vivo* study, the present RFA findings suggest that LPT might have a favourable effect on healing and attachment of titanium orthodontic miniscrews.

Introduction

Photobiomodulation is a current therapeutic approach in which exposure to low level laser (LLL) or light-emitting diode (LED) light is proposed to have beneficial effects on enhanced tissue regeneration and tissue growth, including effects on fibroblastic (Almeida-Lopes et al., 2001) and chondral (Schultz et al., 1985) proliferation, collagen synthesis (Majaron et al., 2000), wound healing (Lowe et al., 1998), and nerve regeneration (Anders et al., 2004). It is easy to perform and uses 30 equipments that can be used for several different orthodontic treatments in the clinical practice, such as accelerating tooth movement (Kawasaki and Shimizu, 2000), reduction of orthodontic post-adjustment pain (Lim et al., 1995), accelerating the process of bone regeneration during the consolidation phase after distraction osteogenesis (Hübler et al., 2010), or in the treatment of oral ulcers promoted by the fixed orthodontic appliances (Rodrigues et al., 2002).

LED radiation is a monochromatic red-to-near infrared (NIR) radiation (Karu *et al.*, 2005). Light in the NIR range 630–1000 nm generated by using LED arrays has been shown to improve retinal function in an animal model of

mitochondrial dysfunction (Eells *et al.*, 2004). The difference between LED and LLL radiation is that the latter is a laser with the characteristic of coherency; whereas LED light is not coherent, therefore expected to result in fewer side-effects (Karu *et al.*, 2005). LED radiation can also be produced at a lower cost compared to the LLL and it can safely be applied to a larger area of the body surface (Karu *et al.*, 2005). LED photobiomodulation therapy (LPT) has been shown to stimulate the intracellular production of adenosine triphosphate (ATP), particularly in cells that are ischaemic or wounded (Brawn *et al.*, 2008).

Temporary anchorage devices (TADs) have the advantages of low cost, simple surgical placement, and ease of removal. They are small enough to be placed in any space in the alveolar bone, even interdental areas (Lim *et al.*, 2009). Both clinical and experimental studies have demonstrated that these miniscrews were basically able to provide sufficient and stable anchorage for tooth movement during the entire time period of orthodontic therapy (Büchter *et al.*, 2005). However, although miniscrew stability should be ensured for clinical practice, they can loosen during treatment.

Unlike the standard dental implants used as orthodontic anchorage after osseointegration, miniscrews can be loaded immediately because stability is supported by the mechanical retention between the implant and the bone tissue (Umemori *et al.*, 1999). However, since magnitude, direction, and pattern of orthodontic force could influence stability, it is necessary to assess stability after stratifying the before and after orthodontic force applications to identify any associated factors. The success of any TADs in providing definitive anchorage depends on its stability. Failure rates of orthodontic TADs are varied between 3 and 19 per cent after loading (Cornelis *et al.*, 2007). Most clinical reports suggest that TADs are stable with applied forces ranging from 50 cN (Park *et al.*, 2001) to 450 cN (Kyung *et al.*, 2003).

It is important to ensure the initial stability of an orthodontic miniscrew because most failures occur during the initial stage (Cha *et al.*, 2010). Furthermore, stability of the miniscrew in the initial stage reduces its micromovement, thus allowing for an appropriate environment that supports the healing process surrounding the bone (Cha *et al.*, 2010). By histomorphometrical evaluation and energy dispersive X-ray microanalysis, Khadra *et al.* (2004) demonstrated that LLL applications had a positive effect on the functional attachment of titanium implants to bone. The irradiated implants showed a better bone attachment than non-irradiated controls. In a human model, Brawn *et al.* (2008) demonstrated more rapid achievement of dental implant stability as a result of the biostimulation effect of LPT.

To speed up the rehabilitation and progression process of orthodontic miniscrews under heavy forces is still a challenging and important clinical aim. Studies on the evaluation of the LPT effect on miniscrew stability under different load conditions were not found in the reviewed literature. The aim of this experimental study was to evaluate the effect of LPT on the stability of immediately loaded miniscrews under different force levels, as assessed by resonance frequency analysis (RFA). For the purposes of this study, the null hypothesis assumed that there is no significant difference between the orthodontic miniscrew stability of LED-irradiated and non-irradiated rabbits under different force levels.

Materials and methods

Implant designs

Screw-shaped titanium orthodontic miniscrews with a length of 8 mm and a diameter of 1.4 mm (14-JA-008H, Dual Tops; Jeil Medical Corporation, Seoul, Korea) were used in the current study (Figure 1)

Experimental animals

A total of fifteen, 8-month-old New Zealand white adult male rabbits weighing 3000–3500 g were used for the study.



Figure 1 Screw-shaped titanium orthodontic miniscrews with a length of 8 mm and a diameter of 1.4 mm (Dual Tops; Jeil Medical Corporation).

The rabbits were kept in standard cages, one in each, subjected to a 12 hour light-dark cycle at the constant temperature of 23°C. Pelleted rabbit diet (with normal nutritional levels) and distilled water were provided ad libitum. Permission was obtained from the Ercives University; Ethics Committee of Experimental Animals after the Research Scientific Committee at the same institution had approved the experimental protocol. The experiments were carried out in the Hakan Cetinsaya Experimental and Clinical Research Centre. The experiments were performed in accordance with the Animal Welfare Act of 20 December 1974, No. 73, Chapter VI Sections 20-22 and the Regulation on Animal Experimentation of 15 January 1996. Care was taken to avoid unnecessary stress and discomfort to the animal throughout the experimental period.

All procedures were performed under sterile conditions in a surgical operating room. The animals were sedated with an intramuscular injection of ketamine (10 mg/kg), atropine (0.06 mg/kg), and stresnil (0.03 mg/kg). In the areas exposed to surgery, 4 ml of local anaesthesia (2 per cent lidocaine with 12.5 mg/ml epinephrine, xylocain/adrenalines; Astra, Södertälje, Sweden) was injected.

All procedures for miniscrew insertion done by the same experienced surgeon (OE). A 3 cm incision was made on the proximal–anterior part of each tibia. The incision penetrated the epidermis, dermis, and the fascial layers. Lateral reflection of these tissues exposed the underlying periosteum. An additional medial–anterior incision was made through the periosteum. The periosteum was elevated and retained by a self-retaining retractor. When the tibial bone of the rabbit was used, four implants were placed in each animal, two in each proximal tibia (Figure 2). Two

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Figure 2 Corresponding implants inserted in a standard distance of 15 mm.

Table 1 Experimental design. LED, light-emitting diode.

Group		Sample size (miniscrew)			End of observation (days)
Group 1	5	10	0	(+)	21
	3			()	
Group 2		10	0	(-)	21
Group 3	5	10	150	(+)	21
Group 4		10	150	(-)	21
Group 5	5	10	300	(+)	21
Group 6		10	300	(-)	21



Figure 3 Orthodontic miniscrew stability was monitored using resonance frequency analysis (Ostell; Integration Diagnostics AB) device.

corresponding implants were inserted by prefabricated distance holders in a standard distance of 15 mm.

In the first and second groups, no force was applied on miniscrews. In the remaining four groups, after insertion, the screws were immediately loaded with transverse forces. The load was provided by Sentalloys (GAC, Grafelfing, Germany) superelastic tension coil springs, which develop a virtually constant force of 150 and 300 cN (Table 1). After coil application, the skin and the fascia periosteum were closed in separate layers with single resorbable sutures (Vicryl 4-0; Ethicon, Norderstedt, Germany). Subcutaneous antibiotic was administered (2.5 ml benzylpenicillin/dihydrostreptomycin; Tardomycels, Bayer Vital, Leverkusen, Germany) preoperatively and then once a day for 7 days.

LED photobiomodulation therapy

For LPT, OsseoPulse® LED device (Biolux Research Ltd, Vancouver, Canada) was used in the present study. The wavelength was 618 nm and the output power was 20 mW/cm². Irradiation was performed under general anaesthesia to ensure the immobility of the test animal for 20 minutes once a day. Treatment was initiated immediately after surgery and carried out daily for 10 consecutive days. A light probe delivered the LED beam and the irradiation was administered by placing the treatment array in light contact with the area to be treated. All irradiation procedures were applied at the same distance. Irradiations were done by the same operator (AE). After activation, the device was automatically deactivated when application time is over.

Clinical follow-up

The animals were inspected after the first few post-operative days for signs of wound dehiscence or infection and weekly thereafter to assess general health. Loading periods of 21 days were used for the miniscrews. At day 21 after RFA measurements, all corresponding miniscrews were removed from the tibia and the skin flap was closed primarily.

Methods of analysis

The orthodontic miniscrew stability was monitored using RFA (Ostell; Integration Diagnostics AB, Göteborg, Sweden) according to Meredith *et al.* (1996). The RFA records were performed at implant insertion session (T1) and 21 days after surgery (T2). At each measurement session, custom-made healing cap had been placed over the miniscrew in order to provide connection and analyse stability values by Ostell device (Figure 3). To avoid excessive torque moments and thus loosening of a miniscrew, a standardized torque of 10 Ncm was applied with a torque-controlled ratchet when connecting the transducer (Smart Peg; Integration Diagnostics AB) to the miniscrew. RFA produced an implant stability quotient (ISQ) value according to a mathematical relation, which was recorded five consecutive

times on each miniscrew at every time interval. The primary outcome value was the change in ISQ from the mean baseline measurement for each miniscrew. All measurements were carried out by one blinded investigator (HA).

Statistical analysis

All data were analysed with the statistical package for social sciences, 15.0 (SPSS for Windows; SPSS Inc., Chicago, Illinois, USA). The normality test of Shapiro–Wilks and Levene's variance homogeneity test were applied to the response variable ISQ (with values between 0 and 100 like a percentage). The data were not normally distributed and there was no homogeneity of variance between the groups. Thus, the statistical evaluation was performed using non-parametric tests. Descriptive statistics were given as quartiles [25th, 50th (median) and 75th]. All baseline values and inter-group differences were compared by Mann–Whitney *U*-test. Intra-group changes during observation period were evaluated by Wilcoxon test. When the *P*-value was less than 0.05, the statistical test was determined as significant.

Results

The baseline ISQ values of all groups were compared and found no statistically significant differences (Table 2). All

60 miniscrews could be inserted with high primary stability (range of median ISQ values: 49.25–55.00). Thus, it is accepted that initial primer stability of all miniscrews was similar in all groups at the start of the experimental procedure.

Descriptive values and statistical comparisons of ISQ changes during observation period under different force levels in control and LPT groups were shown in Table 3. According to Wilcoxon paired samples test, statistically significant changes were determined in ISQ values of LPT group under 0 cN (P = 0.007), 150 cN (P = 0.005), and 300 cN (P = 0.005) force levels. ISQ values of all miniscrews were found higher than the baseline values in all LPT groups at the end of the observation period. Miniscrews in the control group showed lower ISQ values than the baseline, at the end of follow-up period; but only statistically significant decrease in ISQ values were found under 300 cN force (P = 0.005).

Changes in ISQ values of LED-irradiated and control miniscrews under different force levels were compared by Mann–Whitney U-test and demonstrated in Table 4 and Figure 4. Statistically significant differences were found for changes in ISQ values between LPT group and the control (0 cN, P = 0.001; 150 cN, P < 0.001; and 300 cN, P < 0.001). Significant increase was found in LPT applied miniscrews under 0 cN (+11.63 ISQ), 150 cN (+10.50 ISQ),

Table 2 Descriptive statistics and comparisons of baseline ISQ values in both groups (control and LED-photobiomodulated). LED, light-emitting diode; ISQ, implant stability quotient.

Group	n	Force level (cN)	Photobiomodulation application	Quartiles (ISQ values)			P-value
				25th	50th (median)	75th	
Group 1	10	0	LED (+)	50.25	52.50	59.75	0.74
Group 2	10		LED (-)	51.63	54.25	56.75	
Group 3	10	150	LED (+)	47.75	49.25	55.75	0.53
Group 4	10		LED (-)	46.63	55.00	60.25	
Group 5	10	300	LED (+)	48.00	55.00	59.00	0.58
Group 6	10		LED (-)	47.25	53.75	56.38	

Table 3 Descriptive statistics and comparisons of changes in ISQ values during observation period under different force levels in both groups (control and LED-photobiomodulated). LED, light-emitting diode; ISQ, implant stability quotient.

Groups	n	Force level (cN)	Photobiomodulation application	Baseline (T1) ISQ values			End of observation period (T2) ISQ values			Wilcoxon test, <i>P</i> -value
				25th	50th (median)	75th	25th	50th (median)	75th	
Group 1	10	0	LED (+)	50.25	52.50	59.75	53.75	65.00	69.00	0.01
Group 2	10		LED (-)	51.63	54.25	56.75	49.13	52.75	57.50	0.84
Group 3	10	150	LED (+)	47.75	49.25	55.75	57.38	60.50	65.25	0.01
Group 4	10		LED (-)	46.63	55.00	60.25	42.75	50.00	58.50	0.28
Group 5	10	300	LED (+)	48.00	55.00	59.00	58.00	65.50	69.25	0.01
Group 6	10		LED (-)	47.25	53.75	56.38	40.50	45.75	53.25	0.01

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Table 4 Descriptive statistics and inter-group comparisons (control and LED-photobiomodulated groups) of changes in ISQ values under different force levels. LED, light-emitting diode; ISQ, implant stability quotient.

Group	n	Force level (cN)	Photobiomodulation application	Difference (T2–T1) quartiles (ISQ values)			Mann–Whitney <i>U</i> -test, <i>P</i> -value
				25th	50th (median)	75th	
Group 1	10	0	LED (+)	12.00	11.63	11.00	0
Group 2	10		LED (-)	1.50	-1.38	3.38	
Group 3	10	150	LED (+)	9.50	10.50	10.00	< 0.001
Group 4	10		LED (-)	1.00	-6.00	-2.50	
Group 5	10	300	LED (+)	5.25	7.00	8.00	< 0.001
Group 6	10		LED (-)	-4.88	-9.13	-11.50	

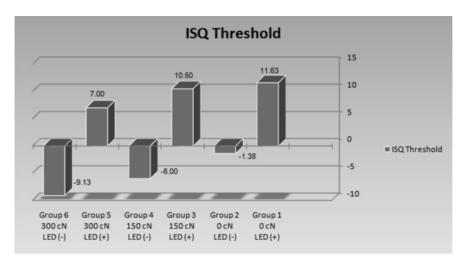


Figure 4 Changes in implant stability quotient (ISQ) values of light-emitting diode (LED) irradiated and non-irradiated control miniscrews under different force levels.

and 300 cN (+7.00 ISQ) force during observation period. By the increase of applied force levels (0–300 cN), ISQ values of miniscrews in control group decreased. Thus, according to increased stability values in LPT group, the null hypothesis of this study was rejected.

Discussion

Many therapeutic alternatives are being studied to promote cell biostimulation and improving the regenerative capacity (Lim et al., 1995; Lowe et al., 1998; Kawasaki and Shimizu, 2000; Majaron et al., 2000; Anders et al., 2004; Khadra et al., 2004; Brawn et al., 2008; Hübler et al., 2010). Hence, photobiomodulation therapy applied by LLL or LED is considered an alternative for the treatment of clinical conditions that require tissue regeneration. In this blinded controlled experimental study, we investigated the effect of LPT with an OsseoPulse® LED device on improving or accelerating the attachment of orthodontic miniscrews. To our knowledge, this is the first study in orthodontics that shows the positive effect of LPT on attachment and stability of orthodontic miniscrews in bone.

The wavelength delivered by LED in the present study is close to that used in LLL (600–1000 nm) with similar energy (Eells *et al.*, 2004). It has been shown that both the LED and the LLL radiation result in photobiomodulation effects (Eells *et al.*, 2004; Karu *et al.*, 2005). LED radiation can be produced at a lower cost compared to the LLL and it can be safely applied to a larger area of the body surface with less side-effects and higher efficacy.

The animal experiment seems to be a useful method for estimating the tissue reactions to the bioactive material (Khadra *et al.*, 2004). Different animal models have been described to evaluate the bone healing procedure. Swennen *et al.* (2002) indicated that rat, rabbit, cat, dog, sheep, minipig, and monkey could be used as an animal model to study the effects of mechanical stimulation on bone. However, the results obtained from an experimental animal model cannot necessarily be extrapolated to humans.

We used in the surgical procedures a closed flap technique. It differs from the clinical procedure in which no closed flap is used (Büchter *et al.*, 2005). In a routine clinical practice, depending on the localization, the miniscrews are either inserted through attached gingiva or the screw head is

covered again with mucosa letting only the wire or coil spring penetrate the mucosa (Büchter *et al.*, 2005). In any case, there is an increased infection risk compared with a closed flap technique thus in turn increasing the risk for screw failure. To eliminate this infection risk, we used the closed flap technique in the present study.

Additionally, in the present study, the load was provided by Sentalloys (GAC) superelastic tension coil springs, which develop a virtually constant force. Thus, we thought that present flap design has no adverse effects on the force system.

Miniscrew stability was measured by using the RFA device together with the wireless SmartPeg attached to the custom-made head of the miniscrew. The technique was utilized without contact and was non-invasive. The RFA device created a variable frequency magnetic pulse and measured the resonance frequency of vibration of the miniscrew to arrive at the ISQ value, which was indicative of primary stability. ISQ is scaled from 1 to 100; the higher the ISQ value, the more stable the miniscrew (Sul et al., 2002). This technology was proven to be capable of characterizing alterations in implant stability during early healing and is sensitive enough to identify differences in longitudinal implant stability based on bone density at the implant recipient site (Barewal et al., 2003). The technique has been demonstrated to be an accurate method for early assessment of osseointegration (Huang et al., 2003). It has been found that ISQ measurements show a high degree of repeatability (<1 per cent variation for individual implants; Meredith et al., 1996).

The significantly wider range in the ISQ values shown by the miniscrews over time might be explained by unscrewing of the miniscrew during the early healing period on installing the transducer. All the miniscrews, however, were stable at all time points and no movement was detected while performing the measurements. At the baseline, it was determined that all miniscrews could be inserted with high primary stability and accepted that initial stability of all was similar in experimental and control groups.

In a previous qualitative report (Ohmae *et al.*, 2001), loading was suggested to increase bone—metal contact. Although bone—implant contact was shown to be slightly increased in loaded screw implants compared with unloaded ones (Ohmae *et al.*, 2001), none of the studies that evaluated this phenomenon found statistically significant differences in the osseointegration index (Deguchi *et al.*, 2003). At the end of follow-up period, we observed no significant difference between the ISQ values of loaded and unloaded implants. This finding was not in accordance with Woods *et al.* (2009) that indicate immediate loading could be beneficial due to the fact that only unloaded control miniscrew implants showed mobility.

The changes in miniscrew stability expressed by ISQ value differences over time might reflect the biologic events associated with the bone–miniscrew interface. In the current

study, ISQ values of all miniscrews were found higher in LPT group at the end of observation period. By the application of LPT, ISQ values of all miniscrews were become higher than the baseline values under different loading levels. These results are consistent with a previous research showing an increase in retention of laser-treated implants in the rabbit model (Khadra *et al.*, 2004) and from the implant study, which demonstrated the acceleration of stability of LED-treated dental implants compared to controls (Brawn *et al.*, 2008).

Regarding to these findings, the authors of this study assumed that the application of LPT can stimulate bone regeneration around orthodontic miniscrew and increase stability. During the insertion of miniscrews, normal sequence of initial resorption next to the surface of the miniscrew followed by new bone deposition may be improved in the control, and when LED treatment is applied after placement, this initial resorption phase may be eliminated and miniscrews may achieved greater stability sooner.

The mechanism by which LPT promotes bone formation is not fully understood. The absorption of laser and LED photons by the respiratory chain enzyme Cytochrome c oxidase (Hawkins and Abrahamse, 2006) is response from increasing of ATP production. Cytochrome c oxidase allows a better cell function especially in cells with a suboptimal metabolic condition. ATP accelerates mitoses; improves tissue repair; stimulates bone repair; balances the production of fibroblasts, with normalization of collagen and elastic fibbers deposited in the repairing tissue; and increases peripheral blood circulation, improving anti-inflammatory action and tissue healing (Kawasaki and Shimizu, 2000). Photobiostimulation is the energy deposited in tissues that produces stimulation effects that release substances, such as serotonin, histamine, and bradykinin, and, furthermore, activates the production of arachidonic acid, converting prostaglandins into prostacyclin (Angeletti et al., 2010). Because osteoblasts may be recruited along the bone edges from undifferentiated precursor cells around the miniscrew, LED irradiation could potentially stimulate the recruitment and/or maturation of osteoblasts.

In an animal study, Doi (2006) applied continuous forces to miniscrews for 6 weeks and found that the success rates of 6 mm miniscrew implants loaded with 300 or 600 g were similar. He did not use any photobiomodulation therapy. This suggests that force levels simply must be low enough not to crush the surrounding bone before stability is affected.

In most of the long-term clinical studies, implant failures have been attributed to overloading or excessive loading when no peri-implantitis phenomena were present (Büchter *et al.*, 2005). Most of the implants losses were considered to be the result of excessive strains and stresses at the bone/implant interface (Adell *et al.*, 1981). Studies by Isidor (1996, 1997) revealed that excessive lateral loading of

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conventional osseointegrated implants resulted in a high risk for the loss of osseointegration. The fact that the degree of loading has a direct influence on the stability of implants confirms the superior influence of loads generated by function. The present study shows that all the miniscrews installed into tibial bones of rabbits were successfully loaded and remained stable throughout the entire duration of the study in photobiomodulation and control groups under different force levels (0–300 cN). This finding is in agreement with earlier studies (Roberts *et al.*, 1989, 1994) demonstrating that implants remained stable without any biostimulation when subjected to tip forces ranging from 100 to 300 cN.

However, from a different perspective, it was determined that statistically significant lower ISQ values detected at the end of observation period in control miniscrews under 0 cN (-1.38 ISQ change), 150 cN (-6.00 ISQ change), and 300 cN (-9.13 ISQ change) forces. It can be speculated that loading may induce microfractures in the surrounding bone and decreased the stability. We thought that positive changes in ISQ values under loading might be a result of the biostimulation effect of LPT. In a recent study, Brawn *et al.* (2008) also demonstrated more rapid achievement of dental implant stability as a result of the biostimulation effect of LPT in a human model.

The ability to characterize the tissue miniscrew interaction is an important tool to clarify the biological mechanisms of LPT. Therefore, tissue–miniscrew interactions should also be evaluated in a histological perspective and cell culture system in order to characterize the cellular responses of irradiated osteogenic cell lines to orthodontic miniscrew surfaces.

Although other studies, such as the effects of different irradiation dosages, the prolonged use of irradiation, or both, on tooth movement, implant stability, and bone remodelling are still required for clinical use consideration, the introduction of LPT on miniscrew stability under different load conditions (0–300 cN) seems feasible and may be of great therapeutic benefit to the success rate of orthodontic TADs. When compared to osseointegrated dental implants, the ISQ values of orthodontic miniscrews were not high enough and that therefore statistically interpretation of the values should be done with great concern.

Conclusions

Within the limits of this 21 days *in vivo* study, the results of this investigation have demonstrated that orthodontic miniscrews that irradiated by LED photobiomodulation had greater stability than the non-irradiated control miniscrews; at 21 days, post-screw placement had greater stability in cortical tibia bone of rabbit. LPT might have a favourable effect on healing and attachment of titanium orthodontic miniscrews.

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