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ORIGINAL ARTICLE

Effect of photobiomodulation on the stability and displacement of orthodontic mini-implants submitted to immediate and delayed loading: a clinical study

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Abstract

The aim of this study was to evaluate the effect of photobiomodulation (PBM) on the stability and displacement of orthodontic mini-implants (MIs) submitted to loading. Forty-eight and 35 mini-implants (1.5 × 8 × 1 mm) were assessed for stability and displacement, respectively (19 patients). MIs were allocated according to the intervention in 1—PBM + immediate loading (IL), 2—PBM + delayed loading (DL) (four weeks after implantation), 3—IL only, and 4—DL only. PBM (Therapy XT, DCM) was implemented using a red emission (660 nm, 4 J/cm^2, 0.1 W, 20 s) immediately after implantation (day 0) and infrared emissions (808 nm; 8 J/cm^2, 0.1 W, 40 s) in the following appointments every 48–72 h during two weeks (days 2, 4, 7, 9, 11, and 14). Loading of 150 gF was applied during three months for all MIs. The stability was assessed by resonance frequency analysis (Osstell ISQ), and images from Cone beam computed tomography were evaluated to determine the amount of the displacement of the MI's head. MIs from the PBM groups presented lower loss of stability ($P = 0.0372$). When the analysis considered the loading protocol as an additional variable, group two showed the lowest loss of stability, being significantly different from groups that did not receive PBM ($P = 0.0161$). There was no difference between groups two and four during the period without loading ($P > 0.05$). DL groups presented lower loss when the effective period of loading was assessed, independently of the application of PBM ($P < 0.0001$). All groups showed displacement of the MIs head without significant differences ($P > 0.05$). DL potentiated the effect of PBM, decreasing the loss of stability.

Keywords Bone screws · Low-level light therapy · Cone beam computed tomography · Orthodontics

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Introduction

Orthodontic mini-implants (MIs) are temporary anchorage devices widely used to support load intended for orthodontic movement of teeth [1]. Advantages of MIs will be exploited if they are maintained at the implantation site without mobility. A few years ago, it was thought that endosseous anchorage devices remained absolutely stationary when loading was applied; however, it is now known that MIs can lose stability [2] and be displaced [3]. It has been established that physiological repair processes and a consequent decrease in bone density, caused by surgical injury during implantation, could favor these events [4].

The bone around MIs could significantly increase its density only 3 months after implantation [5]. Because of this, some authors suggest that some weeks of repair time are needed before applying load on MIs [6, 7]. Although, immediate loading protocols are widely recommended in clinical practice [1], the literature on this subject still remains unclear. It has been demonstrated that the stability of MIs is subject to changes during the repair process and, that after an initial decrease, the stability values remain stable only after the fourth week [7].

Photobiomodulation (PBM) has been proposed to improve the stability of MIs [8–11]. This therapy utilizes non-ionizing light sources in the visible and near-infrared spectrum promoting non-thermal biological processes over tissues [12, 13]. Light emissions used for PBM have the ability to scatter into tissues stimulating wound healing, among other benefits [14]. PBM promotes an increase of the vascularization; modulation of the inflammatory processes; a proliferation of fibroblasts, keratinocytes, chondrocytes, and osteoblasts; and cytokine expression that induce matrix synthesis, improving the bone repair process [14–18]. Therefore, this therapy was successfully used, for example, for midpalatal suture regeneration after rapid maxillary expansion or to enhance osseointegration after dental implant's insertion [19, 20].

Currently, there are only a few studies, mainly in the animal model, describing the effect of PBM on the stability of MIs [8–11, 21]. Although with different methodologies, results seem to be promising, suggesting PBM as a clinical adjuvant to improve the success of devices [21, 22]. Therefore, we hypothesized that PBM therapy could contribute to an enhancement of stability and reduction of the displacement of MIs and that a delayed loading could be more beneficial to improve the effect of PBM. The aim of this study was to evaluate the effect of PBM on these outcomes. The influence of the loading protocol, as modifying factor of the PBM effect, was also assessed.

Materials and methods

Participants' screening and sample definition

The research ethics committee of the School of Dentistry of Ribeirão Preto, University of São Paulo, approved the protocol of this study (56108316.0.0000.5419). Patients in treatment from the graduate clinic in Orthodontics at the School of Dentistry of Ribeirão Preto, University of São Paulo, were assessed for eligibility. Inclusion criteria are presented in Table 1. Participants and/or caregivers were invited to participate and informed consent was obtained before interventions.

MIs were considered sample units for posterior analyses. A sample size calculation was performed in the statistical software SAS 9.2 (SAS Institute Inc., Cary, NC, USA). Considering the data reported by Alves et al. [23] regarding the displacement of MIs (mean = 0.78 mm, SD = 0.43), a formula was implemented considering a study power of 80% and a 95% confidence interval, for an estimation of displacement's reduction by 50% (0.39 mm) using PBM. Calculations resulted in 22 MIs per group (PBM vs. no PBM). Considering an anticipated 10% dropout rate, at least 48 MIs would be necessary.

Allocation of groups and placement of mini-implants

MIs were divided into two groups according to the application of PBM. Each group was subdivided according to the loading protocol received, in the following way: 1—PBM + immediate loading (IL), 2—PBM + delayed loading (DL), 3—IL only, and 4—DL only. It was not possible to comply with adequate randomization due to the impossibility of some patients to receive the entire PBM protocol (duration of two weeks). Therefore, a quasi-experimental design was followed (non-random allocation). Patients were allocated depending on their possibility to comply with the protocol. It was not a split-mouth study; thus, all MIs placed in each participant would receive the same PBM intervention. On

Table 1 Selection criteria

Inclusion criteria
Permanent dentition
Planning of use of MIs in the buccal upper and/or lower posterior regions
MIs that would receive a measurable loading
Adequate oral hygiene
Exclusion criteria
Systemic and/or oral diseases
Use of medication
Consumption of alcohol or tobacco in a chronic way

MIs: Orthodontic mini-implants

the other hand, those MIs could be randomly allocated to receive different loading protocols.

Self-tapping MIs (1.5 × 8 × 1 mm; Ti-6AL-4 V alloy; Conexão, Arujá, SP, Brazil) were inserted on buccal-attached gingiva with a manual instrument through the pilot hole made by the previous lanceting. All devices were inserted by the same operator (FLR) in a blinded way.

Interventions

Application of PBM

Laser emissions were applied over MIs' head, covering tissues around devices. The diameter (8 mm) of the irradiated area (0.5 cm^2) was based on the sum of the approximate amount of displacement towards each side that devices could suffer [3] and the MI's diameter (Fig. 1a). A diode (GaAlAs) laser equipment (Therapy XT, DCM, Kearny, NJ) was used for the application of PBM therapy. Red laser emission (energy density 4 J/cm^2) was applied immediately after the insertion of mini-implants (day 0). In the following appointments, the same laser equipment was regulated to apply infrared emissions (energy density 8 J/cm^2) every 48–72 h during two weeks (days 2, 4, 7, 9, 11, and 14). A self-constructed device (0.9-mm SS' wire) was adapted to the equipment's tip to standardize the distance between the light source and tissues, and consequently, the irradiated area size (Fig. 1b). Equipment's potency was confirmed before laser irradiation (Powermaster/field meter, Coherent, Auburn, CA). The laser set up is detailed in Table 2.

Application of the loading protocol

Standardized continuous loading of 150 gF was applied on MIs using elastomeric chains for three months. Force magnitude was measured by a precision tensiometer (Zeusan, Campinas, SP, Brazil) and renewed every two weeks. Two types of orthodontic mechanics were included in the study (space closure mechanics and molar intrusion mechanics with anchoring in MIs), as long as the applied load is standardized. MIs on groups one and three received loading at the day of implantation (immediate loading), while MIs on groups two and four received load only four weeks after implantation (delayed loading). Intervention protocols are shown in Fig. 2. Patients received prophylaxis on each control and they were instructed to follow an appropriate oral hygiene protocol (leaflets).

Outcomes' assessment

Stability

Stability assessment was performed by resonance frequency analysis (RFA). A self-constructed and validated adapter was used for allowing the junction between the RFA transducer and MI's head (Fig. 3). Stability values (ISQ—implant stability quotient) were recorded in two directions (towards apical and distal) using a resonance frequency analyzer Osstell ISQ (Osstell, Göteborg, Sweden). Three measurements were performed for each direction, finally using only the mean value.

Stability was assessed immediately after implantation (T_0), four weeks after implantation and before loading for groups two and four (T_1), and after three months of loading (T_2) (Fig. 2). Unique previously calibrated evaluator (GAMV) performed all analyses.

Displacement

For displacement analysis, cone beam computed tomography (CBCT) scans were obtained using an Orthopantomograph OP300 equipment (Instrumentarium, Nahkelantie, Tuusula, Finland) with the following set up: regional scans (FOV 6 × 8 cm) with 120 kV, 5 mA, 0.4-mm voxel size, 40 s of scanning and standard resolution. DICOM files were rendered into a volumetric image using Avizo software (Avizo 8.1.1,

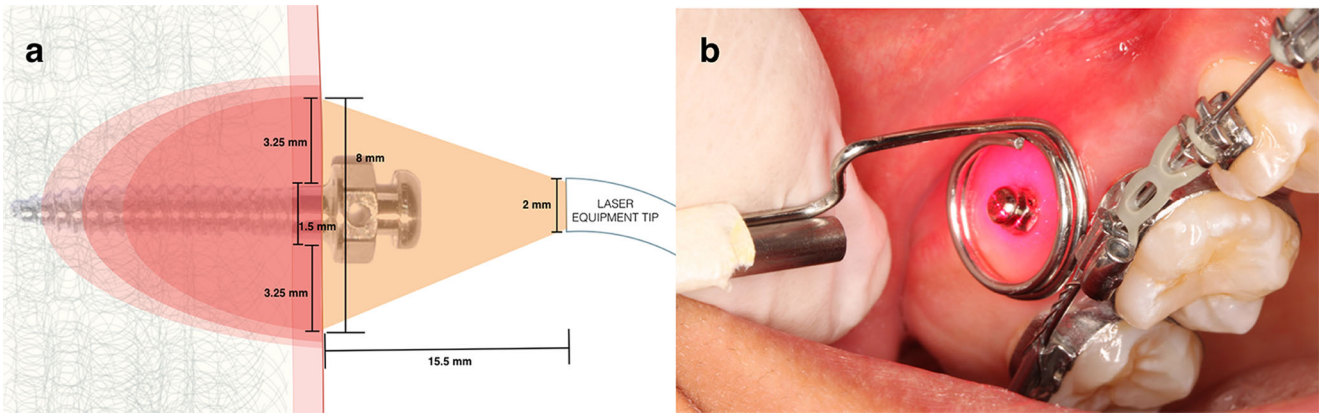

Fig. 1 **a** Diagram showing the diameter of the irradiated area and standardized light-source distance. **b** Laser irradiation using self-constructed device adapted to the laser equipment

Table 2 Laser set up

	Immediate postoperative period	Mediate postoperative period
Clinical factors		
Energy density (J/cm^2)	4	8
Power density (mW/cm^2)	0.2	0.2
Irradiation time (s)	20	40
Irradiated points	1	1
Irradiation method	Noncontact	Noncontact
Rate irradiation	Uniform	Uniform
Number of applications	1	6
Physical factors		
Wavelength (nm)	660 (± 10)	808 (± 10)
Emission	Continuous	Continuous
Power (W)	0.1 (± 20%)	0.1 (± 20%)
Diameter of optical fibers (um)	600	600
Spot size (beam diameter on tissue) (mm)	8	8
Irradiated area (cm^2)	0.5	0.5
Source-tissue distance (mm)	15.5	15.5

Visualization Sciences Group, Burlington, MA), in which the 3D and slice visualizations were respectively accomplished using isosurface and orthoslice functions, in order to locate defined landmarks (Table 3, Fig. 4). Mathematical three-dimensional reference planes were constructed based on them.

For analysis of MIs that mainly received an anteroposterior load vector (space closure mechanics anchored in MIs), frontal plane was used as reference (using PNS, RIH, LIH; and, RMF, LMF, LF on the maxilla or mandible, respectively; Table 3), whereas for those that mainly received a vertical load vector (molar intrusion mechanics anchored in MIs), axial plane was used as reference (using RPO, LPO, OIF; and, LF, RIDO, LIDO on the maxilla or mandible, respectively; Table 3). Orthogonal distances (mm) from the MI's head to the selected reference plane were determined (Fig. 5). CBCT scans were performed at two times: TC_0—immediately after implantation and TC_1—after three months of loading (Fig. 2). Differences between the two distances were calculated to indicate the amount of displacement (mm) (Fig. 5). One evaluator (GAMV) performed all analyses and he was blinded to the intervention received for each MI.

Statistical analysis

Statistical analyses were performed using two-tailed tests ($\alpha = 0.05$) on SAS 9.2 (SAS Institute Inc., Cary, NC) or SAS callable SUDAAN 11.0.0 (RTI International, Research Triangle Park, NC) when necessary. Descriptive statistics was used to present characteristics for each study group. Parametric and non-parametric analyses were performed as appropriate for specific distributions of data, taking into consideration individual clustering. Adjustment for age, sex, and site of implantation was performed for the outcome analyses. When a statistical difference between groups was evidenced, post-test (false discovery rate) was applied for multiple comparisons. Test assumptions were verified for all analyses.

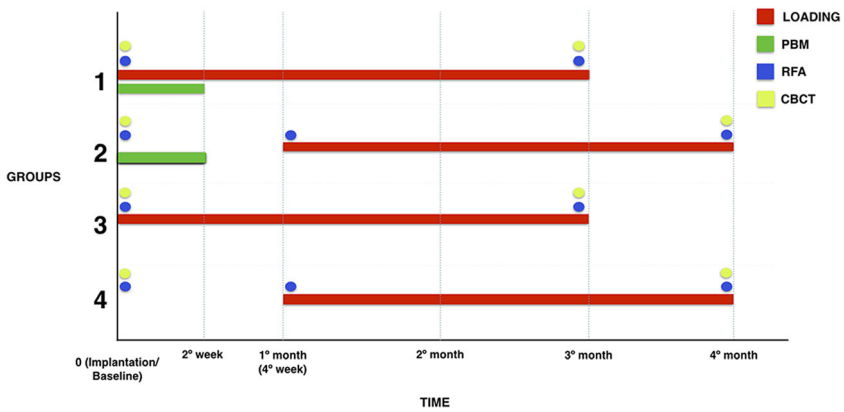

Fig. 2 Intervention protocols. PBM: photobiomodulation; RFA: resonance frequency analysis; CBCT: cone beam computed tomography

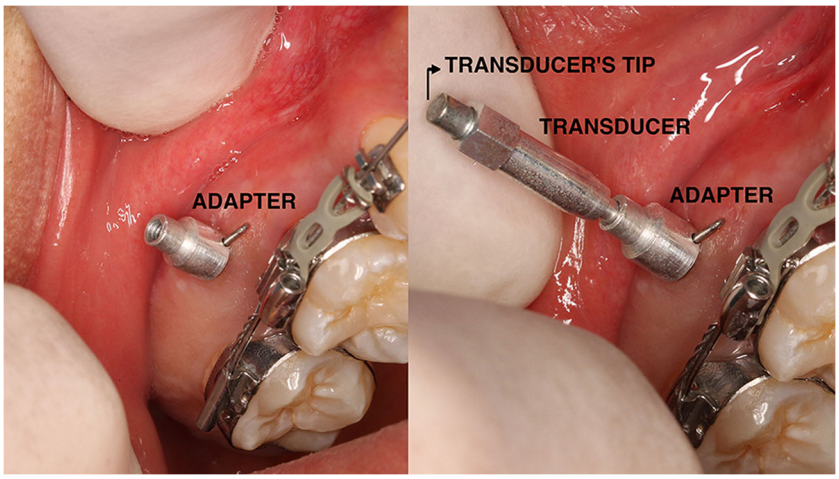

Fig. 3 MI-adapter-transducer system

Reliability of the assessment methods was analyzed by the intraclass correlation coefficient (ICC) by a single evaluator (GAMV). The reliability of the RFA method using the self-constructed adapter was assessed in an in vitro model. Repeated stability measurements (two-week interval) of 30 MIs inserted in artificial bone models showed high reliability ($r = 0.997$; $P < 0.0001$). For the method for identification of landmarks in CBCT, 10 images were randomly selected (five for maxilla and five for mandible) which were evaluated three times (one-week intervals). Repeated measurements also showed high reliability; ICC ranged from 0.792 to 1 ($P < 0.0001$) for all landmarks in the maxilla and mandible.

Results

Fifty-eight MIs started the study. Ten MIs did not continue during the follow-up (four were lost, and six missed the follow-up control). Group four (DL) presented greater loss of MIs (three devices). Only 35 were evaluated for displacement due to the presence of artifacts making the analyses difficult.

The descriptive statistics is presented in Table 4; no statistically significant differences were found between groups according to the sex, age, and implantation site. MIs from the PBM groups presented lower loss of stability ($P = 0.0372$) (Table 5). When the analysis considered the loading protocol as an additional variable, group two (PBM + DL) showed the lowest loss of stability, being significantly different from the groups that did not receive PBM ($P = 0.0161$). There was no difference between groups two and four (DL groups) in the repair period without loading (from T_0 to T_1), but the group two (with PBM) presented lower values of loss of stability. When the effective period of loading was evaluated (three months for all the groups), groups two and four (with DL) showed the lowest loss of stability, independently of the received PBM intervention. Group two (PBM + DL) remained with the lowest loss values (Table 6). Regarding displacement, no significant difference was observed among the groups (Tables 5 and 6).

Discussion

Although it has been suggested in animal studies, the positive effect of PBM on bone repair around MIs [9–11], favoring stability [10] and mechanical retention [8]; controlled clinical studies are still needed to determine its effect not only on stability but also on displacement in the medium and long term. We conducted a quasi-experimental study. Despite not having complied with randomization, groups did not present significant differences according to the sex, age, or implantation site. In any case, the statistical analysis considered covariates adjustment, with the aim of reducing the risk of bias due to the lack of randomization.

The present study did not follow a split-mouth design. Although this is often used for controlled studies with PBM, it can produce false negatives due to systemic effects that this therapy produces [24, 25]. Regarding irradiation protocol, it is difficult to compare studies about the exact mechanism of action of PBM in bone tissue and to establish a correct dose due to different experimental models. Dosimetry characteristics on the present study were based on that better tissue regeneration results are obtained when cells are irradiated with energy of 1–4 J, with intervals of 48 h in initial stages. A literature review that analyzed 137 articles concluded that positive biological responses are observed with energy densities up to 10 J/cm^2 [17]. Our protocol used a dose of 4 and 8 J/cm^2 for the laser irradiation on the day of implantation and for the mediate postoperative period applications, respectively. Because the irradiations were not simultaneous, the laser dose that tissues received always remained within the desired parameters.

Our PBM protocol included the application of two laser wavelengths at different moments. Red emission on the day

Table 3 Landmarks defined on CBCT images

Anatomic/mini-implant landmarks		Landmark definition	Identification of landmark on CBCT image section		
			Axial	Coronal	Sagittal
Maxilla	Posterior nasal spine (PNS)	Tip of the sharp posterior end of the nasal crest of the hard palate.	Mid-posterior point	Inferior-most point	Posterior-most point
	Right palatine orifice (RPO)	Middle-inferior point of right palatine orifice (entrance of the right posterior palatine canal).	Middle-most point	Middle-inferior-most point	Middle-inferior-most point
	Left palatine orifice (LPO)	Middle-inferior point of left palatine orifice (entrance of the left posterior palatine canal).	Middle-most point	Middle-inferior-most point	Middle-inferior-most point
Mandible	Right inferior hamulus (RIH)	Middle-inferior most point of right medial pterygoid process.	Middle-inferior-most point	Inferior-most point	Inferior-most point
	Left inferior hamulus (LIH)	Middle-inferior most point of left medial pterygoid process.	Middle-inferior-most point	Inferior-most point	Inferior-most point
	Oral incisive foramen (OIF)	Middle-inferior most point of oral orifice of the nasopalatine canal.	Middle-most point	Middle-most point	Middle-inferior-most point
	Right mental foramen (RMF)	Middle-lateral most point of the right mental foramen.	Middle-lateral-most point	Middle-lateral most point	Middle-most point
	Left mental foramen (LMF)	Middle-lateral most point of the left mental foramen.	Middle-lateral-most point	Middle-lateral most point	Middle-most point
	Lingual foramen (LF)	Middle-superior most point at the entrance of lingual orifice of the lingual canal.	Middle-posterior most point	Middle-most point	Middle-posterior most point
	Right inferior dental orifice (RIDO)	Middle-superior point of right orifice of the inferior dental canal.	Middle-medial most point	Middle-medial most point	Middle-most point
	Left inferior dental orifice (LIDO)	Middle-superior point of left orifice of the inferior dental canal.	Middle-medial most point	Middle-medial most point	Middle-most point
Mini-implant	H point (Head)	Middle-exterior most point on the head of the device.	Middle-exterior most point	Middle-exterior most point	Middle-most point

of implantation was applied based on the following: (1) it has been reported that tissues surrounding screws, even well-positioned, present mild inflammation [26]; (2) inflammation has been suggested to prevent normal healing in the early period after insertion [27]; (3) orthodontic literature has been stated that inflammation affects the success of MIs [28]; (4) it has been demonstrated that PBM has an inhibitory effect on the inflammatory process [29]; and (5) red emission has been previously reported for this purpose [30, 31]. Therefore, a laser wavelength of 660 nm was applied to improve the immediate postoperative period, aiming to accelerate the repair of the recently injured mucosa and favor the modulation of the initial inflammatory process. On the other hand, infrared emissions were applied on the next days based on the following assumptions: (1) a decrease in the bone density occurs after a surgical injury [4]; (2) the bone repair process around MIs may take a few months [5]; (3) the stability of these devices is subject to changes during this period [7]; (4) it has been demonstrated that PBM favors the bone repair process and partial osseointegration of dental implants on the mediate postoperative period [20]; and (5) higher wavelengths penetrate tissues in greater depth than visible red light [32]. For these reasons, infrared laser emissions of 808 nm were applied in order to reach the bone tissue of the depth around MIs and accelerate the repair process. Besides that, different energies of 2 and 4 J were delivered to the tissues in the immediate and mediate laser application periods, respectively.

This is a novel laser protocol, so further research should be performed to confirm our findings. The laser equipment used does not allow selecting different modes of laser emission. On the other hand, the manufacturer does not provide the Gaussian profile mode of the emission for the equipment. We assume that the equipment emits a conventional laser beam represented by a Gaussian profile with a typical symmetric bell-shaped curve. In that case, the laser energy emitted would be concentrated in the center of the beam. Unfortunately, this was not possible to be confirmed.

It is important to mention that the center of the laser beam was applied over the MIs head and that the peripheral regions of the beam are the ones that acted in the tissues surrounding the device. Therefore, we hypothesize that, probably, the effect size of PBM could be higher if a "top-hat shaped" or "multi-mode transverse" modes are used, guaranteeing more homogeneous radiation across the beam profile. Considering that the irradiated area was partially occupied by the MI head, the real dose received by the tissues was lower than planned. We suggest testing different strategies including irradiation of more points around the mini-implants in further studies.

Regarding stability of MIs, a few studies using PBM and evaluating the stability [10, 21, 33, 34], by the RFA or percussion test, have been reported. Omasa et al. [10], in an animal study, found an improvement in the stability of MIs, evidencing decreased PTV values (assessed by the Periotest method) after

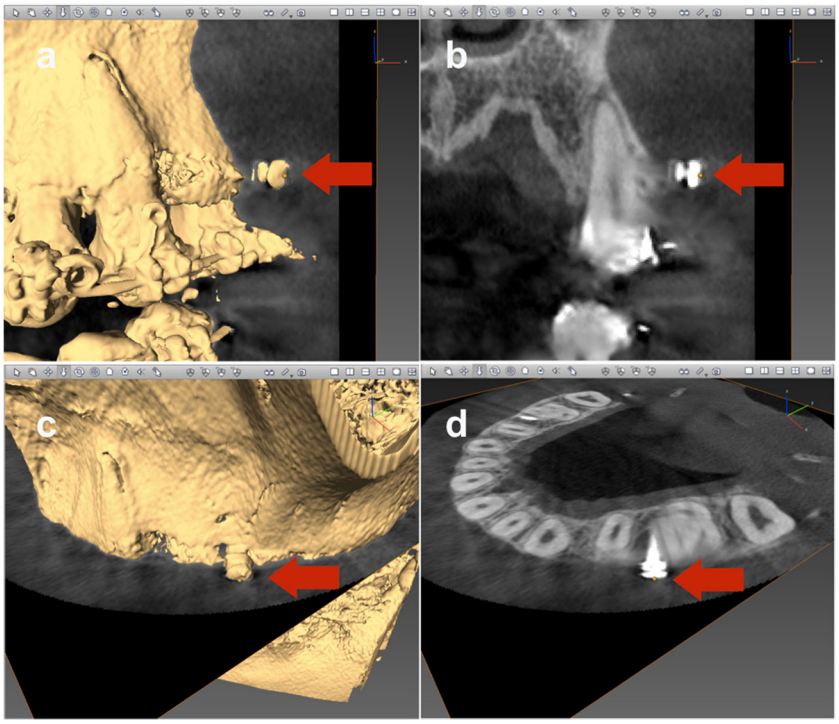

Fig. 4 Spherical marker on MI's head. **a** and **b** Confirmation of landmark position, 3D visualization, and coronal CBCT slice using isosurface and orthoslice functions, respectively; **c** and **d** The same procedure assessing axial CBCT slice

35 days of implantation on the group that received PBM. Osman et al. [21] in an RCT, using the same evaluation method (Periotest), demonstrated that the group treated with PBM lost less stability when compared with the control group, although this difference was not statistically significant. Our results confirmed these findings. PBM did not add stability to devices for any of the groups evaluated (ISQ values during the follow-up for all groups were always lower than the initial values); however, when comparing the loss of stability, groups receiving PBM showed a lower loss. The difference found has clinical relevance due to the tendency of values to worsen during follow-up. Considering that MIs are most often used for periods longer than three months, loss of stability should be monitored and controlled.

Uysal et al. [33] found stability gains in MIs placed on rabbit tibias when LED was applied. Similarly, Ekizer et al. [34] in an RCT reported small gains in MIs' stability when LED was applied with load application and slight losses when therapy was not applied during a three-month follow-up. Although changes in ISQ values were small, the difference between groups was statistically significant when compared in the second and third follow-up months. These results are

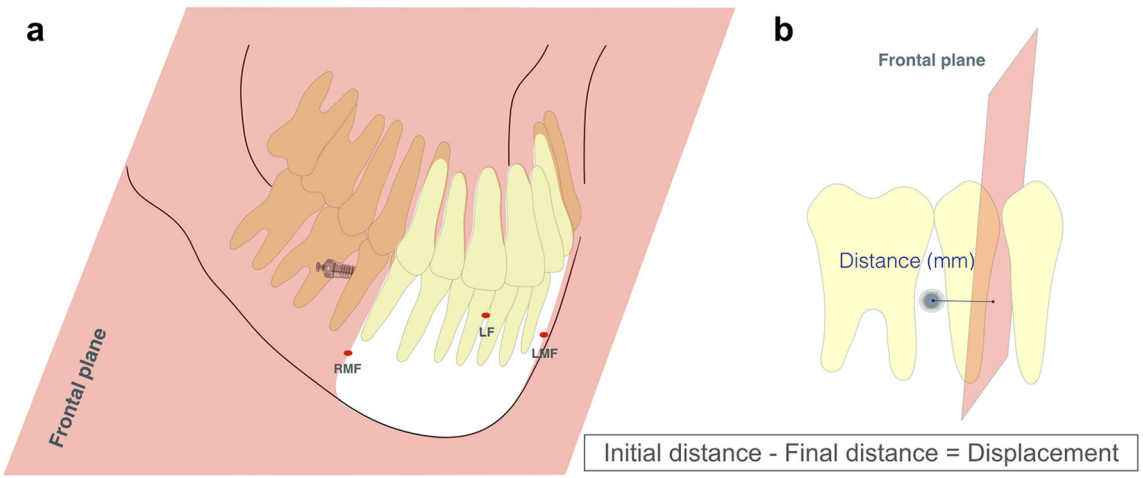

Fig. 5 Diagram explaining method for displacement measurement. **a** Reference frontal plane (passing through the RMF, LMF, and LF landmarks) to evaluate the anteroposterior displacement of a MI placed in the mandible. **b** Distance from the MI's head, in orthogonal direction, to the reference plane

Table 4 Groups' characterization

	Group 1 (PBM + IL)	Group 2 (PBM + DL)	Group 3 (IL)	Group 4 (DL)	*P* value
Assessed for stability					
MIs (*n*)	12	12	12	12	
Sex *n* (%)					
Male	3 (25)	4 (33.3)	2 (16.7)	3 (25)	0.7910
Female	9 (75)	8 (66.7)	10 (83.3)	9 (75)	
Age					
Mean (SD)	16.4 (6.8)	16.2 (4.3)	21.6 (13.6)	15.0 (3.5)	0.6609
Median (Q1–Q3)	15 (12.5–15)	15 (13.5–18.5)	18 (14–20)	15 (12–15)	
Implantation site *n* (%)					
Maxilla	8 (66.7)	5 (41.7)	7 (58.3)	4 (33.3)	0.3255
Mandible	4 (33.3)	7 (58.3)	5 (41.7)	8 (66.7)	
Assessed for displacement					
MIs (*n*)	6	8	12	9	
Sex *n* (%)					
Male	1 (16.7)	2 (25)	2 (16.7)	0	0.4132
Female	5 (83.3)	6 (75)	10 (83.3)	9 (100)	
Age					
Mean (SD)	13.2 (1.5)	15.6 (4.2)	21.6 (13.6)	15.0 (4.1)	0.2225
Median (Q1–Q3)	12.5 (12–15)	15 (12–18.5)	18 (14–20)	13 (12–15)	
Implantation site *n* (%)					
Maxilla	5 (83.3)	5 (62.5)	7 (58.3)	3 (33.3)	0.2363
Mandible	1 (16.7)	3 (37.5)	5 (41.7)	6 (66.7)	

PBM photobiomodulation, *IL* immediate loading, *DL* delayed loading, *SD* standard deviation, *Q1* quartile 1, *Q3* quartile 3

Chi-square or Kruskal Wallis tests were used for distributions or means comparisons

contradictory with those presented in our study because although slight differences were found between the groups, there was always a loss of stability.

As previously mentioned, it has been demonstrated that the stability of MIs is subject to changes during the repair process [7]. Considering that the bone repair process around MIs may take a few months [5], we assumed that the moment of the initial application of loading could influence the outcomes assessed and modify the effect produced by the PBM therapy. We aimed to assess whether loading (a stress situation around devices) applied together with or after PBM would influence our results. In that sense, the magnitude of the loading was standardized for all devices, rather than the direction of the loading or the type of orthodontic mechanics used.

Table 5 Loss of stability and displacement of mini-implants according to the application of PBM

	PBM (groups 1 and 2)	No PBM (groups 3 and 4)	*P* value
Loss of stability (ISQ values)			
MIs (*n*)	24	24	
$T_2 - T_0$ md (SE)	−7.0 (0.5)	−8.6 (0.6)	0.0372*
Effective time of loading† md (SE) (3 months of loading)	−5.8 (0.4)	−7.0 (0.6)	0.0954
Displacement (mm)			
MIs (*n*)	14	21	
$TC_1 - TC_0$¥ md (SE)	0.75 (0.2)	0.73 (0.1)	0.9356

PBM photobiomodulation, *ISQ* implant stability quotient, *md* mean difference, *SE* standard error

† T_2-T_0 for groups 1 and 3; T_2-T_1 for groups 2 and 4

¥ Absolute values for mean differences are reported

*Statistically significant difference

Individual clustering was considered

Regression analysis adjusted by age, sex, and implantation site was used

Table 6 Loss of stability and displacement of mini-implants according to the application of PBM and loading protocol

	Group 1 (PBM + IL)	Group 2 (PBM + DL)	Group 3 (IL)	Group 4 (DL)	*P* value
Loss of stability (ISQ values)					
MIs (*n*)	12	12	12	12	
T_1–T_0 md (SE)	–	−2.4 (0.3)	–	−3.1 (0.4)	0.1146
T_2–T_0 md (SE)	−7.8 (0.8)^{ab}	−6.2 (0.5)^{a}	−8.7 (0.7)^{b}	−8.5 (0.6)^{b}	0.0161*
Effective time of loading† md (SE) (3 months of loading)	−7.7 (0.7)^{a}	−3.9 (0.3)^{b}	−8.7 (0.7)^{a}	−5.4 (0.6)^{c}	<0.0001*
Displacement (mm)					
MIs (*n*)	6	8	12	9	
TC_1-$TC_0$$^{¥}$ md (SE)	0.56 (0.2)	0.88 (0.3)	0.95 (0.1)	0.46 (0.2)	0.1379

PBM photobiomodulation, *IL* immediate loading, *DL* delayed loading, *ISQ* implant stability quotient, *md* mean difference, *SE* standard error

† T_2–T_0 for groups 1 and 3; T_2–T_1 for groups 2 and 4

$^{¥}$ Absolute values for mean differences are reported

*Statistically significant difference

Different letters show statistically significant difference between groups (false discovery rate)

Individual clustering was considered

Regression analysis adjusted by age, sex, and implantation site was used

Only one animal study used photobiostimulation and different magnitudes of loading on MIs [33]. The authors concluded that when loading applied on devices was greater, the stability gain of devices that received LED therapy was lower and the loss of stability in the control group was greater. Although, using a different methodology, we also demonstrated that load could modify the photostimulatory effect. PBM alone may not significantly influence the stability of MIs. This is supported by the comparison between groups two and four from T_0 to T_1 without loading intervention. Conversely, results showed that the PBM group with application of DL presented a lower loss of stability, both from T_0 to T_2 and when it was assessed only the effective period of loading. DL potentiated the effect of PBM. We assume that PBM therapy and DL are interventions that act synergistically to achieve this goal. Probably, the stress caused by loading decreases the effect of PBM on bone repair around MIs.

Regarding displacement analyses, although CBCT has been shown to be a more accurate method for evaluating MIs positioning, the high-radiation dose and cost are disadvantages that still prevent its routine use. The American Academy of Oral and Maxillofacial Radiology recognizes this method as being clinically useful in identifying optimal site location for positioning of MIs; however, it gives certain recommendations for the reduction of the radiation dose [35]. In the present study, CBCT was used to assess the amount of displacement of MIs. We believe that the use of CBCTs in the evaluated period would also offer benefits to the patient regarding the monitoring of the position and relation of the devices with adjacent structures. With a radio-protector point of view, we based our procedures on the ALARA principle ("as low as reasonably achievable"), and although the exact radiation dose was not calculated, we used regional images with reduced FOV avoiding high resolutions. For this reason, it was not possible to perform more sophisticated superposition methods.

There were no significant differences between the groups. Group three had the highest values of displacement. Contrary to expectations, group two also presented a large amount of displacement, although it presented the lowest loss of stability for the present study. As previously reported in the literature, we assume that PBM enhances the bone condition around mini-implants, maybe by stimulation of partial osseointegration [9–11]. We hypothesize that this improvement did not have an impact on the amount of displacement of devices probably due to that MIs could displace through the alveolar bone by remodeling processes guided by mechanical load exerted on these devices [36], maintaining the bone integrity around MIs. It is important to mention that the follow-up time was only three months. Considering that MIs are mostly used for longer periods of time, it is possible that PBM has only a long-term effect on the displacement of these devices. Methodological limitations could also have affected these results. The absence of statistical difference between the groups for this outcome could be due to a type 2 error, where a possible real effect of the intervention is denied because the calculated sample size was not reached.

Results presented in the literature concerning the effect of PBM on the stability of MIs should be carefully evaluated due to the great variability of methodological designs and PBM protocols used. Additional basic biology investigations are still necessary to determine the real effect of this therapy on

tissues around mini-implants. We recommend carrying out further investigations to design shorter protocols that focus on the delivered doses and adequate selection of the emission Gaussian profile. Similarly, further clinical studies with larger sample sizes and longer follow-up time should be provided to increase knowledge on this subject. Present results cannot be generalized for different types of MIs or for long-term follow-up greater than 3 months with loading.

Conclusions

Delayed loading potentiated the effect of the photobiomodulation therapy. The mini-implants that received these two interventions together presented the lowest loss of stability. Neither photobiomodulation therapy nor the loading protocol influenced the displacement of devices.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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