Human Interleukin-1β Profile and Self-Reported Pain Monitoring Using Clear Aligners with or without Acceleration Techniques: A Case Report and Investigational Study

Selma Pascoal,1,2 Aline Gonçalves,1,2 Andreia Brandão,1 Duarte Rocha,1 Sofia Oliveira,2,3 Francesca Monteiro,2,3,4 Oscar Carvalho,2,3 Susana Coimbra,5,6 and Teresa Pinho1

1UNIPRO—Oral Pathology and Rehabilitation Research Unit, University Institute of Health Sciences (IUCS), CESPU, Porto, Portugal
2Center for Microelectromechanical Systems (CMEMS), University of Minho, Campus Azurém, Braga, Portugal
3LABBELS—Associate Laboratory, Braga, Guimarães, Portugal
4ICVS/3B’s—PT Government Associate Laboratory, Braga, Guimarães, Portugal
5UCIBIO, REQUIMTE, Laboratory of Biochemistry, Portugal
6TOXRUN—Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, Gandra, Portugal
7IBMC—Instituto Biologia Molecular e Celular, I3S—Inst Inovação e Investigação em Saúde, Universidade Do Porto, Porto, Portugal

Correspondence should be addressed to Teresa Pinho; teresa.pinho@iucs.cespu.pt

Received 21 June 2022; Revised 10 August 2022; Accepted 11 August 2022; Published 31 August 2022

Copyright © 2022 Selma Pascoal et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction. There is a growing demand for more aesthetic, comfortable, and faster orthodontic treatments, and clear aligners emerged as a solution to fulfill this need. However, the effectiveness of clear aligners to treat complex malocclusions is yet contentious. The use of acceleration methods could improve the efficacy of clear aligners by stimulating cells’ mechanobiology through numerous pathways, but this hypothesis is still poorly explored. Objective. We aimed to monitor the release profile of an inflammatory marker—the interleukin-1β—and to evaluate its relationship with self-reported pain scores with and without the use of acceleration techniques during an orthodontic treatment requiring difficult tooth movements with clear aligners. Case Report. Here, we report a case of a 46-year-old female patient who presented functional and aesthetic complaints. Intraoral examination revealed a diminished overjet and overbite, rotation of teeth 45 and 24, absence of teeth 25, 35, and 36, buccolingual dislocation of tooth 21, a tendency to a Class III malocclusion, and a 2 mm left deviation of the lower midline. This study is divided into three stimulation phases: no stimulation, mechanical vibration stimulation, and photobiomodulation. Interleukin-1β levels in gingival crevicular fluid samples from the pressure side of six selected teeth were evaluated at four time points after the orthodontic treatment onset. Pain monitoring in those teeth was performed using a visual analogue scale at the same time points. Results. Interleukin-1β protein production peaked 24 h after treatment onset. Complex movements were associated with increased self-reported pain. Conclusion. Clear aligners show limitations in solving complex tooth movements, even when combined with acceleration. The development of customized and programmable stimulation microdevices integrated into “smart aligners,” which could be designed to specifically stimulate the direction of movement and stimulation parameters and could constitute a solution to optimize the orthodontic tooth movement with clear aligners.
1. Introduction

Unlike physiological tooth movement, orthodontic tooth movement (OTM) mechanically induces a biological response that disrupts the equilibrium of the dentoalveolar complex [1]. Recently, several histological studies on OTM have demonstrated that each tooth moves in the periodontal space, creating pressure and tension areas as a consequence of mechanical stress. The pressure maintained on the teeth is the fuze of its movement into a different position within the periodontal space, compressing in some areas while stretching it in others [2–5]. This leads to structural modifications of the periodontal ligament accompanied by alterations of the biochemical environment in the gingival crevicular fluid (GCF) [6]. Among others, the release of proinflammatory molecules is one of the primary biological responses induced by the OTM. In particular, interleukin-1β (IL-1β) is secreted by osteoclasts as an immediate response (IL-1β responses induced by the OTM over time).

This sense, this biomarker works as a reliable proxy for the evaluation of the inflammatory response and bone formation induced by the OTM over time.

To shorten the treatment duration and limit the side effects of the OTM, different acceleration techniques have been investigated. Two noninvasive, painless, and effective stimulatory approaches to accelerate the OTM have stood out—photobiomodulation (PBM) and mechanical vibrations (MV) [9]. PBM is a light-based low-cost and noninvasive approach that involves the exposure of tissues to red or near-infrared light (NIR) to promote a therapeutic effect in multiple biomedical applications, such as cartilage regeneration and transcranial stimulation therapies against neurological disorders [10–12]. Wavelengths from 600 to 1200 nm are used, and photon absorption by hemoglobin and water is reduced, allowing it to reach deeper tissues and the alveolar bone. PBM stimulates the proliferation of osteoclasts, osteoblasts, and fibroblasts, thereby, affecting bone remodeling and accelerating tooth movement [13–15].

Similarly, recent literature suggests that MV presents numerous therapeutic advantages in different scientific fields [10, 16, 17], including orthodontics [18, 19]. Several hypotheses have been proposed to explain how MV enhances the rate of orthodontic tooth movement. It has been reported that MV may stimulate the differentiation of osteoclasts from hematopoietic cells by increasing blood flow. These signals may be mediated in response to direct effects on the cell membrane, changes in ion transport, activation of stretch-activated channels, activator changes in the attachments between skeletal bones and extracellular matrix, or modification of intracellular signals that regulate gene expression to promote bone remodeling and, consequently, an increased movement rate [18, 20, 21]. Recently, both preclinical and clinical studies have found that MV may enhance the OTM via a mechanism related to the induction of inflammatory mediators [14]. In addition, studies have demonstrated that AcceleDent®, a device with low-intensity vibration frequencies (30 Hz), accelerates the OTM and reduces the patient’s discomfort [22].

The most notable orthodontic advance in the last decade was the introduction of digitally fabricated clear aligners to move the teeth in small and progressive sequences [23]. Currently, the largest provider of clear aligners is Invisalign® [24]. These orthodontic appliances consist of a series of plastic aligners exchanged every 7 to 14 days that deliver forces to the tooth surface, promoting a sequenced OTM [25]. It offers superior advantages regarding the comfort and hygiene of the oral cavity [26]. Moreover, different products with effects against microbial plaque that forms on clear aligners have recently reached the market, reducing the number of cavities, and consequently improving patients’ gingival and periodontal health [27–29]. Clear aligners are also less uncomfortable and more aesthetic compared to conventional fixed appliances [23, 30]. This started by treating mild malocclusions only (e.g., minor crowding and space closure) but it rapidly evolved with the introduction of optimized attachments, allowing the control of more complex tooth movements [25, 28, 31, 32]. However, there are still some studies reporting problems in controlling difficult movements with clear aligners [25, 33–35]. The occurrence of unpredictable movements leads to maladjustment of the clear aligner which prolongs and compromises the treatment [36]. Therefore, the use of clear aligners to promote the OTM in complex dental problems requires more sequenced and controlled treatment.

1.1. Aim. The present case study aims to investigate the IL-1β release profile and the self-reported pain over an orthodontic treatment with clear aligners accompanied by PBM and MV. The analysis of the relationship established between these variables and the OTM complexity was also envisioned.

2. Case Report

The current report describes the orthodontic treatment applied to a 46-year-old female patient who presented functional and aesthetic complaints. Intraoral examination revealed a diminished overjet and overbite, rotation of the lower right second premolar (45) and upper left first premolar (24), absence of the upper left second premolar (25), an edentulous space between the lower left first premolar and the second molar (absence of the teeth 35 and 36, where it was decided to open space only for one teeth), positive buccolingual misposition of the upper left central, a tendency to a Class III malocclusion, and a deviation of the lower midline 2 mm to the left (see the X-ray imaging in the Supplementary Figure S1).

After a thorough examination of the clinical status of the patient, the Invisalign® Diamond provider and specialist in orthodontics, who supervised the orthodontic treatment
(TP), decided to employ an orthodontic treatment using Invisalign® clear aligners. The evolution of the treatment was monitored through digital models acquired with the iTero Element® intraoral scanner (Copenhagen, Denmark) and intraoral photographs, before the orthodontic intervention and at each set of additional clear aligners.

Teeth 21, 24, 26, 34, 37, and 45 were selected for analysis due to their problematic and abnormal positions, and, thus, the need for more complex movements. More specifically, according to Invisalign® guidelines and classification considerations, at the beginning of this treatment, there were the following “complex/advanced” movements to perform: tooth 24 needed 55.3° of rotation, tooth 26 needed 38° of rotation, and tooth 45 needed 72° of rotation. Considering “moderate movements,” tooth 21 needed 6° of root inclination, tooth 34 needed 0.6 mm of intrusion, and tooth 37 needed 0.6 mm of extrusion. Therefore, the main goals of this orthodontic treatment were as follows: (i) in the second quadrant, a derotation with opening space between teeth 24 and 26 and root translation of teeth 11 and 21; (ii) in the third quadrant, opening space between 34 and 37; and (iii) in the fourth quadrant, a derotation of tooth number 45. The amount of tooth movement predicted by the ClinCheck® software (Align Technology, San Jose, California, USA) for each tooth is presented in Supplementary Tables 1 and S2.

Initially, only clear aligners were being used, without any kind of auxiliaries (Supplementary Figure S2). The complexity of the rotation movements, particularly the derotation of round teeth such as premolars and canines [19, 23–25], the clinicians decided to use orthodontic auxiliaries, namely, buttons with elastics and sectional fixed appliances, to aid the movement in the second and fourth quadrants. After a year of treatment, the first set of additional clear aligners was requested. Previous movements were maintained but with less amplitude (Supplementary Table S3). However, even after this adjustment, the clinicians noticed that the clear aligners were continually maladapted to change clear aligners every 5 days. The orthodontic intervention and at each set of additional clear aligners, since the required movements were difficult to be achieved and the continuous aligner misfit was observed. The first phase corresponds to the control moment, in which no stimulation was used. The patient was instructed to change the clear aligners every 7 days. The second phase comprised the delivery of MV, whereas the third phase included the application of PBM along with the clear aligner treatment. During phases 2 and 3, after GCF collection, the patient was instructed to change clear aligners every 5 days. The orthodontic intervention timeline is depicted in Figure 1.

The teeth associated with the most difficult movements were selected (Table 1) and three samples of GCF (per tooth) were collected to analyze the concentration of IL-1β. Digital records were made over time. In addition, pain monitoring was performed using a visual analogue scale (VAS) at all time points for each tooth.

The samples were collected in four moments—T0 (baseline, before the placement of the clear aligner), T1 (24 h after the clear aligner placement), T2 (72 h), and T3 (7 days), in all stimulation phases (i.e., no stimulation, MV, and PBM). After IL-1β collection and pain measurement at the baseline, the coil-spring between the teeth 24 and 26 was (re) activated by the length of a bracket (see Supplementary Figure S5 for further information on spring-coil specificities).

3. Intervention

3.1. Stimulation Procedures. During the second phase of the study, the patient was instructed to use the MV device AcceleDent by Propel Orthodontics® (Milpitas, California, USA) for 20 min/day. This device operates with a frequency and applied force of 30 Hz and 0.25 N, respectively [37].

In the third phase, the PBM device OrthoPulse by Biolux® Research (Vancouver, Canada) was used for 5 min per arch, totaling 10 min/day. This stimulation device operates at a fluence of 90 mW/cm² and it uses 850 nm near-infrared (NIR) light to promote bone formation around the tooth roots being dislocated [9].

3.2. GCF Collection and IL-1β Protein Quantification. The GCF was collected from the pressure side of the selected teeth. The site was isolated using cotton rolls and any supragingival plaque was removed using a cotton bud. The cervical area was dried using an air syringe, and GCF was collected using standardized sterile absorbent paper point (Roeko Paper Points 0.06 °30 by Coltene/Whaledent Inc, Germany). Paper strips were inserted 1 to 2 mm into the gingival sulcus around each tooth and remained in situ for 30 s. Blood-contaminated strips were discarded.

After GCF collection, the strips were transferred to sterile tubes. The GCF volume was determined by weighing based on the difference in the weight of the paper before and after GCF collection and assuming a GCF density value of 1, as previously described [28]. The GCF absorbed in each paper strip was diluted in 250 μL of phosphate buffer saline (PBS, pH 7.4), centrifuged (13,000g at 4°C for 15 min), and stored at −80°C. The GCF samples were assayed to evaluate IL-1β concentration
using an enzyme-linked immunosorbent assay (Human IL-1β/IL-1F2 Quantikine HS ELISA Kit by R&D Systems, Minnesota, USA), following the manufacturer’s instructions. The IL-1β concentration (pg/μL) was calculated by dividing the amount of IL-1β by the volume of GCF for each sample [38].

3.3. Statistical Analysis. Statistical analysis was performed using the IBM SPSS® Statistics 27 (Armonk, New York, USA). First, a Shapiro–Wilk test was used to ascertain the data normality. The data did not follow a normal distribution and, thus, nonparametric tests were used. For the analysis of IL-1β levels and self-reported pain index throughout time, the Wilcoxon signed rank test was conducted. To compare the IL-1β levels and self-reported pain index between different levels of stimulation and type of movement, the Kruskal–Wallis test was used. P values lower than 0.05 were considered statistically significant. Graphical analysis and reporting were performed using GraphPad Prism version 6.0 for Windows (GraphPad Software Inc, San Diego, California, USA).

4. Treatment Results
4.1. Overall Orthodontic Movement Evaluation. With the constant maladjustment, the second set of additional clear aligners was carried out and the vibration device AcceleDent® was introduced. At T0, the space in the second quadrant was open, but some essential moves were still missing (Supplementary Table S4), namely, the 1.4 mm extrusion and 3.0° mesial rotation of tooth 24, as well as a distal rotation of 26.7° of tooth 45. The clear aligner misadjusted increased over time during vibrational stimulation (Supplementary Figure S6). After 4 months of vibration, the clear aligner misfit in the second quadrant continued and intrusion of tooth 24 occurred.

Hence, the third set of additional clear aligners was necessary to induce a 0.5 mm mesial translation movement and 2.1 mm extrusion of tooth 24, and 0.1 mm distal translation movement of tooth 26. Both teeth were supposed to perform a 11° distal rotation. In the mandibula, 0.8 mm mesial translation of tooth 34, distalization movement of tooth 37, and a crown and root distal rotation of 45° of tooth 45 were envisioned (Supplementary Table S5). During this phase, OrthoPulse® was used to apply PBM. Initially, the clear aligners were fully adjusted, but, after 3 months, there was a misfit in the second and fourth quadrants. This was probably because teeth 45, 24, and 26 still require rotational movements, and tooth 24 needs to be extruded. Hence, the clear aligner showed many difficulties in gripping the tooth (Supplementary Figure S7).

The clinical case was completed successfully (Supplementary Figure S8), although it was necessary to resort to
auxiliaries in more complex movements to achieve the treatment goal (see Figure 2 with pre- and posttreatment photographic recording).

4.2. Longitudinal Analysis of IL-1β Profile and Pain. To evaluate the effect of time on IL-1β levels, independently from the type of stimulation or complexity of the orthodontic movement, the data were grouped according to the measurements’ time point. The obtained graph and statistical analysis are depicted in Figure 3(a). IL-1β levels reached a maximum 1 day after the beginning of the study stages, which were significantly different from the levels measured at baseline and days 3 and 7. Compared to baseline, the expression of IL-1β was significantly increased after 1 and 3 days of activation/reactivation, demonstrating an augmented inflammatory response at these timepoints, more prominent on day 1.

A similar analysis was performed for self-reported pain using the VAS (Figure 3(b)), for which no significant differences were observed with time during the first week after the beginning/reactivation of the orthodontic treatment.

4.2.1. Effect of Light and Vibration Stimulation on IL-1β Levels and Pain over Time. At this point, the effect of three levels of stimulation on IL-1β expression and pain was assessed, independently of the type of movement induced by the OTM. Figure 4 displays the profile of IL-1β and self-reported pain over time according to the type of stimulation employed. Despite there being a small tendency for an increase in the levels of IL-1β on day 1 after both PBM and MV, and on day 3 after MV, the great variability of the data disabled us to find a significant difference among the type of stimulation for any time point (Figure 4(a)).

In the case of the pain scores, Figure 4(b) suggests that the vibration stimulus was able to slightly reduce the self-reported pain index 1 and 3 days after stimulation when compared to the nonstimulated group. Importantly, the PBM and MV groups showed some level of pain at the baseline as it coincides with the introduction of a new set of clear aligners. Thus, the graph depicted in Figure 4(b) must be preferably analyzed by comparing the data over time within each stimulation group.

4.2.2. Impact of the Type of Movement on IL-1β Expression and Pain over Time. We aimed to address how different types of movement and their inherent complexity could impact IL-1β levels and self-reported pain. For that, two groups of teeth were considered: (i) teeth with complex movements, comprising the measurement related to teeth 24, 26 (translation movement with a coil-spring), and 45 (rotation and extrusion movements), where the clear aligners were continuously maladapted; and (ii) teeth with moderate (i.e., less complex) movements, namely, teeth 21 (torque movement), 34, and 37 (translation movement without coil-spring). The corresponding data on IL-1β and pain are displayed over time in Figure 5. For IL-1β expression, similar results to those observed for different types of stimulation were found. Due to the great variability of the data (mainly for days 1 and 3), no statistically significant differences were detected between complex and moderate movements, although the graph suggests that slightly augmented levels of IL-1β were detected in GCF samples from teeth subjected to complex movements compared to moderate movements on day 3 (Figure 5(a)).

In line with this observation, Figure 5(b) shows a statistically significant difference between the pain felt in teeth describing complex and moderate movements on day 3. Overall, and independently from the type of stimulation, complex movements were associated with increased pain levels on the third day after orthodontic reactivation, according to the discomfort reported by the patient.

5. Discussion

The present case report and investigational study analyze the profile of IL-1β production and self-reported pain during the
first week that follows the activation/reactivation of the orthodontic appliances. The orthodontic treatment started without any additional treatment, and IL-1β and pain data were collected at the second set of additional clear aligners (phase 1). During the third set of additional clear aligners of the orthodontic treatment, the teeth and surrounding tissues were stimulated using MV (phase 2) and PBM (phase 3), in which IL-1β levels were measured and pain scores were recorded. We noticed that IL-1β increased on the first day, regardless of the type of tooth and stimulation, and there is no relationship between increased IL-1β and stronger pain.

5.1. Inflammatory Response on Clear Aligner-Strained Teeth. Multiple reports on the analysis of GCF of patients undergoing a conventional orthodontic treatment demonstrate that several biochemical changes occur during the first weeks of treatment [3, 6, 38]. In accordance, we detected time-dependent variations in IL-1β levels during the first days after reactivation.

In our study, regardless of the type of tooth and stimulation, IL-1β was greatly increased in the 24 h that followed the beginning of the treatment. At baseline, IL-1β was much more reduced than on the first and third days, but there were no statistical differences compared to the seventh day, showing that it returned to baseline values. In line with these findings, Grant and colleagues and García-Lopez et al. have previously reported an increase in IL-1β and TNF-α levels from 2 to 24 h after the beginning of orthodontic treatment, returning to control levels from 24 to 48 h [22, 39]. These data confirm the activation of inflammatory mediators during the orthodontic treatment, a very early response to orthodontic stress.

In addition, we assessed how different types of movement and their inherent complexity could impact the IL-1β level. Grant et al. found IL-1β increased levels in the canines subjected to inclination using MBT prescription brackets (3M Unitek, UK) at 4 h, 7 days, and 6 weeks, while no significant changes in IL-1β levels were found among time points [39]. This observation suggests that different teeth...
with distinct movements present different IL-1β expression profiles. Based on such evidence, we would expect an increase in IL-1β between the 24 h and 3 days follow-ups, mainly for complex movements when compared to the moderate ones. However, we found no statistically significant differences between complex and moderate movements, although the data suggest that slightly augmented levels of IL-1β were detected in GCF samples on day 3 (Figure 4(a)). Importantly, this inflammatory behavior was previously observed in patients using fixed orthodontic appliances [3, 38].

The misadjustment observed during the first and second sets of additional clear aligners led the clinicians to consider acceleration methods. MV have been proven to stimulate osteoclasts’ differentiation from hematopoietic cells by increasing blood flow [19] and improving OTM rate via a mechanism related to the induction of inflammatory mediators [19, 40]. Similarly, PBM has been proved to stimulate the proliferation of periodontium cells to modulate the orthodontically induced inflammatory response in the periodontium due to the multiple biomechanisms, including the release of inflammatory cytokines, affecting bone remodeling and accelerating tooth movement [41–44].

Here, the effect of the three stimulation phases (i.e., no stimulation, MV, and PBM) on IL-1β secretion showed a small tendency for an increase on day 1 after MV and PBM, and on day 3 after MV. The great variability of the data disabled us to find significant differences between the type of stimulation for any time point, which could be caused by the distinct movements that were evaluated. Importantly, some teeth had more than one movement. In such cases, the movement with the greatest amplitude in ClinCheck® planning was considered. In addition, teeth 24, 26, and 45 required the utilization of auxiliaries, namely, brackets and a coil-spring, which could have also impacted the overall analysis.

5.2. Pain Indexes along the Orthodontic Treatment Using Clear Aligners. During the various phases of orthodontic treatment, pain develops in response to the tension and pressure
zones generated in the periodontal ligament following the application of force [45, 46].

Here, the self-reported pain index did not change significantly with time during the first week after the beginning or reactivation of the orthodontic treatment. A statistically significant difference between the pain felt in teeth describing complex and moderate movements on day 3 was observed. Overall, and independently from the type of stimulation, complex movements tended to be associated with increased pain levels on the third day after orthodontic reactivation.

Previous studies have demonstrated that the pain reaches its peak 24 h after the beginning of the orthodontic stress, which gradually decreases over the following 7 days [47, 48]. Specifically, White et al. [44] and Bondemark et al. [49] found that pain peaked between the first and third days and gradually decreased over the fourth and fifth days. It is important to note that the current case report required the utilization of auxiliary appliances, which may have influenced the level of pain felt by the patient. This could have contributed to the different results found compared to the existing literature.

No correlation between IL-1β levels and pain indexes was found. In GCF, proinflammatory mediators such as IL-1β, prostaglandin E2 (PGE2), and neuropeptides (e.g., substance P) were associated with pain and discomfort [50, 51]. Giannopoulou et al. reported that pain intensity at the 1 h follow-up was associated with increased PGE2 levels, whereas pain intensity at 24 h was associated with increased IL-1β levels [50]. Sampaio et al. reported an insignificant increase in IL-1β levels 24 h after the onset of the force application compared to baseline [51].

MV have been suggested as a very helpful stimulation modality to simultaneously activate different nerve fibers that conduct nonnoxious stimuli, and to reduce compression of the periodontal ligament [52], which have been associated with pain relief [44, 45]. Here, we found no

---

**Figure 5:** Graphical display of (a) interleukin-1β levels in gingival crevicular fluid samples; and (b) pain scores reported by the patient, at the baseline and after 1, 3, and 7 days, grouped according to the complexity of the movement promoted by the orthodontic appliances (i.e., complex and moderate movements) ($p < 0.05$).
differences in pain scores after MV stimulation, although MV were able to slightly reduce self-reported pain index 24 h and 3 days after stimulation. This is in line with previous reports where similar levels of pain were observed in groups with and without vibration [53, 54]. On the contrary, Lobre et al. reported significantly lower pain scores during the fourth month in the group treated with AcceleDent® compared to controls [55].

Similar to what has been reported for IL-1β expression, the application of PBM has been effective in alleviating orthodontic pain without apparent side effects [14, 15]. PBM is thought to reduce pain by increasing local blood flow, inhibiting inflammatory substance secretion, inducing neurotransmitter release, altering the conduction and excitation of peripheral nerves, and stimulating endorphin release [14, 15]. However, data from this case report showed no significant differences in pain scores reported by the patient after PBM application compared to the other stimulation regimens. The use of different methods of pain assessment and the parameters of PBM may explain the disparity of the obtained results when compared to previous data [14, 15].

5.3. Effectiveness of the Idealized Orthodontic Movement. From a clinical perspective, this case report presented several clear aligner mismatches in teeth 24 and 45 due to the complex movements to be performed. Using the same clear aligner (approximately 10 months after the first collection), the vibratory stimuli were introduced in the treatment. During this phase, there was a slight misadjustment of 0.2 mm on tooth 24, with the remaining teeth being adjusted. When introducing the use of MV, it was deduced that the stimulus could somehow recover this maladjustment, but it did not happen. During the vibration phase, which lasted approximately 3 months, the tooth was misadjusted even more.

Hence, new clear aligners were made, and PBM therapy was provided over 3 months. At the end of this phase, both teeth 24 and 45 ended up misfit.

Therefore, the stimulation techniques used for movement acceleration were ineffective in this case report. Despite the long treatment time, the clear aligners always ended up misfitting on teeth that required more complex movements. Thus, we deduce that these stimulation protocols could be effective in correcting simpler clinical situations.

6. Conclusion and Future Perspectives

Interleukin-1β increased on the first day regardless of the type of tooth and stimulation. Although PBM and MV have been proven to accelerate OTM and modulate the patients' inflammatory response, the current study found no statistically significant differences between the IL-1β levels and pain between stimulated and nonstimulated teeth. Besides, no correlation between increased IL-1β and pain indexes was found. Clinically, even after stimulation, the clear aligner maladjustment remained in teeth requiring more complex movements. We found that IL-1β increased at the 24 h follow-up regardless of the type of tooth and stimulation, and the pain was increased on day 3. There is no correlation between increased IL-1β and pain indexes, but complex movements were associated with increased self-reported pain.

Overall, we observed that clear aligners show some limitations when complex tooth movements are required, even when the orthodontic treatment is combined with acceleration techniques. To counteract the considerable mismatches associated with more complex movements, cellular mechanisms of action must be well-defined before clinical application. Besides, future studies in the field must investigate optimal MV and PBM protocols (in terms of, e.g., dose, wave properties, and the application technique) to address specific types of movement and teeth. The development of customized and programmable stimulation devices designed in a way to specifically stimulate the required direction of movement could also constitute an optimized solution to achieve an effective, rapid, and painless OTM.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Authors’ Contributions

All authors contributed to the drafting of the current manuscript. TP was the orthodontist responsible for the orthodontic treatment. The literature review and initial manuscript writing were performed by SP, FM, AB, and SO. SP, AG, SC, and AB were responsible for the clinical and laboratorial part of the included studies. FM and SO performed the statistical part. DR, OC, SC, and TP provided the needed guidance to write and finalize the manuscript. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

This study was supported by UNIPRO—Oral Pathology and Rehabilitation Research Unit, University Institute of Health Sciences (IUCS), CESPU, through the project OrthoAlign-PI-4RL-CESPU-2019, National FCT (Fundação para a Ciência e Tecnologia) funding in the scope of the grants SFRH/BD/09375/2020 and UI/BD/150951/2021, and the projects UIDB/04436/2020 and UIDP/04436/2020.

Supplementary Materials

Supplementary Figure S1. X-ray imaging of the case report before the orthodontic treatment obtained using the Cranex 3+ Panoramic X-ray device by Soredex Orion Corporation (Helsinki, Finland; September 20th, 2018). Supplementary Figure S2. Photographic record at the start of treatment (only wearing clear aligners, November 22nd, 2018). Supplementary Figure S3. Photographic record of the clear aligners misfit after placing sectional fixed auxiliaries to aid the translation movement before the application of photobiomodulation and vibration.
References

[1] G. Isola, G. Matarrese, G. Cardoso, L. Perillo, and L. Ramaglia, “Mechanobiology of the tooth movement during the orthodontic treatment: a literature review,” Minerva Stomatologica, vol. 65, no. 5, pp. 299–327, 2016.

[2] V. Krishnan and Z. Davidovich, “Cellular, molecular, and tissue-level reactions to orthodontic force,” American Journal of Orthodontics and Dentofacial Orthopedics, vol. 129, no. 4, pp. 463–483, 2006.

[3] T. Castrollo, E. F. Gamero, G. P. Caviglia, and A. Deregibus, “Biochemical markers of bone metabolism during early orthodontic tooth movement with aligners,” The Angle Orthodontist, vol. 87, no. 1, pp. 74–81, 2017.

[4] S. Kaya, M. Çifter, A. Çekici, V. Olgaç, H. İlsėver, and G. Isik, “Effects of orthodontic force magnitude on cell apoptosis and RANKL-induced osteoclastogenesis,” Journal of Orofacial Orthopedics/Fortschritte der Kieferorthopädie, vol. 81, no. 2, pp. 100–112, 2020.

[5] A. Schröder, P. Käppler, U. Nazet et al., “Effects of compressive and tensile strain on macrophages during simulated orthodontic tooth movement,” Mediators of Inflammation, vol. 2020, Article ID 2814015, 10 pages, 2020.

[6] F. d’Apuzzo, L. Nucci, I. Delfno et al., “Application of vibrational spectroscopies in the qualitative analysis of gingival crevicular fluid and periodontal ligament during orthodontic tooth movement,” Journal of Clinical Medicine, vol. 10, no. 7, p. 1405, 2021.

[7] Z. Davidovich, O. F. Nicolay, P. W. Ngan, and J. L. Shanfeld, “Neurotransmitters, cytokines, and the control of alveolar bone remodeling in orthodontics,” Dental Clinics of North America, vol. 32, no. 3, pp. 411–435, 1988.

[8] L. Feller, R. A. G. Khammisa, I. Scheckter, G. Thomadakis, J. Fourie, and J. Lemmer, “Biological events in periodontal ligament and alveolar bone associated with application of orthodontic forces,” The Scientific World Journal, vol. 2015, Article ID 876509, 7 pages, 2015.

[9] M. Jedliński, U. Romeo, A. Del Vecchio, G. Palaia, and G. Gallicco, “Comparison of the effects of photobiomodulation with different lasers on orthodontic movement and reduction of the treatment time with fixed appliances in novel scientific reports: a systematic review with meta-analysis,” Photobiomodulation, Photomedicine, and Laser Surgery, vol. 38, no. 8, pp. 455–465, 2020.

[10] F. Monteiro, S. Oliveira, M. Gasik et al., “How does the Alzheimer’s disease brain respond to optomechanical stimuli? A narrative review,” Brain Stimulation, vol. 14, no. 6, pp. 1667–1668, 2021.

[11] S. Oliveira, R. Andrade, J. Espregueira-Mendes et al., “In vitro and in vivo effects of photobiomodulation on cartilage repair for knee osteoarthritis: a systematic review,” Cartilage, vol. 13, 2021.

[12] F. Monteiro, Ó Carvalho, N. Sousa, F. S. Silva, and I. Sotiropoulos, “Photobiomodulation and visual stimulation against cognitive decline and Alzheimer’s disease pathology: a systematic review,” Alzheimer’s & Dementia: Translational Research & Clinical Interventions, Article ID e12249, 2022.

[13] H. Tury, L. Maltz, and U. Oron, “Low-level laser irradiation (LLL) promotes proliferation of mesenchymal and cardiac stem cells in culture,” Lasers in Surgery and Medicine, vol. 39, no. 4, pp. 373–378, 2007.

[14] M. Cronshaw, S. Parker, E. Anagnostaki, and E. Lynch, “Systematic review of orthodontic treatment management with photobiomodulation therapy,” Photobiomodulation, Photomedicine, and Laser Surgery, vol. 37, no. 12, pp. 862–868, 2019.

[15] G. Isola, M. Matarrese, F. Bruguglio et al., “Effectiveness of low-level laser therapy during tooth movement: a randomized clinical trial,” Materials, vol. 12, no. 13, p. 2187, 2019.

[16] F. Monteiro, I. Sotiropoulos, Ó Carvalho, N. Sousa, and F. S. Silva, “Multi-mechanical waves against Alzheimer’s disease pathology: a systematic review,” Translational Neurodegeneration, vol. 10, no. 1, pp. 36–28, 2021.

[17] S. Oliveira, R. Andrade, C. Valente et al., “Mechanical-based therapies may reduce pain and disability in some patients with knee osteoarthritis: a systematic review with meta-analysis,” The Knee, vol. 37, pp. 28–46, 2022.

[18] C. Leethanakul, P. Phusuntornsakul, and A. Pravitharangul, “Vibratory stimulus and accelerated tooth movement: a critical appraisal,” Journal of the World Federation of Orthodontists, vol. 7, no. 3, pp. 106–112, 2018.

[19] M. Katchooi, B. Cohanim, S. Tai, B. Bayirli, C. Spiekerman, and G. Huang, “Effect of supplemental vibration on orthodontic movement with aligners: a randomized trial,” American Journal of Orthodontics and Dentofacial Orthopedics, vol. 153, no. 3, pp. 336–346, 2018.

[20] M. Alikhani, S. Alansari, M. A. Hamidaddin et al., “Vibration paradox in orthodontics: anabolic and catabolic effects,” PLoS One, vol. 13, no. 5, Article ID e0196540, 2018.

[21] P. Phusuntornsakul, S. Jitpukdeebodintra, P. Pavasant, and J. Fourie, and J. Lemmer, “Biological events in periodontal ligament and alveolar bone associated with application of orthodontic forces,” The Scientific World Journal, vol. 2015, Article ID 876509, 7 pages, 2015.
[23] Y. Ke, Y. Zhu, and M. Zhu, "A comparison of treatment effectiveness between clear aligner and fixed appliance therapies," *BMC Oral Health*, vol. 19, no. 1, p. 24, 2019.

[24] K. Ojima and C. H. Kau, "A perspective in accelerated orthodontics with aligner treatment," *Seminars in Orthodontics*, vol. 23, no. 1, pp. 76–82, 2017.

[25] J. Hennessy and E. A. Al-Awadhi, "Clear aligners generations and orthodontic tooth movement," *Journal of Orthodontics*, vol. 43, no. 1, pp. 68–76, 2016.

[26] G. Mampieri and A. Giancotti, "Invisalign technique in the treatment of adults with pre-restorative concerns," *Progress in Orthodontics*, vol. 14, no. 1, p. 40, 2013.

[27] F. d’Apuzzo, L. Perillo, C. K. Carrico et al., "Clear aligner treatment: different perspectives between orthodontists and general dentists," *Progress in orthodontics*, vol. 20, no. 1, p. 10, 2019.

[28] T. Weir, "Clear aligners in orthodontic treatment," *Australian Dental Journal*, vol. 62, pp. 58–62, 2017.

[29] A. Meto, B. Colombari, A. Castagnoli, M. Sarti, L. Denti, and E. Blasi, "Efficacy of a copper–calcium–hydroxide solution in reducing microbial plaque on orthodontic clear aligners: a case report," *European Journal of Dermatology*, vol. 13, no. 3, pp. 478–484, 2019.

[30] B. S. Garnett, K. Mahood, M. Nguyen et al., "Cephalometric comparison of adult anterior open bite treatment using clear aligners and fixed appliances," *The Angle Orthodontist*, vol. 89, no. 1, pp. 3–9, 2018.

[31] A. Papadimitriou, S. Mousoulea, N. Gkantidis, and D. Kloukos, "Clinical effectiveness of Invisalign® orthodontic treatment: a systematic review," *Progress in Orthodontics*, vol. 19, no. 1, p. 37, 2018.

[32] S. J. Bowman, "Improving the predictability of clear aligners," *Seminars in Orthodontics*, vol. 23, no. 1, pp. 65–75, 2017.

[33] G. Rossini, S. Parrini, and T. Castroflorio, "Efficacy of clear aligners in controlling orthodontic tooth movement: a systematic review," *Smile Dental Journal*, vol. 10, no. 1, p. 36, 2015.

[34] N. D. Kravitz, B. Kusnoto, E. BeCole, A. Obrez, and B. Agran, "How well does Invisalign work? A prospective clinical study evaluating the efficacy of tooth movement with Invisalign," *American Journal of Orthodontics and Dentofacial Orthopedics*, vol. 135, no. 1, pp. 27–35, 2009.

[35] L. Galan-lopez, J. Barcia-Gonzalez, and E. Plasencia, "A systematic review of the accuracy and efficiency of dental movements with Invisalign," *The Korean Journal of Orthodontics*, vol. 49, no. 3, pp. 140–149, 2019.

[36] T. Pinho, M. Santos, Y. Agarwal, M. Goyal, M. Kumar, and A. Kaur, "Skeletal open bite treated with clear aligners and miniscrews," *American Journal of Orthodontics and Dentofacial Orthopedics*, vol. 159, no. 2, pp. 224–233, 2021.

[37] D. Jing, J. Xiao, X. Li, Y. Li, and Z. Zhao, "The effectiveness of vibrational stimulus to accelerate orthodontic tooth movement: a systematic review," *BMC Oral Health*, vol. 17, no. 1, p. 143, 2017.

[38] C. Leethanakul, S. Suamphan, S. Jitpudeebodintra, U. Thongudomporn, and C. Charoemratro, "Vibratory stimulation increases interleukin-1 beta secretion during orthodontic tooth movement," *The Angle Orthodontist*, vol. 86, no. 1, pp. 74–80, 2016.

[39] M. Grant, J. Wilson, P. Rock, and I. Chapple, "Induction of cytokines, MMP9, TIMPs, RANKL and OPG during orthodontic tooth movement," *The European Journal of Orthodontics*, vol. 35, no. 5, pp. 644–651, 2013.

[40] P. Keerthana, R. Diddige, and P. Chitra, "RETRACTED: performance comparison of vibration devices on orthodontic tooth movement - a systematic review and meta-analysis," *Journal of Oral Biology and Craniofacial Research*, vol. 10, no. 4, pp. 814–823, 2020.

[41] I. Andrade Jr., A. B. Sousa, G. G. Silva, and G. G. Silva, "New therapeutic modalities to modulate orthodontic tooth movement," *Dental Press Journal of Orthodontics*, vol. 19, no. 6, pp. 123–133, 2014.

[42] S. J. S. Kim, M. Y. M. Y. Chou, and Y. G. Y. G. Park, "Effect of low-level laser on the rate of tooth movement," *Seminars in Orthodontics*, vol. 21, no. 3, pp. 210–218, 2015.

[43] A. J. A. J. Swidi, R. W. R. W. Taylor, L. P. L. P. Tadlock, and P. H. H. Buschang, "Recent advances in orthodontic retention methods: a review article," *Journal of the World Federation of Orthodontists*, vol. 7, no. 1, pp. 6–12, 2018.

[44] D. W. White, K. C. Julien, H. Jacob, P. M. Campbell, and P. H. Buschang, "Discomfort associated with Invisalign and traditional brackets: a randomized, prospective trial," *The Angle Orthodontist*, vol. 87, no. 6, pp. 801–808, 2017.

[45] M. M. E. Almallah, W. H. Almahdi, and M. Y. Hajeer, "Evaluation of low level laser therapy on pain perception following orthodontic elastomeric separation: a randomized controlled trial," *Journal of Clinical and Diagnostic Research*, vol. 10, no. 11, pp. ZC23–ZC28, 2016.

[46] V. Krishnan, "Orthodontic pain: from causes to management—a review," *The European Journal of Orthodontics*, vol. 29, no. 2, pp. 170–179, 2007.

[47] W. T. Kim, M. Bayome, J.-B. Park, J. H. Park, S.-H. Baek, and Y.-A. Kook, "Effect of frequent laser irradiation on orthodontic pain," *The Angle Orthodontist*, vol. 83, no. 4, pp. 611–616, 2013.

[48] S. Patel, S. P. McGorray, R. Yezierski, R. Fillingim, H. Logan, and T. T. Wheeler, "Efects of analgesics on orthodontic pain," *American Journal of Orthodontics and Dentofacial Orthopedics*, vol. 139, no. 1, pp. e53–e8, 2011.

[49] L. Bondemark, K. Fredriksson, and S. Ilros, "Separation efect and perception of pain and discomfort from two types of orthodontic separators," *World Journal of Orthodontics*, vol. 5, no. 2, pp. 172–176, 2004.

[50] C. Giannopoulos, L. D. Dudic, and S. Kiliaridis, "Pain discomfort and crevicular fluid changes induced by orthodontic elastic separators in children," *The Journal of Pain*, vol. 7, no. 5, pp. 367–376, 2006.

[51] F. A. Sampaio, C. R. A. Sampaio, C. O. Cunha et al., "The efect of orthodontic separator and short-term fixed orthodontic appliance on inflammatory mediators and somatosensory function," *Journal of Oral Rehabilitation*, vol. 46, no. 3, pp. 257–267, 2019.

[52] R. Melzack and P. D. Wall, "Pain mechanisms: a new theory," *Science*, vol. 150, no. 3699, pp. 971–979, 1965.

[53] K. Taha, R. S. Conley, P. Arany, S. Warunek, and T. Al-Jewair, "Evaluation of mechanical vibrations on maxillary canine retraction and perceived pain: a pilot, single-center, randomized-controlled clinical trial," *Odontology*, vol. 108, no. 2, pp. 321–330, 2020.

[54] P. Miles, H. Smith, R. Weyant, and D. J. Rinchuse, "The efects of a vibrational appliance on tooth movement and patient discomfort: a prospective randomised clinical trial," *Australian Orthodontic Journal*, vol. 28, no. 2, pp. 213–218, 2012.

[55] W. D. Lobre, B. J. Callegari, G. Gardner, C. M. Marsh, A. C. Bush, and W. J. Dunn, "Pain control in orthodontics using a micropulse vibration device: a randomized clinical trial," *The Angle Orthodontist*, vol. 86, no. 4, pp. 625–630, 2016.