

Changes in maxillary molar pulp blood flow during orthodontic intrusion

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Introduction/Objective: The aim of this prospective study was to evaluate the effects of maxillary first molar intrusion on pulpal blood flow (PBF) in humans as recorded by laser Doppler flowmetry (LDF).

Materials and methods: Maxillary first molars of 16 participants were divided into two groups. In the study group, 20 teeth in 10 participants were subjected to an intrusive force of 100 g delivered from mini-implants for 6 months. A control group of 6 subjects (12 teeth) received no orthodontic treatment. LDF measurements were recorded at baseline and at 3 days, 3 weeks, 3 months and 6 months during intrusion. Data was analysed using the Wilcoxon Signed Rank and Mann-Whitney U tests, with a level of $p < 0.05$ considered statistically significant.

Results: No significant changes in PBF perfusion units (PU) were observed in the control group over the course of the study. However, PBF in the study group was significantly higher at T0 (8.7 ± 0.9 PU) when compared with T1 (6.1 ± 0.6 PU, $p < 0.001$) and T2 (6.0 ± 0.6 PU, $p < 0.001$). PBF did not vary significantly between T1 and T2 ($p = 0.073$) or between T3 and T4 ($p = 0.262$). Moreover, PBF at the end of the study (T4) was similar to baseline PBF values for both groups (study group: $p = 0.687$; control group: $p = 0.525$).

Conclusions: Despite significant short-term regressive changes in pulpal tissue during continuous molar intrusion with mini-implants and an applied force of 100 g, blood vessel function was maintained throughout intrusion, as indicated by LDF measurements of PBF, which tended to return to baseline values by the end of the observation period. These results highlight the changes that can occur in molar vascularity, especially during six months of intrusion.

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Introduction

Orthodontic forces are known to produce tissue damage and an inflammatory response in the periodontium¹ as well as cell damage, inflammatory changes and circulatory disturbances in the dental pulp.² Although several studies have suggested that orthodontic forces can adversely affect pulpal microcirculation,^{2–6} there is no convincing data which demonstrates actual changes in pulpal microvasculature after force application. Changes in pulpal vasculature and blood flow are the parameters most commonly used to study tissue response to an orthodontic force. Pulpal response to an applied force may also be evaluated by histological observation,⁴ fluorescent microsphere injection⁷

and measurement of pulp tissue respiration rates;³ however, all of these methods may only be applied following tooth extraction. In contrast, laser Doppler flowmetry (LDF) is a noninvasive method which can be used to obtain repeated measurements of pulpal blood flow (PBF) without causing tissue damage.⁸

Molar intrusion is frequently a desired treatment option for the correction of an anterior skeletal open bite. Evident from animal^{5,6} and histological studies, intrusion is one of the most damaging to the dental pulp when applied to mature teeth.^{3,4} As a result, there is limited information available on the effects of intrusive forces on pulpal blood flow in humans,^{9–12} and the current information, in itself, is conflicting.

Whereas Sano et al.⁹ found a significant reduction in PBF during the application of a continuous intrusive force, Brodin et al.¹¹ and Ikawa et al.¹² both also reported a brief intrusive force caused a significant reduction in PBF. However, Barwick and Ramsay found no change in PBF during the application of a brief intrusive force.¹⁰ All of the studies were performed on human incisors and to date, there are no published studies evaluating blood flow in the dental pulp of molars during orthodontic intrusion. Therefore, the present study was conducted using LDF to evaluate blood flow changes in pulp tissue during orthodontic intrusion of human maxillary first molars.

Materials and methods

Subjects and intrusive force application

The study was approved by the Institutional Review Board and Ethics Committee of the Turkish Ministry of Health's Kecioren Training and Research Hospital (Study No: B.10.4.ISM.4.06.68.49/180) and complied with the principles of the Helsinki Declaration. Informed consent was obtained from all participants. Sixteen healthy patients (age 18 - 25 years; mean age: 21.7 years) who had an anterior open bite of more than 2 mm and a mild Class II skeletal relation were selected to participate in the study. Initial periapical radiographs were taken and patients with missing maxillary first molar teeth, a history of trauma, caries, restorations, periapical radiolucency, root resorption, or previous orthodontic treatment were excluded from the study. The teeth were considered healthy if they were free of caries, restorations, defects, and discolouration. The gingiva was healthy if it had a normal appearance, the depth of the gingival sulcus was less than 2 mm, and there was no tooth mobility. Participants were enrolled in one of two groups. In the study group (10 participants, N = 20 teeth), maxillary left and right first molars were intruded using 4 mini-implants (2 per tooth) for 6 months, whereas the control group (6 patients, N = 12 teeth) received no orthodontic treatment.

Intrusion

Orthodontic bands with buccal tubes were cemented onto the maxillary molars and a continuous 0.016 x 0.022 inch stainless steel wire was placed in the

maxillary arch in preparation for mini-implant insertion. On the left and right sides, one mini-implant (AbsoAnchor; Dentos, Taegu, Korea; diameter, 1.3 mm; length, 6 mm) was inserted into the buccal alveolus between the maxillary first and second molars and one mini-implant was placed in the paramedian palatal area 2 mm from the midpalatal suture and close to the imaginary midline between the second premolar and first molar. Standard periapical radiographs were taken to check the position of the mini-implants in relation to the neighbouring roots. One week after placement, the mini-implants were loaded with an applied intrusive force of 100 g. Maxillary molar intrusion was performed using elastic power chains attaching the alveolar mini-implant to the main archwire and the palatal mini-implant to a lingual button on the maxillary molar. The magnitude of the intrusive force was checked and calibrated using a gram-force gauge (Correx; Ortho Care, Saltaire, UK) during initial activation (baseline) and at monthly appointments. In the present study, 6 months was considered a sufficient period for evaluating long-term changes in PBF. Although intrusion was not completed in some patients, PBF measurements were discontinued. The mini-implants were removed when the intrusion was accomplished.

Laser Doppler Flowmeter

PBF was measured using a Laser Doppler Flowmeter (Periflux PF 4001, Perimed, Järfälla, Sweden) which recorded backscattered light. The LDF output signal voltage is linearly related to red-blood-cell flow (number of cells x average velocity) which is recorded in perfusion units (PU) to provide a relative measurement of blood flow. The LDF used in the present study employed a 1 mW He-Ne laser with a wavelength of 632.8 nm. A straight probe (PF 416, Perimed, Järfälla, Sweden) with a diameter of 2 mm was used to conduct a light beam of 125 µm (fibre to-fibre distance: 500 µm) to the measurement site within the dental pulp and to record the backscattered light in the flowmeter. The flowmeter was calibrated prior to each data collection session. The narrow band was adjusted to read zero voltage when the probe was placed against a motionless object, while a commercially available motility standard was used to calibrate the flowmeter on the wide band to a specific value of 250 PU (Perimed, Järfälla, Sweden).

Recording procedures

LDF measurements were recorded just prior to molar intrusion (T0) and at Day 3 (T1), Week 3 (T2), Month 3 (T3) and Month 6 (T4) of intrusion. The accuracy and reproducibility of measurements were achieved by providing each patient with a custom-fabricated splint of self-curing acrylic resin that was used to secure the probe in the appropriate position. Each splint had a hole, 2.0 mm in diameter and with its centre approximately 2 mm from the gingival margin (Figure 1) on the buccal side and at right angles to the mesio-distal centre of the tooth surface. Prior to PBF measurement, the archwire and molar bands were temporarily removed from the teeth, and rubber-dam and the acrylic splint were positioned in the patient's mouth to eliminate any signal artifact derived from periodontal blood flow. The tip of the LDF probe was inserted into the hole in the splint and recording was started following a 10 minute patient rest period. All measurements were taken by the same unit and PBF was measured in the right and left molar teeth at the same session. All measurements were performed by the same operator under standardised clinical conditions. Attempts were made to minimise bias due to movement of the subject and probe, and pulse rate and blood pressure were recorded throughout the measurement sessions.

None of the participants reported pain or discomfort during the procedure. After obtaining a constant reading, the splint and rubber dam were removed and the archwire and molar bands were replaced. At each measurement session, the mean PU for each tooth was calculated based on the phase of stable

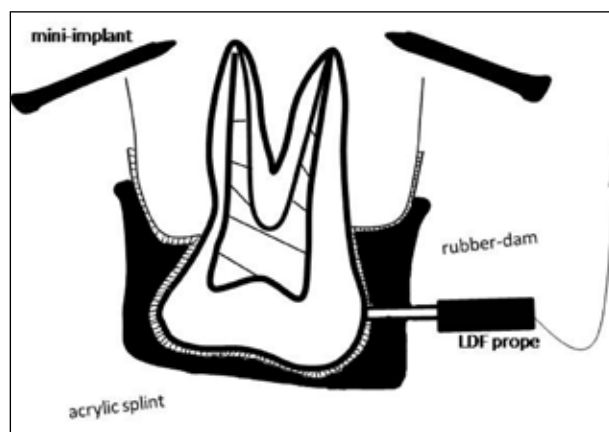


Figure 1. Schematic drawing of LDF probe in the appropriate position 2 mm from the gingival margin on the buccal face. For PBF measurement, the arch and molar bands were temporarily removed from the teeth for the study group. (LDF probe location on a tooth using an acrylic splint) (Schematic diagram of the measurement of pulpal blood flow).

values, excluding peaks attributable to movement artefacts. The LDF data was transferred to a computer connected to the RS-232 port of the flowmeter using the system's own software (PeriSoft for Windows, Perimed) and stored for later analysis.

Statistical analysis

Statistical analysis was performed using the MedCalc 13.0 software program (MedCalc Software bvba, Ostend, Belgium; <http://www.medcalc.org>; 2014). Changes in PBF within and between groups were assessed by the Wilcoxon Signed Rank and Mann-Whitney U tests, respectively, with statistical significance set at $p < 0.05$.

Results

After 6 months of orthodontic treatment delivered by the mini-implant system, the maxillary molars intruded 1 - 2 mm (mean intrusion was 1.76 mm). As the aim of the present study was to evaluate PBF of the molars over a 6-month period, there was no need to wait for the completion of intrusion and overall treatment. Of the 40 mini-screws inserted, mobility was observed in two implants, which produced an overall success rate of 95%. One implant was lost in the third month of the study and was replaced, and another was lost in the fifth month, and was removed but not replaced as the study was nearing completion. None of the subjects complained of pain or tooth discolouration during the study period and periapical radiographs of the study patients showed no signs of root resorption.

Table I shows the maxillary molar PBF values at T0, T1, T2, T3 and T4 for the study and control groups. Whereas no significant changes in PBF were observed in the control group, the Wilcoxon Signed Rank test showed that the mean PBF in the study group decreased significantly from T0 [8.7 ± 0.9 perfusion units (PU)] to T1 (6.1 ± 0.6 PU, $p < 0.001$) and remained significantly lower than baseline at T2 (6.0 ± 0.6 PU, $p < .001$). However, the difference in PBF at T1 and T2 was not statistically significant ($p = 0.073$), nor were there any statistically significant differences in PBF at T0 and T3 ($p = 0.211$), T0 and T4 ($p = 0.525$), or T3 and T4 ($p = 0.262$) (Table II). The changes in molar PBF over time in the study group compared with the relatively constant PBF in the control group are illustrated in Figure 2.

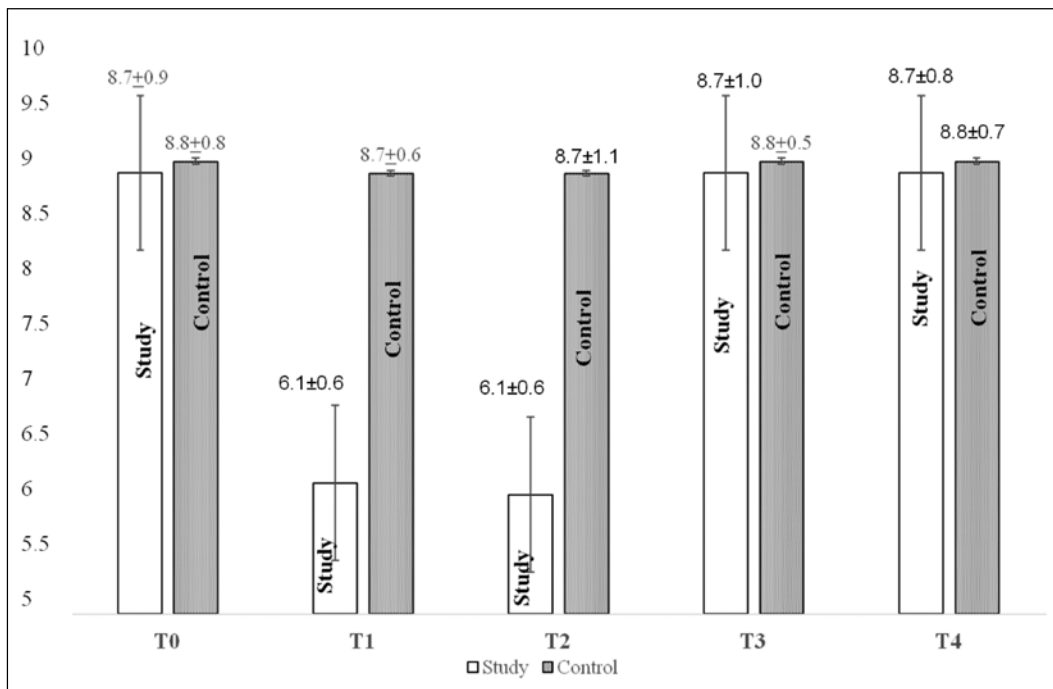


Figure 2. Mean PBF changes for both study and control groups.

Table I. Mean perfusion units and SDs of measurements taken from the maxillary first molars at each of the five sessions.

Groups	T0	T1	T2	T3	T4
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Study group (N = 20)	8.7 ± 0.9	6.1 ± 0.6	6.0 ± 0.6	8.7 ± 1.0	8.7 ± 0.8
Control group (N = 12)	8.8 ± 0.8	8.7 ± 0.7	8.7 ± 1.1	8.8 ± 0.5	8.8 ± 0.7
p*	0.803	<0.001	<0.001	0.346	0.687

*Mann-Whitney U test

Discussion

The present study found PBF values in maxillary first molars were significantly affected by orthodontic intrusion. No differences between the baseline PBF values of the study and control groups were identified and no significant changes in PBF were observed in the control group at any point during the study. However, PBF values in the study group were lower at all time points following the initiation of intrusion, with severe reductions observed at 3 days (69%) and at 3 weeks (70.1%) when compared with the mean baseline levels of 7.1 - 10.7 PU. The initial decrease in PBF in the study group may be a result of a significant compression of the blood-supplying vessels caused by transient apical displacement of the tooth as a result of the intrusion.⁹ The initial decrease in PBF was followed by a gradual recovery after 3 weeks of intrusion in which PBF values at 3 months and 6 months attained levels similar to those measured prior to force application. Due to the study design,

Table II. Comparison of PBF values between different time points (Wilcoxon signed rank test).

	Study group	Control group
T0-T1	<0.001	0.695
T0-T2	<0.001	0.695
T0-T3	0.211	0.937
T0-T4	0.525	0.969
T1-T2	0.073	0.756
T1-T3	<0.001	0.929
T1-T4	<0.001	0.875
T2-T3	<0.001	0.388
T2-T4	<0.001	0.533
T3-T4	0.262	0.814

it was not possible to determine the exact point at which PBF began to recover. The stable temporal pattern of blood flow observed in the control group was considered an indication of the consistency of the LDF testing method and ruled out the possibility that

the changes in PBF observed in the study group were the result of repeated measurements or alterations in flowmeter calibration or sensitivity.

An explanation for the severe reduction in the PBF of molars noted at Day 3 and Week 3 following intrusion is possibly related to molar anatomy. PBF rates are affected by the degree of blood vessel constriction and circulatory disturbance, which is, in part, determined by the anatomy of the root apex. Studies have shown that, in comparison with incisors and premolars, molars have smaller apical foramina¹³⁻¹⁵ and the decrease in apical diameter may account for an increase in circulatory disturbance. Stenvik and Mjor⁴ reported more severe histological disturbances in teeth with smaller apical foramina. Conversely, larger vessels entering the pulp play a major role in minimising a decrease in blood flow during the application of an orthodontic force, which could explain Labart's finding that pulpal respiration increased in the continuously erupting incisors of rats.¹⁶ In addition, root anatomy affects stress distribution and the greater root surface area of molars compared with other teeth would mean a wider distribution of a given stress at the root surface. A lesser amount of stress could be expected to produce a lesser impact on blood flow. Therefore, the reversible vascular changes seen in the molar pulp at months 3 and 6 in the present study could be due to the wider stress distribution effects on the molar root.

The effects of orthodontic forces, including intrusion on dental pulp, have been examined in several previous studies.¹⁷⁻²⁰ However, the majority of these studies were conducted in animal models and the results are conflicting with regard to the effects on vascularity, as well as the ability of the pulp to recover over time.^{5,7,11,12} Anstendig and Kronman⁶ found a decrease in the number of blood vessels following the application of orthodontic forces in dogs. Guevara and McClugage¹⁸ also observed an initial decrease in blood flow in rats using *in vivo* microscopy. Konno et al. evaluated changes in pulpal morphology and blood flow in response to intrusion in an animal model and found that pressure generated by an intrusive force reduced the number of capillary blood vessels below the apical foramen, which subsequently produced slight degenerative changes in pulp tissue.¹⁹ These findings support those of the present study. In contrast, a histomorphometric study on rats conducted by Nixon et al.¹⁷ reported an increase in the number of functional pulpal vessels after the

application of an orthodontic force. Kvinnsland et al.⁷ utilised fluorescent microspheres and showed a substantial increase in blood flow in the dental pulp of rat molars following mesial tipping, and Labart et al.¹⁶ reported increased respiration rates (1 - 2 times greater than in controls) in the pulp of rat incisors subjected to orthodontic stress over 72 hours.

In the present study, PBF was shown to return to near-baseline levels after 3 months of intrusion. This finding agrees with Konno et al.,¹⁹ who indicated that the pulp could recover from degenerative changes after the removal of an applied orthodontic force. Similarly, Grünheid et al.²⁰ showed that force-induced movement of molar teeth in rats caused trauma to pulpal tissue which subsequently affected early wound-healing. However, while the trauma was extensive, it was also shown to be temporary. A further study in a rat model also found that inflammatory vascular reactions subsided within 3 weeks in all tissues.²¹ In contrast, there are many studies which have indicated that injury to the dental pulp can be permanent and lead to an eventual loss of vitality.^{3,6,18,22,23}

Despite the number of animal studies concluding that intrusive orthodontic forces can have an iatrogenic effect on pulpal circulation, it is difficult to correlate past findings with those of the present study, as physiological and/or methodological differences exist. Only a few experimental studies have evaluated the response of the dental pulp to intrusive forces in humans using LDF.^{9-12,24} Ikawa et al.¹² reported that a brief intrusive force significantly reduced PBF, and Brodin et al.¹¹ showed that orthodontic intrusion evoked a temporary reduction in PBF. In a study examining the effects of the continuous application of an intrusive force on dental pulp, Sano et al.⁹ showed that PBF was significantly reduced and was found to have decreased significantly at 3 days but returned towards baseline values at 3 weeks following intrusion. These findings match those of the present study. In contrast, Barwick and Ramsay¹⁰ indicated that the application of a brief intrusive force did not alter PBF; however, their study was limited by a small sample size and a short observation period. It should be noted that while studies demonstrating detrimental changes in pulp subjected to orthodontic force tend to support the findings of the present study, all the previous studies in the literature using LDF to examine PBF in humans following orthodontic intrusion were conducted on incisors, whereas the present study is the first to assess the molars.

Intrusion methodology

Differences in reported circulatory changes in the pulp occurring in response to intrusion may also be attributed to differences in intrusion methodology,^{25,26} magnitude of force,²⁶⁻²⁸ duration of treatment^{25,27,28} and patient age.²⁹ Several treatment options have been developed for intruding molar teeth and correcting an anterior open bite in adult patients. These have included tooth extraction,³⁰ the use of bite-blocks,³¹ multiple-loop edgewise archwire (MEAW) therapy,³² fixed appliances with vertical elastics³³ and extra-oral appliances.³⁴ However, most have proven unsatisfactory as a result of poor skeletal-aesthetic patterns and a strong tendency for relapse. Surgical correction involving repositioning of the maxilla and the mandible³⁵ has been found to achieve satisfactory results; however, the complexity, risks and costs of orthognathic surgery have prompted a search for alternative treatment options.

Recently, fixed-anchorage devices including osseo-integrated dental implants, titanium mini-plates and mini-implants have been introduced to control orthodontic forces and intrusion.³⁶ Unfortunately, osseo-integrated implants and mini-plates require more extensive, often surgical, intervention for placement and removal which precludes their use in many patients. Of all the fixed-anchorage devices currently available for intrusion, mini-implants are the least invasive, most conservative relative to placement and removal, most flexible with respect to implantation site selection and the least expensive. Mini-implants are especially well suited for noncompliant patients. For these reasons, the present study chose mini-implants for molar intrusion. Moreover, in order to counteract the buccal tipping of molars (which occurs when intrusion is performed by only applying an apically-directed force to the buccal tooth attachment), intrusive forces were applied from buccal and palatal aspects simultaneously and PBF measurements commenced three days after loading. Loading was delayed for one week after the insertion of the mini-implants in order to facilitate soft tissue healing.³⁷

The study found mini-implants loaded with forces of 100 g (each mini-implant receiving 50% of the force applied to a tooth) to have excellent stability. The failure rate in this study (5%) was substantially lower than the rate reported for mini-implants loaded immediately upon insertion (13% - 20%).³⁸⁻⁴⁰ Whereas

placement difficulties (causing failure in primary stability) and inflammation of the soft tissue around the mini-implant were the main reasons reported for failure in previous studies, the high success rate in the present study can be attributed to the achievement of primary stability, minimal peri-implant inflammation and the controlled experimental environment.

Magnitude of force

While Umemori et al.⁴¹ recommend a force of 500 g, Park et al.⁴² used 200-300 g and Kalra et al.⁴³ used 90 g of force, the optimal magnitude of force for molar intrusion has not yet been established. Given that the application of a higher force has been shown to have minimal effect on the rate of intrusion,^{25,44} the present study applied a relatively light force (100 g) during molar intrusion in order to minimise the possibility of root resorption.

Duration of treatment

PBF measurements started 3 days after mini-implant loading⁴⁵ and continued for 6 months. Measurements were delayed because it was expected that any signs of inflammation would appear gradually and, given the length of time required for molar intrusion, measurements continued for 6 months. Cooper et al.⁴⁶ reported that histological changes in rat incisors did not become evident until an orthodontic force had been applied for 7 months, and both Oppenheim⁴⁷ and Butcher and Taylor^{5,48} demonstrated that vascular changes persisted 7.5 months after orthodontic force was discontinued. In the present study, 6 months was considered sufficient time for evaluating long-term changes in PBF. Although intrusion and orthodontic treatment was not completed in some patients, PBF measurements were discontinued.

Subject age

PBF is known to decrease with age. Histological findings have shown that, not only do the number of blood vessels decrease with age, the pulpal area also decreases due to increases in calcified tissue.⁴⁹⁻⁵² In the present study, the effects of age were eliminated by limiting participation to subjects in the same age group (18 - 25 years) and initial periapical radiographs were taken to ensure that none of the subjects had pulp stones.

LDF testing

Thermal and electric pulp tests are the tests most commonly used to obtain information about pulp condition.⁵³ However, in addition to the unpleasantness and occasional pain involved in patient response to thermal and electric sensory stimulation, the diagnostic usefulness of these tests is limited by the subjectivity of patient response. Furthermore, conventional pulp tests rely on indirect monitoring of pulp vitality by measuring neural response rather than vascular circulation⁵³ which provides a more accurate picture of pulp vitality.⁵⁴ Tests which rely on a neural response may falsely report a lack of pulp vitality in teeth which have temporarily or permanently lost their sensory function. This is observed in teeth undergoing orthodontic treatment which can disrupt the pulp's sensory response system for up to nine months.⁵⁵ The present study therefore chose to evaluate pulp status by using LDF to directly measure PBF. The advantages of LDF for human research are that it is an accurate, noninvasive, reproducible, reliable method of assessing blood flow in microvascular systems.^{56,57} Although the value of this method has been well documented, its high cost and difficulty of use in clinical practice have delayed its wide-scale introduction.

While LDF may be the most appropriate method for accurately determining pulp vitality, LDF assessment of PBF is highly susceptible to environmental and technical factors, such as flowmeter characteristics,^{56,57} the gingival isolation device,^{58,59} ambient temperature, the position of the probe and patient position and rest status.^{56,58,60,61} In addition, there are other patient-related factors related to stress, medication and age-related changes.^{29,62-64} Moreover, although lasers with longer wavelengths produce higher flux readings (probably due to their greater penetration through tooth tissue), the inclusion of non-pulpal blood flow within the signal may reduce the vital-to-nonvital signal ratio.^{57,65} For this reason, the present study used a 632.8 nm laser source rather than a 780 nm or 810 nm laser. Furthermore, a custom-made acrylic-resin splint was used to stabilise the probe, maintain it in contact with the tooth and create a reproducible position for follow-up measurements. In a technique successfully employed in earlier studies,⁶⁶ black rubber-dam was used in conjunction with the splint in order to minimise the contribution of neighbouring pulp and gingiva on the flux signal. To further ensure the validity of the measurements, special care was taken

to maintain ambient temperatures and patient-related factors such as position, rest and stress levels.

LDF assessment is widely understood to be easier in anterior teeth than in posterior teeth, as probe placement is more difficult in the posterior region. However, it has been suggested that the greater dentine thickness also make it difficult to obtain LDF readings in molar teeth. In a study examining the effects of power increases on signals recorded from human teeth, Sasano et al.⁶⁷ found that first premolars produced no signal at laser outputs of 2, 5, 7, or 10 mW. The authors suggested that the thick dentine prevented the photons from deeply penetrating and passing through the tooth towards the receiving fibre. In contrast to this assertion, Vongsavan and Matthews⁶⁵ found LDF capable of passing through enamel and dentine of 2 - 3.5 mm in thickness to measure blood flow in dental tissue, and Ikawa et al.⁶⁸ showed that laser light was capable of penetrating roots to a depth of 6 mm. Additionally, Polat et al.⁶⁹ observed that laser light penetrated approximately to a depth of 4 mm of dense root and 13 mm depth less dense tissue. In the present study, at no time during any of the observation periods was there an absence of PBF, indicating that enamel thickness was not a significant issue in measurement. The finding of Sasano et al.⁶⁷ could be attributed to their use of an LDF probe comprised of two glass optical fibres, one of which was used for transmitting light onto the labial surface, and the other for receiving it at the palatal surface of the same tooth.

Despite the precautions taken to eliminate possible problems associated with LDF measurements of maxillary molars, contralateral molars in the same subject could not be used as control teeth because of the malocclusion being treated. Therefore, molars from different patients were used as controls. Considering that numerous biological factors (i.e. patient-related characteristics) can influence pulpal response, extrapolation of results to the experimental molars may be problematic. This is an important aspect that requires further study.^{51,52}

Conclusions

Despite significant short-term regressive changes in pulpal tissue during continuous molar intrusion with mini-implants, blood vessel function was maintained throughout intrusion. At the end of the observation

period, pulpal blood flow tended to return to baseline values, indicating that changes in pulpal blood flow are reversible. While the present study used LDF to demonstrate vascular responses, further research is needed to evaluate physiological changes following molar intrusion and identify which factors have the greatest influence on pulpal outcomes. Moreover, future studies should assess PBF at closer time intervals, especially between Week 3 and Month 3 of intrusion, in order to identify, more precisely, the point at which PBF begins to recover following orthodontic intrusion.

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