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Evaluation of pulpal blood flow during orthodontic space closure: Prospective clinical trial

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Introduction: Orthodontic tooth movement (OTM) is a biological process that can affect the vascularization of the dental pulp. The forces exerted on the teeth may increase periapical pressure that could compress the arterioles, which in turn affects pulpal blood flow (PBF). The study aimed to investigate how OTM affects PBF during orthodontic space closure. **Methods:** A total of 22 adolescent participants who required orthodontic space closure in mandibular posterior sectors were enrolled in a prospective clinical study. The same sliding mechanics, wires, and active elements were used. Patients were observed before OTM, after leveling before space closure, and at the 4th, 7th, 21st, and 28th during active space closure. PBF was measured with laser Doppler (LD) flowmetry. Dental models were obtained with an intraoral scanner. **Results:** The LD flow values decreased significantly during the observation period (2-way repeated measures analysis of variance, P <0.001). There was a significant difference in LD flow between tooth categories (2-way repeated measures analysis of variance, P <0.001). During space closure, the most pronounced LD flow reduction was observed in single-rooted teeth closest to the residual space. A higher speed of OTM was associated with a greater decrease in LD flow on day 4 of OTM (Pearson correlation, $P = 0.0299$). **Conclusions:** Orthodontic space closure reduced PBF; it was lowest in the early stages of space closure and showed a tendency to increase during the first month. Anterior teeth closer to the interdental space that experiences more OTM and teeth that move faster during initial OTM had a higher risk of reduced blood flow. (Am J Orthod Dentofacial Orthop 2024;166:549-60)

Orthodontic tooth movement (OTM) is a biological process that can alter the physiology of the dental pulp and affect its vascular system. The dental pulp is a highly vascularized tissue whose vasculature is largely under local neural and endothelial control, although it may be affected by systemic conditions such as age or systolic blood pressure.[1] An effective pulpal vasculature is essential for the supply of oxygen and nutrients, which enables healing and tissue defense.[2,3]

There is ample evidence that orthodontic forces acting on the teeth can exert forces that increase periodontal and especially periapical pressure, which could affect pulpal arterioles and impair pulpal blood flow (PBF). These changes may affect the metabolism of the dental pulp and its histologic structure.[4]

Accurate assessment of pulp vitality is crucial in dentistry as it can influence clinical decision-making. Unfortunately, sensitivity tests provide only indirect information that depends firstly on the person's perceived response to the stimuli and secondly on the clinician's interpretation of the patient's perception of sensitivity. Compared with pulpal sensitivity, pulpal blood perfusion is a more accurate indicator of pulp vitality because it reflects its vascularization.[5] A useful clinical test to monitor pulpal perfusion is laser Doppler (LD) flowmetry. It is a simple, noninvasive measurement that allows direct, semiquantitative, continuous monitoring of PBF in real time and detects dynamic changes in blood flow in tissues, including the dental pulp.[6]

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Previous studies have shown that leveling and aligning, extrusive, intrusive, and expansive types of OTM affect blood flow in the dental pulp.[7,8] However, there is very little data on how the translation of teeth during space closure affects the dynamics of blood flow in the dental pulp, especially in anchoring posterior teeth. Furthermore, empirical knowledge about which risk factors and how they modulate the vascular response of the pulp is limited; to the best of our knowledge, no trial has studied the effect of the amount or the speed of OTM on dental pulp blood perfusion.[9] To date, only 2 studies have investigated the effects of orthodontic space closure on the dental pulp.[10,11] Both studies examined only the anterior teeth with controversial results. In these studies, PBF was not measured in the anchoring posterior teeth, which, in the case of extraction treatment with space closures, undergo a notable amount of OTM.

As space closure is a translational OTM of the entire tooth crown and root, which is subjected to the greatest apical and cervical stress, it can take a relatively long time and requires greater forces, which could impair the blood supply to the dental pulp.[12] Knowing not only how the pulp circulation changes but also at what time and where in the dental arch the effects are more pronounced could lead to more informed decision-making that also takes into account the lowest possible risk to the dental pulp. This could be of great clinical benefit, especially for teeth whose pulp vitality is already compromised. Therefore, our clinical study aimed to evaluate dental PBF during leveling and alignment, monitor it during the space closure phases, evaluate the role of certain controlled risk factors, and identify parameters that could influence PBF during OTM.

MATERIAL AND METHODS

Twenty-two adolescent patients aged 11.1-19.3 years were included in the prospective clinical trial. The National Medical Ethics Committee approved the study under protocol no. 0120-659/2016/6. All participants and their parents were informed about the study in a language and manner they could understand. Written informed consent was obtained. Inclusion and exclusion criteria are shown in Table I.[13]

In all these clinical situations, the orthodontic treatment plan for linear closure of the interdental space in the mandibular dental arch was made. The use of nonsteroidal anti-inflammatory drugs during active OTM was discouraged.

Orthodontic brackets with preadjusted tooth tip and torque according to the Roth prescription were used (RadiancePlus, American Orthodontics, Sheboygan, Wisconsin). In the first phase of orthodontic treatment with a fixed appliance, only leveling and alignment of the teeth were performed before an active orthodontic force was applied to close the interdental spaces (Fig 1).

The subsequent retraction phase is one of the phases of orthodontic treatment that was performed in a controlled manner. When the position of the teeth allowed the placement of the 0.019 × 0.025-in square stainless steel archwires, active horizontal orthodontic forces were applied to begin sliding mechanics-translational linear movements of the teeth to close the space. Active tiebacks using a polyurethane elastomeric module were used (Dentaurum GmbH, Ispringen, Germany). Patients were monitored across 6-time points: beginning (before possible extractions and orthodontic treatment, and after leveling and aligning phase right before space closure onset) (T0), 4th day of space closure (T4), 7th day of space closure (T7), 21st day of space closure (T21), and 28th day of space closure (T28). The elastic modulus was prestretched at each orthodontic force application (T0-T28), and the force was calibrated to 250 cN per side. Orthodontic forces that cause reciprocal movement of the posterior and anterior anchorage units "en masse" against each other (Fig 2, *A* and *B*). The frequent (on average, 1 week) application of new elastic modules thus compensated for the decrease in force caused by the aging of the elastomeric material and the shortening of the residual space (Fig 1). This enabled an almost standardized closure of the space with the same guide arches and active elements.

The typical positions of the teeth after the leveling and alignment phase are shown schematically. The teeth were categorized according to their position in relation to the residual space (1 tooth next to the residual space, 2-second tooth to the residual space, and 3-tooth third or further to the residual space) and according to the type of tooth (Fig 2, *A* and *B*).

The dental arch models were obtained using the iTero 3-dimensional intraoral scanner (iTero Element, Align Technology, San Jose, Calif). The maxillary and mandibular dental arches were scanned, and their corresponding positions were registered. Dental arches were scanned at the beginning, at T0, T4, T7, and T28. The distances between the teeth were measured between the lateral ridges of the 2 neighboring teeth. The speed of movement of the teeth during active orthodontic space closure between different time points was calculated in millimeters per day (Fig 2, *A* and *B*).

LD flowmetry measurements (Periflux P5000; Perimed Company, Järfälla, Sweden) that use a diode

Table I. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Severe (dental arch discrepancy of 3-6 mm) and very severe dental crowding in the mandibular dental arch (dental arch discrepancy >6 mm) with a treatment plan for premolar extraction	Systemic or local diseases that influence the movement of the teeth
Tooth agenesis in the posterior region of the mandibular dental arch (eg, premolar agenesis) and gaps of at least 5 mm in width caused by the missing tooth with a treatment plan for space closure	Systemic medications that influence bone metabolism (eg, bisphosphonates)[13]
Gap because of tooth extraction in the posterior region in the mandibular dental arch (eg, extraction because of severe caries, endodontic complications, or severe hypomineralization) with a missing tooth space of at least 5 mm and a treatment plan for space closure	Inability to lie still for a longer period Cardiovascular diseases

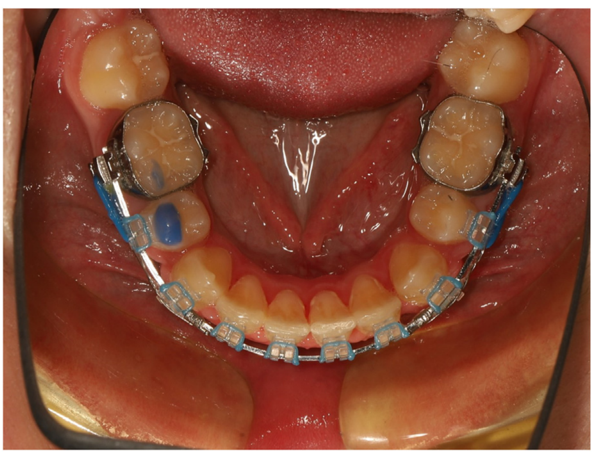

Fig 1. Intraoral status in the mandibular arch during orthodontic space closure.

laser with a wavelength of 780 nm were performed by a single examiner (A.G.), as described in previous studies.

The act of measuring LD flow itself does not influence the rate of measured LD flow.[14] A beam of red or infrared laser light is directed at the pulp. Part of the light that hits a moving object is scattered back into a photodetector, shifting the wavelength according to Doppler. Because red blood cells represent most moving objects in the dental pulp, measurements of their density and velocity are interpreted as PBF by the Doppler-shifted, backscattered light. LD flowmetry uses the ratio between the Doppler-shifted and nonshifted light within the backscattered light to measure blood flow through the tissue.[8,15,16] LD flow is expressed in arbitrary perfusion units (PU) by consensus.[16,17]

To measure the LD flow, the patients were placed in a semirecumbent position. Before the measurement, all orthodontic elements that could interfere with the measurement, such as archwires, molar bands, and residues of glass ionomer cement, were removed. During the measurement, participants were asked to lie still, breathe normally through the nose, and avoid moving any oral or perioral muscles. An angled probe with a 0.125-mm core diameter and 0.25-mm fiber separation was used for the measurement. It was stabilized on the same dental arch. The LD probe was placed on the lingual side of the tooth surface for single-rooted teeth (premolars and anterior teeth), as the brackets were on the buccal side, and on the buccal side for multirooted teeth (molars) to reduce unwanted movement of the probe because of the proximity of the tongue (Fig 3, *A* and *B*). The probe with the laser emitter was placed between the central and gingival third of the tooth crown in which the pulp tissue of the tooth is projected.

The pulpal LD flow value in PU was recorded. The LD flow in the tooth of each participant was measured for at least 1 minute. An average value and a standard deviation (SD) in PU of the measurements within the measurement period were then calculated for each tooth. During the measurement, the patient's systemic oxygenation and pulse were monitored.

Statistical analysis

The data obtained from the study were recorded and processed using Microsoft Excel (Microsoft Corporation, Redmond, Wash). Statistical analyses were performed using SigmaPlot software (version 14.0; Systat, Palo Alto, Calif). P values <0.05 were considered statistically significant. Unless otherwise stated, data are presented as mean $\pm$ SD. On the basis of preliminary data, the power of the test was estimated to be 0.75-0.85. Sample size calculation was performed by using dependent sample analysis of variance sample size calculation tool with 6 separate time points. To achieve statistical significance ($P <0.05$), the study would require the inclusion of 122-155 teeth at each time point.

A 2-way repeated measures analysis of variance (RM-ANOVA) was used to assess pulpal LD flow at each time

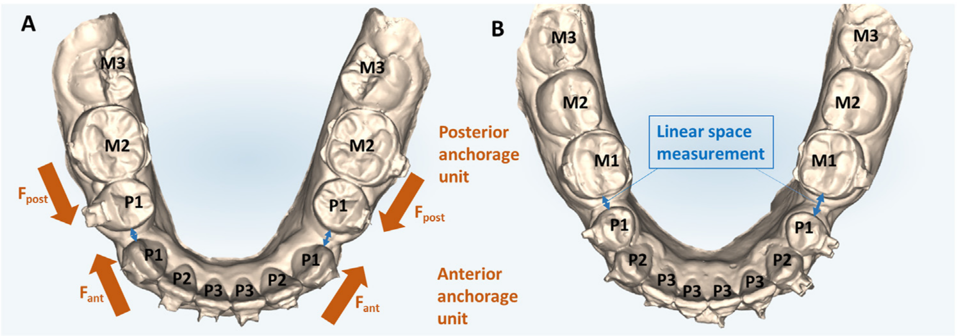

Fig 2. Mandibular dental arch models before active space closure. Tooth categories are marked according to the position in relation to the remaining space (1-3) and the tooth type (P, single-rooted; M, multirooted). Biomechanic situation before space closure with en-masse retraction: **A,** Model with the extraction of first premolars. Parallel force vectors are marked with an *orange* arrow (F_{ant}, anterior force vector; F_{post}, posterior force vector); **B,** Model with the extraction of second premolars. Linear interdental space measurements are marked with *blue* arrows.

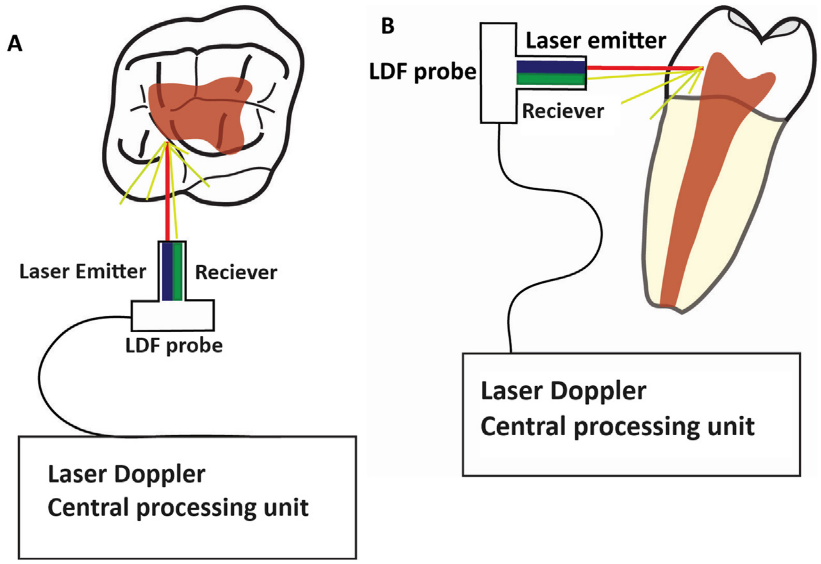

Fig 3. Schematic of LD flow measurement. Part of the laser light emitted by the probe is backscattered by the moving objects in the dental pulp and undergoes a Doppler shift that affects its wavelength. The backscattered light is detected by the receiver in the probe. The central processing unit of the LD device calculates the blood flow from the ratio between the Doppler-shifted and non-shifted light in the backscattered light: **A,** Occlusal view; **B,** Coronal view.

point, controlled for the influence of tooth category (tooth type and position). The Holm-Sidak multiple comparison method was used to distinguish between time points and tooth categories. Tooth type and tooth position were used together as a single factor as they were clinically related. Two-way repeated measures models require a complete data set with no missing values in each category; therefore, all patients or teeth with missing values had to be excluded.

Associations between different numerical variables were tested using linear regression and Pearson correlation. Selected data were presented using scatter plots. If the relationship is statistically significant, a line with a slope coefficient is displayed.

Table II. Numbers and distribution of teeth included in the study

Category	*n (%)*
M2	30 (17.34)
M1	18 (10.41)
P1	48 (27.75)
P2	33 (19.08)
P3	27 (16.61)
M3	17 (9.83)
Total	173 (100.00)

RESULTS

The study included 22 patients (8 boys and 14 girls), and the mean age of the participants was 14.4 ± 1.87 years. Repeated measurements were performed on 173 teeth and 1038 pulp measurements at 6 different time points (Table II). The leveling and alignment phase (between the beginning and T0) lasted an average of 15.3 ± 5.2 months.

The effects of orthodontic treatment on pulpal LD flow were tested with a 2-way RM-ANOVA. The values of PBF changed significantly during treatment (P <0.001), and the power of the test with $\alpha = 0.05$ was 1.0. PBF decreased significantly during the leveling phase and further decreased during space closure (between T0 and T4-28). Although there were no significant differences during the days of space closure, the lowest PBF was observed during the first days of orthodontic space closure. During the month of space closure, there was a tendency toward increased LD flow, but the increase was statistically insignificant. The results are shown in Figure 4 and Supplementary Table I. Further data from the 2-way RM-ANOVA can be found in the Appendix. The duration of the leveling and alignment phase was not associated with mean LD flow values at T0 or the difference in LD flow between the beginning and T0 time points, which was P >0.05 (Pearson correlation).

The 2-way RM-ANOVA model was used to assess LD flow values between tooth categories. There was a significant difference between tooth categories (P <0.001). The power of the test with $\alpha = 0.05$ for the tooth category was 1. There was a significant interaction between tooth category and time of treatment (P = 0.046). Teeth with a root adjacent to the residual space had the lowest LD flow. The reduction in LD flow was most pronounced in teeth closest to the interdental space. The blood flow of multirooted teeth (molars) was less affected than single-rooted premolars.

Initially, there were no differences in the LD flow values between the teeth, with the exception of the teeth in the M3 category, which had higher blood flows. However, during space closure, the differences between the categories were based on the type of tooth and the position in relation to the interdental space. The results (least square means for the tooth category) are shown in Figure 5 and Supplementary Table II. Further data from the 2-way RM-ANOVA can be found in the Appendix.

The relationship between the speed of the OTM during active space closure and the LD flow was modeled using a Pearson correlation. The mean velocity ± SD of OTM during space closure was 0.101 ± 0.070 mm/d at T4, 0.108 ± 0.127 mm/d at T7, and 0.040 ± 0.024 mm/d at T28.

There was a statistically significant negative correlation between the speed of OTM and LD flow (Table III). The variables were only briefly interdependent on T4 (P = 0.030) and did not reach the level of statistical significance on T7 and after that (P = 0.653). The relationship between OTM speed and LD flow at time point T4 is shown in Figure 6. The linear regression showed that, on average, for every mm/day of faster speed of OTM at T4, pulp flow decreased by 6.196 PU. The values for the other correlations between the time points can be found in Table III. The difference in PBF between T0 in each time point followed a very similar pattern (Table III).

The associations between the differences in pulpal LD blood flow values were tested using the Pearson correlation. The Pearson correlation between dLDflow-B-T0 and dPBF-T0-T4 showed a significant negative relationship (P <0.001, r = 0.466). The relationship is shown in Figure 7. A similar relationship was also observed between dLDflow-B-T0 and other intervals (Table IV).

Intraoral models provided 3-dimensional data for the measurement of residual interdental distance. The mean residual distance at T0 was 3.2 mm ± 2.64. There was no correlation between LD flow values, LD flow differences, and residual distances (P >0.05). Patient age and LD flow or difference in LD flow values also did not correlate (P >0.05).

DISCUSSION

The study investigated PBF in anterior and posterior teeth before OTM, after leveling, and during orthodontic space closure using traditional tooth-supported orthodontic mechanics that closely mimic standard orthodontic clinical procedures. The data obtained from the trial showed that the PBF decreased after the leveling phase and continued to decrease during orthodontic space closure. The reduction in PBF was more pronounced in

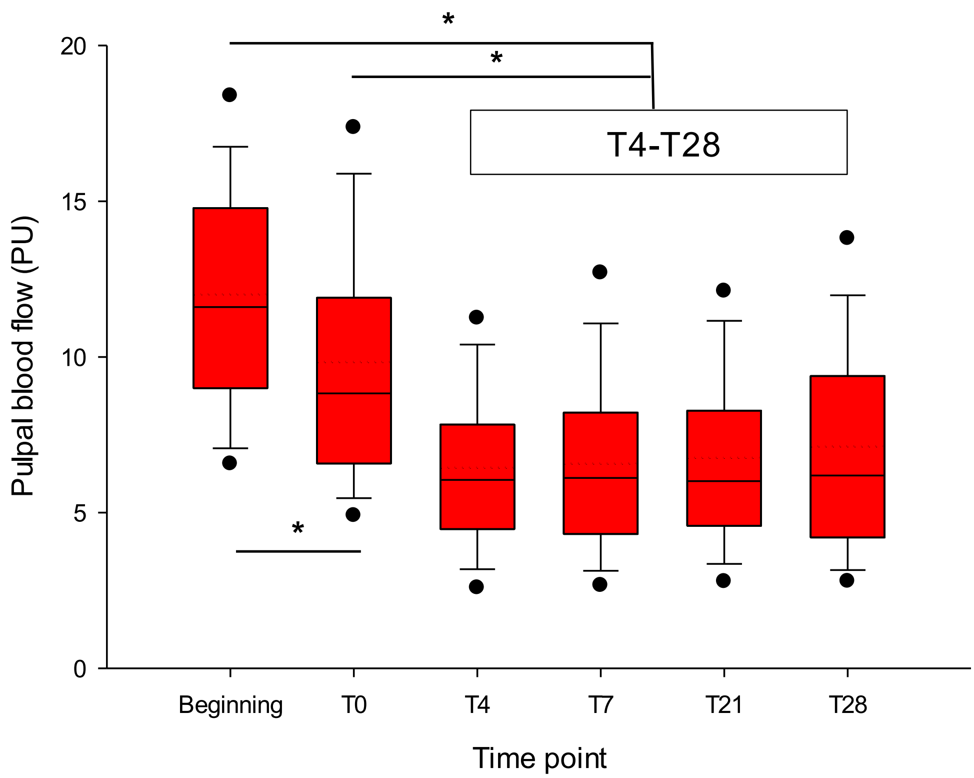

Fig 4. Boxplot of LD blood flow values between time points. *Solid* line, median; *Dotted* line, mean; Boxes, 25th/75th percentiles; Whiskers, 5th/95th percentiles. *Significant differences (P <0.05).

teeth that were closer to the interdental space to be closed. The observed PBF reduction was lower in multirooted teeth than in single-rooted teeth. A higher speed of OTM was associated with a greater reduction in LD flow on T4.

The initial preorthodontic mean LD flow was 12.48 PU, which is comparable to studies (5-15 PU) with participants of similar age.[4,18-20] After the leveling phase of treatment, we observed a significant reduction in PBF. Similarly, reduced PBF during the leveling phase of OTM was also reported in several other studies.[6-8,18,20] During orthodontic space closure, PBF decreased further and was lower compared with the preorthodontic baseline as well as T0 (after the leveling and alignment phase). The PBF was lowest on day 4. There were no significant changes in PBF during the first month of active space closure (T4-28). PBF followed a pattern similar to phases of OTM.[21] The greatest reduction in PBF was observed in the initial phase, likely because of compression of the periodontal ligament and periapical vessels.[22] During the lag phase, only a minor tendency toward an increase in PBF was observed. In the postlag phase, when PBF tends to increase, this is likely because of increased tooth mobility, which is a result of bone resorption (and consequently lower periodontal pressure) and intrinsic vasodilatory mechanisms of the pulp.

Because the leveling phase was completed at time T0, OTM and the forces exerted by the appliance on the teeth were very limited. Although PBF physiologically decreases with age, the decrease in PBF at T0 compared with the initial time point cannot be attributed solely to the fact that the patients became older after the leveling phase, as the average decrease in PBF because of aging is only approximately 0.25 PU per year (unpublished results). Compared with the metadata control of 1238 untreated teeth, in which a reduction in mean PBF was mainly because of 15 months of natural aging of the pulp (−0.3499 PU), a greater difference (compared with baseline) in PBF was observed at T0 (−2.743 PU) and T4 (−5.782 PU). These results are shown graphically in the Forest plot (Fig 8). Compression of the periapical vessels supplying the pulp could be due to the use of active elements for space closure.[23] The effect of OTM on the vessels of the dental pulp with comparable mechanics has only been investigated in 2 previous studies.

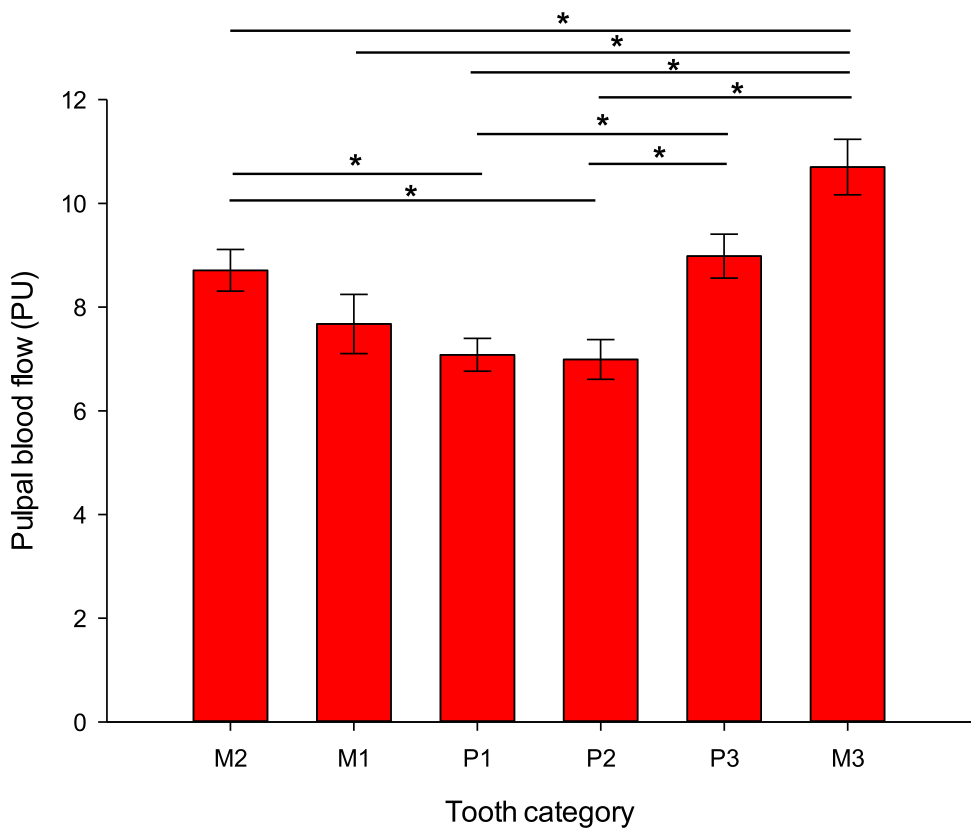

Fig 5. Least square means of LD blood flow among tooth categories; error bars represent the standard error of the mean. *Significant differences (P <0.05).

Table III. Correlation between the speed of the OTM and the LD flow or the difference in LD flows

Time points	*r*	P *value*
Correlation between the speed of OTM and LD flow at:		
T4	−0.155*	0.025*
T7	−0.041	0.547
T28	0.040	0.435
Correlation between the speed of OTM and dLD flow from T0 to		
T4	−0.148*	0.030*
T7	−0.031	0.653
T28	0.049	0.474

*Statistically significant Pearson correlation (P <0.05).

Both studies examined the anterior teeth only. Similar to our results, Sabuncuoglu et al[11] observed a significant reduction in PBF on the third and seventh day of active retraction. In contrast, Guo et al[10] found no changes in PBF. This is probably because of large differences in the measurement time points. Guo et al[10] measured PBF only after the removal of the fixed appliance, thus observing long-term effects on the vasculature, whereas these studies and those of Sabuncuoglu et al[11] focused on short-term changes in the dental pulp.

Blood flow through peripheral tissues is not constant and may fluctuate because of cardiac activity, respiration, or intrinsic regulation of the tissue (myogenic, neurogenic, and endothelial).[24,25] To date, no research work has measured or commented on the variability of LD flow during OTM. The variability (SD) of LD flow within the measurement period increased slightly during leveling (although it just missed the level of statistical significance) and decreased significantly during orthodontic space closure compared with the time points at the beginning or the end of leveling (T0). The results suggest that different types of OTM may influence not only the mean PBF but also its variability.

Several "risk factors" were carefully considered in our study. Teeth were categorized on the basis of known biomechanics in extraction patients.[26,27] To date, empirical clinical knowledge on risk factors that modulate the vascular response of the pulp is limited.[9,28] The tooth

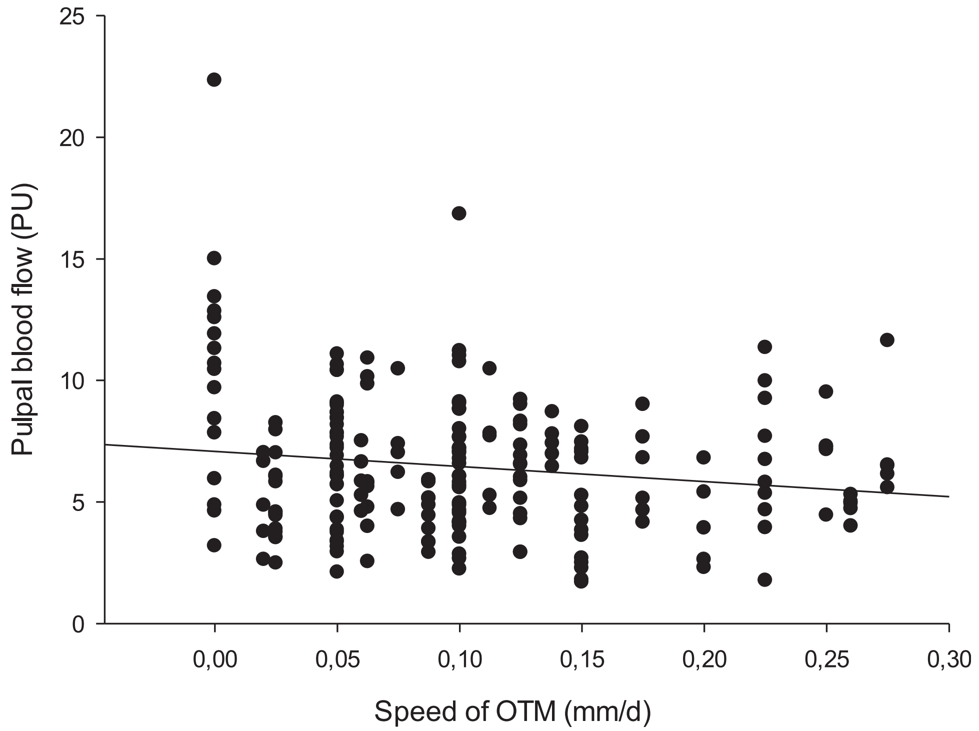

Fig 6. Scatter plot with linear regression of the interdependence of OTM speed and PBF at T4.

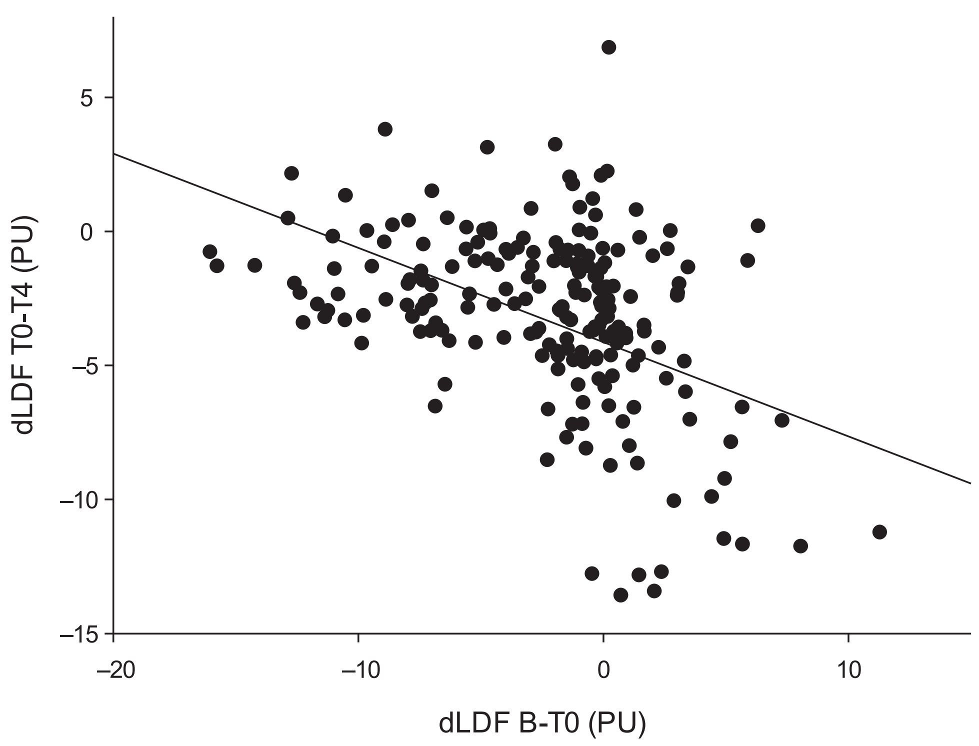

Fig 7. Scatter plot with linear regression between dLDFlow-B-T0 and dLDFlow-T0-T4.

Table IV. Correlation between dLD flow between the start of treatment and T0 and time points during active OTM

Correlation between dPBF-B-T0 and	*r*	P *value*
dPBF-T0-T4	−0.466*	<0.001
dPBF-T0-T7	−0.412*	<0.001
dPBF-T0-T21	−0.412*	<0.001
dPBF-T0-T28	−0.283*	<0.001

*Statistically significant Pearson correlation (P <0.05).

category includes information about the position of the tooth and its type (single-rooted/multirooted). Teeth were categorized because different amounts of OTM are expected depending on their position, and the amount of periodontal surface is much greater in multirooted teeth. A greater reduction in PBF was observed in teeth that were closer to the residual space to be closed than in teeth that were further away (eg, single-rooted teeth that were 1 or 2 sites away from the residual space [P1 or P2] had a significantly lower PBF than single-rooted teeth that were ≥3 sites away from the residual space [P3]), suggesting that pulpal LD flow is also affected by anchoring posterior teeth, albeit to a lesser extent. From a biomechanical point of view, teeth closer to the gap were subjected to longer and more intense OTM. The PBF of multirooted molars was less affected than the PBF of single-rooted anterior teeth. Compared with multirooted molars, single-rooted anterior teeth have larger periodontal surface areas. Therefore, orthodontic force is more evenly distributed across larger surfaces, resulting in lower periapical pressure and stress and, consequently, less reduction in PBF.[12]

Although the forces used for space closure were the same in all patients, the speed of OTM varied among the participants. The mean OTM velocity during the first month of space closure was 1.25 mm/mo, which is within the expected limits of OTM.[29,30] The rate of OTM was highest in the first few days because of periodontal compression.[31] The lag phase of OTM manifested, on average, only as a slower rate of OTM and not as a complete cessation of OTM. The data showed that a higher OTM velocity was significantly associated with a lower PBF; however, this observation was only statistically significant on T4. This means that greater periodontal compression in the initial phases of OTM also has a greater impact on the pulpal vasculature.[31] In later phases of OTM, pulpal compensatory vasodilatation mechanisms probably compensate for part of the reduction in PBF. Blood flow in the pulp is regulated by neuronal release of vasoactive peptides (calcitonin gene-related peptide, vasoactive intestinal peptide, and substance P), control of sympathetic tone,[32,33] and by endothelial local blood flow control through the release of nitric oxide and adenosine.[34,35] These mechanisms are probably upregulated together with the inflammatory response in the later phases of OTM.[3]

We found that there was a significant negative correlation between dLDflow-B-T0 and dLDflow-T0-(T4-T28). If LD flow decreased between the time points at baseline and T0, it increased/decreased less during room closure and vice versa. This relationship has a prognostic value: teeth in that a decrease in LD flow was observed during the leveling and alignment phase are less likely to have a worsening of PBF during orthodontic space closure. The correlation (r >0.4) can be considered moderate.[36] The age of the patient was not associated with the mean LD flow or its change during treatment. This could be due to the narrow age range of the participant group.

In general, PBF is expected to decrease with increasing age. This is due to anatomic and functional changes in the microcirculation, such as increased stiffness, decreased density, impaired organization, and reduced reactivity, as well as the deposition of secondary dentin and cementum, that narrow the apical foramen and pulp canal and reduce the space available for the vessels.[37-39]

Although measurement of blood flow by LD flowmetry is considered the most reliable of the available tests for pulp vitality,[40,41] one of its major drawbacks is that it is a relatively complex and expensive system that is only available to larger clinical and research organizations.[42] It is also the most time-consuming of the pulp tests, which limits its applicability in everyday life.[43]

A limitation of this study is that it does not reach the milestones of a randomized controlled trial. The trial used control measurements instead of a control group. There are several challenges in selecting an appropriate control group. Choosing a control group of participants without malocclusion could lead to bias because of confounding: teeth are in a more optimal position, which could influence the state of the pulp. Furthermore, there is already ample evidence that the pulp condition does not change significantly without the OTM intervention.[4,8,11,44] Conversely, selecting a control group of participants with similar malocclusion would deprive them of treatment at the optimal time. It is known that OTM treatment is more efficient in younger patients with lower periodontal and pulpal risks.[45-47] Contemporary ethical guidelines are based on the principle that the benefit of the participants is paramount. Therefore, it is unacceptable to subject

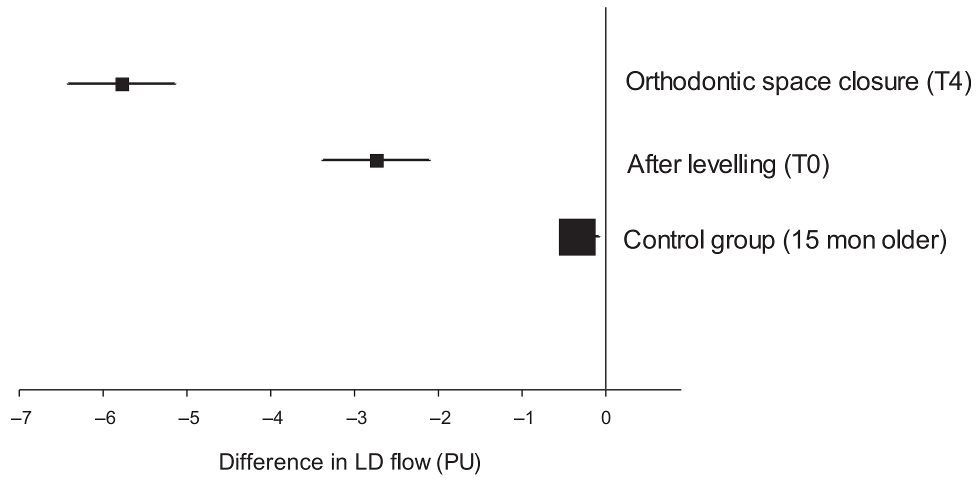

Fig 8. Forest plot of the difference in pulpal LD flow in comparison between the untreated control group (metadata) and the treated experimental group at T0 and T4. The *square* stands for the mean value of the sample, its size for the sample size, and the error bars for the 95% confidence intervals.

participants to examinations for something that is already known or deprive them of treatment at a more optimal time. Because of these ethical considerations and the difficulty of implementation, most human studies have not included a control group.[48,49]

Orthodontic leveling and subsequent space closure reduce PBF. Teeth with smaller anchorage that are closer to the interdental space and exposed to more OTM and teeth that move faster during initial (first few days) OTM have a higher risk of reduced blood flow. OTM temporarily and partially interrupts the pulpal blood supply because of pressure in the periapical area, which may impair pulpal oxygenation and increase the risk of pulpal tissue damage.[23,50] Studies after traumatic dental injuries show that low (3-6 PU) and very low PBF (≤3 PU) were associated with significantly poorer outcomes in terms of clinical vitality of the pulp.[51] Knowledge of the clinical procedures that affect the pulp vasculature is crucial for informed clinical decision-making, especially in patients with teeth with borderline vital pulp. Being able to predict where and when to expect a reduction during OTM could, therefore, be of clinical value. Although there is insufficient data to propose general clinical recommendations, the results suggest that PBF is less impaired when the initial (phase 1) rate of OTM is lower (slow start). Incidentally, this is the time when patients report the greatest discomfort because of OTM.[22] An initial diagnosis of the pulp before treatment could help to create a treatment plan that avoids having to move teeth with compromised pulp over long distances.

CONCLUSIONS

The results of our study show that orthodontic leveling and alignment reduce PBF. Dental PBF is further reduced during active orthodontic space closure. PBF is lowest in the early stages of space closure; it slowly increases during the first month. The blood flow of multirooted teeth and teeth further away from the interdental space is less affected.

A higher speed of OTM in the initial phase of OTM (in the first few days) can further reduce PBF.

There is an inverse relationship between the extent of PBF reduction during leveling and the extent of PBF reduction during space closure.

AUTHOR CREDIT STATEMENT

Aljaž Golež; contributed to conceptualization, methodology, software, investigation, formal analysis, investigation, resources, data curation, original draft preparation, manuscript review and editing, visualization, and project administration; Maja Ovsenik contributed to conceptualization, methodology, investigation, resources, manuscript review and editing, supervision, project administration, and funding acquisition; Ksenija Cankar contributed to conceptualization, methodology, software, investigation, resources, manuscript review and editing, visualization, supervision, project administration, and funding acquisition.

Data are incorporated into the article and the supplementary online material. Some of the data contain sensitive personal information and are therefore protected by national and European union laws (general data protection regulation). However, data that do not jeopardize the privacy of study participants may be made available by the corresponding author (AG) upon reasonable request.

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ajodo.2024.07.017.

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SUPPLEMENTARY MATERIAL

Supplementary Table I. Least-squares mean pulpal LD blood flow and SEM by time point

Time point	*Mean LD flow (PU)*	*SEM*
Beginning	12.476	0.230
T0 (end of leveling)	9.733*	0.231
T4	6.694*,†	0.231
T7	6.761*,†	0.231
T21	7.150*,†	0.231
T28	7.330*,†	0.231

Note. Two-way RM-ANOVA, Holm-Sidak post-hoc test.
SEM, standard error of the mean.
*P <0.05 compared with the beginning time point; †P <0.05 compared with the T0 time point.

Supplementary Table II. Least square means of pulpal LD flow values and SEM by tooth category

Tooth category	*Mean LD flow (PU)*	*SEM*
M2	8.711*	0.402
M1	7.675*	0.572
P1	7.082*,†,‡	0.318
P2	6.992*,†,‡	0.383
P3	8.983	0.424
M3	10.701†	0.534

Note. Two-way RM-ANOVA, Holm-Sidak post-hoc test.
SEM, standard error of the mean.
*P <0.05 compared with M3 category; †P <0.05 compared with M2 category; ‡P <0.05 compared with P3 category.

APPENDIX

Two-Way RM-ANOVA (1-factor repetition).
General Linear Model.

Dependent Variable: LDF-mean.

Source of variation	*DF*	*SS*	*MS*	*F value*	P *value*
Tooth category	5	1,440.209	288,042	10.163	<0.001
ID zoba (tooth category)	168	4,883.913	29,071		
Interval	5	4030.063	806.013	100,417	<0.001
Tooth category × interval	25	308.132	12,325	1.536	0.046
Residual	834	6694.255	8.027		
Total	1037	18,177.156	17.529		

DF, degrees of freedom; *MS*, mean squares; *SS*, sum of squares.

Main effects cannot be properly interpreted if significant interaction is determined. This is because the size of a factor's effect depends on the level of the other factor.

The effect of different levels of tooth category depends on what level of interval is present. There is a statistically significant interaction between tooth category and interval ($P = 0.046$).

Power of performed test with $\alpha = 0.0500$: for tooth category: 1000.

Power of performed test with $\alpha = 0.0500$: for interval: 1000.

Power of performed test with $\alpha = 0.0500$: for tooth category × interval: 0.484.

Expected MS.

Approximate DF residual for tooth category = 171.337.

Expected MS(tooth category) = var(res) + 5.964 var(ID zoba[tooth category]) + var(tooth category).

Expected MS(ID zoba[tooth category]) = var(res) + 5.758 var(ID zoba[tooth category]).

Expected MS(interval) = var(res) + var(Interval).

Expected MS(tooth category × interval) = var(res) + var(tooth category x Interval).

Expected MS(residual) = var(res).

Least square means for the tooth category.

Group	*Mean*	*SEM*
M2	8.711	0.402
M1	7.675	0.572
P1	7.082	0.318
P2	6.992	0.383
P3	8.983	0.424
M3	10.701	0.534

Least square means for interval.

Group	*Mean*	*SEM*
B	12.476	0.230
T0	9.733	0.231
T4	6.694	0.231
T7	6.761	0.231
T21	7.150	0.231
T28	7.330	0.231

Least square means for tooth category × interval.

Group	*Mean*	*SEM*
M2 × B	12.886	0.517
M2 × T0	10.759	0.517
M2 × T4	6.772	0.517
M2 × T7	7.274	0.517
M2 × T21	7.061	0.517
M2 × T28	7.513	0.517
M1 × B	10.703	0.671
M1 × T0	8.211	0.685
M1 × T4	6.061	0.685
M1 × T7	6.360	0.685
M1 × T21	6.856	0.685
M1 × T28	7.860	0.685
P1 × B	12.003	0.409
P1 × T0	8.695	0.409
P1 × T4	5.205	0.409
P1 × T7	5.718	0.409
P1 × T21	5.561	0.409
P1 × T28	5.307	0.409
P2 × B	11.252	0.493
P2 × T0	8.558	0.493
P2 × T4	5.357	0.493
P2 × T7	5.310	0.493
P2 × T21	5.592	0.493
P2 × T28	5.883	0.493
P3 × B	11.523	0.545
P3 × T0	10.543	0.545
P3 × T4	8.187	0.545
P3 × T7	7.371	0.545

Continued

Group	Mean	SEM
P3 × T21	8.152	0.545
P3 × T28	8.121	0.545
M3 × B	16.490	0.687
M3 × T0	11.628	0.687
M3 × T4	8.582	0.687
M3 × T7	8.533	0.687
M3 × T21	9.678	0.687
M3 × T28	9.295	0.687

SEM, standard error of the mean.

All pairwise multiple comparison procedures (Holm-Sidak method).

Overall significance level $\alpha = 0.05$.

Comparisons for factor: tooth category.

Comparison	Diff of means	t	P values	P <0.050
M3 vs P1	3.619	5.826	<0.001	Yes
M3 vs P2	3.709	5.644	<0.001	Yes
M3 vs M1	3.026	3.867	0.002	Yes
P3 vs P1	1.901	3.591	0.005	Yes
P3 vs P2	1.991	3.485	0.007	Yes
M2 vs P1	1.629	3.180	0.017	Yes
M2 vs P2	1.719	3.095	0.021	Yes
M3 vs M2	1.990	2.979	0.026	Yes
M3 vs P3	1.718	2.521	0.085	No
P3 vs M1	1.308	1.837	0.345	No
M2 vs M1	1.035	1.481	0.531	No
M1 vs P2	0.683	0.992	0.789	No
M1 vs P1	0.594	0.907	0.745	No
P3 vs M2	0.272	0.466	0.872	No
P1 vs P2	0.0895	0.180	0.858	No

Comparisons for factor: interval.

Comparison	Diff of means	t	P values	P <0.050
B vs T4	5.782	17.762	<0.001	Yes
B vs T7	5.715	17.556	<0.001	Yes
B vs T21	5.326	16.361	<0.001	Yes
B vs T28	5.146	15.809	<0.001	Yes
T0 vs T4	3.039	9.311	<0.001	Yes
T0 vs T7	2.972	9.106	<0.001	Yes
B vs T0	2.744	8.428	<0.001	Yes
T0 vs T21	2.583	7.914	<0.001	Yes
T0 vs T28	2.403	7.362	<0.001	Yes
T28 vs T4	0.636	1.949	0.273	No
T28 vs T7	0.569	1.743	0.347	No
T21 vs T4	0.456	1.397	0.508	No
T21 vs T7	0.389	1.192	0.550	No
T28 vs T21	0.180	0.551	0.825	No
T7 vs T4	0.0670	0.205	0.837	No

Comparisons for factor: interval within M2.

Comparison	Diff of means	t	P values	P <0.050
B vs T4	6.114	8.358	<0.001	Yes
B vs T21	5.825	7.963	<0.001	Yes
B vs T7	5.612	7.672	<0.001	Yes
B vs T28	5.373	7.345	<0.001	Yes
T0 vs T4	3.987	5.451	<0.001	Yes
T0 vs T21	3.699	5.056	<0.001	Yes
T0 vs T7	3.486	4.765	<0.001	Yes
T0 vs T28	3.246	4.438	<0.001	Yes
B vs T0	2.126	2.907	0.026	Yes
T28 vs T4	0.741	1.013	0.893	No
T7 vs T4	0.502	0.686	0.966	No
T28 vs T21	0.452	0.618	0.954	No
T21 vs T4	0.289	0.395	0.971	No
T28 vs T7	0.239	0.327	0.934	No
T7 vs T21	0.213	0.291	0.771	No

Comparisons for factor: interval within M1.

Comparison	Diff of means	t	P values	P <0.050
B vs T4	4.642	4.839	<0.001	Yes
B vs T7	4.343	4.528	<0.001	Yes
B vs T21	3.848	4.011	<0.001	Yes
B vs T28	2.844	2.964	0.037	Yes
B vs T0	2.492	2.598	0.100	No
T0 vs T4	2.150	2.219	0.238	No
T0 vs T7	1.851	1.911	0.407	No
T28 vs T4	1.798	1.856	0.410	No
T28 vs T7	1.500	1.548	0.598	No
T0 vs T21	1.356	1.399	0.654	No
T28 vs T21	1.004	1.036	0.833	No
T21 vs T4	0.794	0.820	0.881	No
T21 vs T7	0.496	0.512	0.940	No
T0 vs T28	0.352	0.363	0.920	No
T7 vs T4	0.299	0.308	0.758	No

Comparisons for factor: interval within P1.

Comparison	Diff of means	t	P values	P <0.050
B vs T4	6.798	11.755	<0.001	Yes
B vs T28	6.696	11.578	<0.001	Yes
B vs T21	6.442	11.139	<0.001	Yes
B vs T7	6.285	10.868	<0.001	Yes
T0 vs T4	3.490	6.035	<0.001	Yes
T0 vs T28	3.388	5.859	<0.001	Yes
B vs T0	3.308	5.720	<0.001	Yes
T0 vs T21	3.134	5.419	<0.001	Yes
T0 vs T7	2.977	5.148	<0.001	Yes
T7 vs T4	0.513	0.887	0.941	No
T7 vs T28	0.411	0.711	0.961	No
T21 vs T4	0.356	0.616	0.954	No
T21 vs T28	0.254	0.440	0.961	No
T7 vs T21	0.157	0.271	0.954	No
T28 vs T4	0.102	0.177	0.860	No

Comparisons for factor: interval within P2.

Comparison	*Diff of means*	*t*	P *values*	P *<0.050*
B vs T7	5.942	8.519	<0.001	Yes
B vs T4	5.895	8.452	<0.001	Yes
B vs T21	5.660	8.115	<0.001	Yes
B vs T28	5.369	7.697	<0.001	Yes
T0 vs T7	3.248	4.657	<0.001	Yes
T0 vs T4	3.201	4.590	<0.001	Yes
T0 vs T21	2.966	4.253	<0.001	Yes
B vs T0	2.694	3.862	<0.001	Yes
T0 vs T28	2.675	3.835	<0.001	Yes
T28 vs T7	0.573	0.822	0.958	No
T28 vs T4	0.526	0.755	0.950	No
T28 vs T21	0.291	0.418	0.989	No
T21 vs T7	0.282	0.404	0.969	No
T21 vs T4	0.235	0.337	0.930	No
T4 vs T7	0.0471	0.0676	0.946	No

Comparisons for factor: interval within P3.

Comparison	*Diff of means*	*t*	P *values*	P *<0.050*
B vs T7	4.152	5.385	<0.001	Yes
B vs T28	3.402	4.413	<0.001	Yes
B vs T21	3.371	4.372	<0.001	Yes
B vs T4	3.337	4.327	<0.001	Yes
T0 vs T7	3.172	4.114	<0.001	Yes
T0 vs T28	2.422	3.141	0.017	Yes
T0 vs T21	2.391	3.101	0.018	Yes
T0 vs T4	2.356	3.056	0.018	Yes
B vs T0	0.980	1.271	0.797	No
T4 vs T7	0.816	1.058	0.872	No
T21 vs T7	0.781	1.013	0.845	No
T28 vs T7	0.750	0.972	0.800	No
T4 vs T28	0.0657	0.0853	1.000	No
T4 vs T21	0.0344	0.0447	0.999	No
T21 vs T28	0.0313	0.0406	0.968	No

Comparisons for factor: interval within M3.

Comparison	*Diff of means*	*t*	P *values*	P *<0.050*
B vs T7	7.957	8.188	<0.001	Yes
B vs T4	7.908	8.138	<0.001	Yes
B vs T28	7.195	7.404	<0.001	Yes
B vs T21	6.812	7.010	<0.001	Yes
B vs T0	4.862	5.003	<0.001	Yes
T0 vs T7	3.095	3.185	0.015	Yes
T0 vs T4	3.046	3.135	0.016	Yes
T0 vs T28	2.333	2.401	0.125	No
T0 vs T21	1.951	2.007	0.276	No
T21 vs T7	1.144	1.178	0.806	No
T21 vs T4	1.096	1.127	0.778	No
T28 vs T7	0.762	0.784	0.897	No
T28 vs T4	0.713	0.734	0.845	No
T21 vs T28	0.382	0.393	0.906	No
T4 vs T7	0.0487	0.0501	0.960	No

Comparisons for factor: tooth category within B.

Comparison	*Diff of means*	*t*	P *values*	P *<0.050*
M3 vs P2	5.238	5.161	<0.001	Yes
M3 vs M1	5.787	5.020	<0.001	Yes
M3 vs P3	4.967	4.719	<0.001	Yes
M3 vs P1	4.487	4.677	<0.001	Yes
M3 vs M2	3.604	3.493	0.006	Yes
M2 vs M1	2.182	2.146	0.279	No
M2 vs P2	1.634	1.905	0.411	No
M2 vs P3	1.362	1.511	0.676	No
P1 vs M1	1.300	1.378	0.726	No
M2 vs P1	0.883	1.116	0.842	No
P1 vs P2	0.751	0.977	0.864	No
P3 vs M1	0.820	0.790	0.894	No
P1 vs P3	0.480	0.587	0.913	No
P2 vs M1	0.549	0.549	0.826	No
P3 vs P2	0.271	0.308	0.758	No

Comparisons for factor: tooth category within T0.

Comparison	*Diff of means*	*t*	P *values*	P *<0.050*
M3 vs P1	2.933	3.057	0.034	Yes
M3 vs P2	3.070	3.025	0.035	Yes
M3 vs M1	3.417	2.935	0.044	Yes
M2 vs P1	2.064	2.609	0.106	No
M2 vs P2	2.201	2.567	0.109	No
M2 vs M1	2.548	2.473	0.128	No
P3 vs P1	1.848	2.260	0.198	No
P3 vs P2	1.985	2.250	0.182	No
P3 vs M1	2.332	2.219	0.173	No
M3 vs P3	1.085	1.031	0.885	No
M3 vs M2	0.869	0.842	0.922	No
P1 vs M1	0.484	0.505	0.978	No
P2 vs M1	0.347	0.342	0.981	No
M2 vs P3	0.216	0.240	0.964	No
P1 vs P2	0.137	0.178	0.859	No

Comparisons for factor: tooth category within T4.

Comparison	*Diff of means*	*t*	P *values*	P *<0.050*
P3 vs P1	2.982	3.646	0.004	Yes
M3 vs P1	3.377	3.520	0.006	Yes
P3 vs P2	2.830	3.208	0.018	Yes
M3 vs P2	3.225	3.178	0.018	Yes
M3 vs M1	2.521	2.165	0.291	No
P3 vs M1	2.125	2.023	0.359	No
M2 vs P1	1.567	1.981	0.358	No
M3 vs M2	1.810	1.754	0.486	No
M2 vs P2	1.415	1.650	0.519	No
P3 vs M2	1.415	1.569	0.527	No
M1 vs P1	0.856	0.895	0.902	No
M1 vs P2	0.704	0.695	0.931	No
M2 vs M1	0.711	0.690	0.868	No
M3 vs P3	0.395	0.376	0.914	No
P2 vs P1	0.152	0.198	0.843	No

Comparisons for factor: tooth category within T7.

Comparison	Diff of means	t	P values	P <0.050
M3 vs P2	3.223	3.176	0.023	Yes
M3 vs P1	2.815	2.934	0.047	Yes
P3 vs P2	2.061	2.337	0.228	No
M2 vs P2	1.964	2.290	0.237	No
P3 vs P1	1.653	2.021	0.388	No
M2 vs P1	1.556	1.966	0.399	No
M3 vs M1	2.173	1.867	0.440	No
M3 vs M2	1.260	1.221	0.867	No
M3 vs P3	1.162	1.104	0.889	No
M1 vs P2	1.050	1.037	0.883	No
P3 vs M1	1.011	0.962	0.871	No
M2 vs M1	0.914	0.887	0.848	No
M1 vs P1	0.642	0.671	0.877	No
P1 vs P2	0.408	0.531	0.836	No
P3 vs M2	0.0973	0.108	0.914	No

Comparisons for factor: tooth category within T21.

Comparison	Diff of means	t	P values	P <0.050
M3 vs P1	4.116	4.290	<0.001	Yes
M3 vs P2	4.086	4.026	<0.001	Yes
P3 vs P1	2.591	3.168	0.021	Yes
P3 vs P2	2.560	2.902	0.045	Yes
M3 vs M2	2.617	2.536	0.119	No
M3 vs M1	2.822	2.424	0.146	No
M2 vs P1	1.499	1.895	0.419	No
M2 vs P2	1.469	1.713	0.518	No
M3 vs P3	1.525	1.449	0.673	No
M1 vs P1	1.294	1.352	0.689	No
M1 vs P2	1.264	1.248	0.697	No
P3 vs M1	1.296	1.234	0.625	No
P3 vs M2	1.091	1.210	0.537	No
M2 vs M1	0.205	0.199	0.975	No
P2 vs P1	0.0307	0.0399	0.968	No

Comparisons for factor: tooth category within T28.

Comparison	Diff of means	t	P values	P <0.050
M3 vs P1	3.988	4.157	<0.001	Yes
P3 vs P1	2.814	3.441	0.009	Yes
M3 vs P2	3.412	3.362	0.011	Yes
M2 vs P1	2.206	2.788	0.063	No
M1 vs P1	2.553	2.666	0.083	No
P3 vs P2	2.237	2.536	0.108	No
M1 vs P2	1.976	1.951	0.378	No
M2 vs P2	1.630	1.900	0.379	No
M3 vs M2	1.782	1.727	0.461	No
M3 vs M1	1.435	1.233	0.771	No
M3 vs P3	1.174	1.116	0.785	No
P2 vs P1	0.576	0.750	0.911	No
P3 vs M2	0.608	0.674	0.875	No
M1 vs M2	0.347	0.337	0.931	No
P3 vs M1	0.261	0.249	0.804	No

Diff, difference.

1-way RM-ANOVA.
Dependent variable: LDF-SD.

Treatment name	N	Missing	Mean	SD	SEM
B	173	0	2.943	1.601	0.122
T0	173	0	3.358	2.323	0.177
T4	173	0	2.187	1.504	0.114
T7	173	0	2.125	1.480	0.113
T21	173	0	2.215	1.634	0.124
T28	173	1	2.060	1.396	0.106

SEM, standard error of the mean.

Source of variation	DF	SS	MS	F value	P value
Between subjects	173	1266.889	7.323		
Between treatments	5	248.718	49.744	25.706	<0.001
Residual	858	1660.333	1.935		
Total	1036	3176.890	3.066		

DF, degrees of freedom; *MS*, mean squares; *SS*, sum of squares.

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P \leq 0.001$). A multiple comparison procedure is used to isolate the group or groups that differ from the others.

Power of performed test with $\alpha = 0.050$: 1.000.

Expected MS.

Approximate DF Residual = 858.000.

Expected MS(subject) = var(res) + 5.960 var(Subj).

Expected MS(treatment) = var(res) + var(treatment).

Expected MS(residual) = var(res).

All pairwise multiple comparison procedures (Tukey test).

Comparisons for factor: interval.

Comparison	Diff of means	p	q	P values	P <0.050
T0 vs T28	1.304	6	12.269	<0.001	Yes
T0 vs T7	1.233	6	11.629	<0.001	Yes
T0 vs T4	1.171	6	11.043	<0.001	Yes
T0 vs T21	1.143	6	10.780	<0.001	Yes
T0 vs B	0.425	6	4.007	0.052	No
B vs T28	0.879	6	8.283	<0.001	Yes
B vs T7	0.809	6	7.635	<0.001	Yes
B vs T4	0.747	6	7.049	<0.001	Yes
B vs T21	0.719	6	6.785	<0.001	Yes
T21 vs T28	0.160	6	1.508	0.895	No
T21 vs T7	0.0901	6	0.849	0.991	Do not test
T21 vs T4	0.0279	6	0.263	1.000	Do not test
T4 vs T28	0.132	6	1.245	0.951	Do not test
T4 vs T7	0.0621	6	0.586	0.998	Do not test
T7 vs T28	0.0701	6	0.660	0.997	Do not test

Pearson product moment correlation.

Variables	*dLDF-T0-T4*	*dLDF-T0-T7*	*dLDF-T0-T21*	*dLDF-T0-T28*
dLDF-B-T0				
r	−0.466	−0.412	−0.412	−0.283
P value	2.324×10^{-12}	9.14×10^{-10}	1.72×10^{-8}	4.21×10^{-5}
n	203	204	173	203
dLDF-T0-T4				
r		0.811	0.738	0.674
P value		1.364×10^{-46}	3.146×10^{-31}	2.091×10^{-28}
n		194	174	204
dLDF-T0-T7				
r			0.795	0.738
P value			6.224×10^{-39}	1.079×10^{-34}
n			173	194
dLDF-T0-T21				
r				0.756
P value				1.976×10^{-033}
n				174