Comparison of in vivo and in vitro models to evaluate pulp temperature rise during exposure to a Polywave® LED light curing unit

Objectives: To measure and compare in vivo and in vitro pulp temperature (PT) increase (ΔTEMP) over baseline, physiologic temperature using the same intact upper premolars exposed to the same Polywave® LED curing light. Methodology: After local Ethics Committee approval (#255,945), local anesthesia, rubber dam isolation, small occlusal preparations/minute pulp exposure (n=15) were performed in teeth requiring extraction for orthodontic reasons. A sterile probe of a temperature measurement system (Temperature Data Acquisition, Physitemp) was placed within the pulp chamber and the buccal surface was sequentially exposed to a LED LCU (Bluephase 20i, Ivoclar Vivadent) using the following exposure modes: 10-s low or high, 5-s Turbo, and 60-s high. Afterwards, the teeth were extracted and K-type thermocouples were placed within the pulp chamber through the original access. The teeth were attached to an assembly simulating the in vivo environment, being similarly exposed while real-time temperature (°C) was recorded. ΔTEMP values and time for temperature to reach maximum (ΔTIME) were subjected to two-way ANOVA and Bonferroni's post-hoc tests (pre-set alpha 0.05). Results: Higher ΔTEMP was observed in vitro than in vivo. No significant difference in ΔTIME was observed between test conditions. A significant, positive relationship was observed between radiant exposure and ΔTEMP for both conditions (in vivo: r²=0.917; p<0.001; in vitro: r²=0.919; p<0.001). Conclusion: Although the in vitro model overestimated in vivo PT increase, in vitro PT rise was close to in vivo values for clinically relevant exposure modes.

Keywords: Bicuspids. Volunteers. Dental curing lights. Temperature.
Introduction

Maintenance of pulp safety is an essential challenge for clinicians in many restorative treatments, since heat generated from use of high and low speed handpieces,\textsuperscript{1} restorative materials having exothermic setting reactions,\textsuperscript{2} restoration finishing and polishing,\textsuperscript{3} as well as from application of high power light emitting diode (LED) - based light curing units (LCUs) and laser sources to polymerize resin-based materials\textsuperscript{4} may cause pulp temperature (PT) to rise to values considered harmful for the pulp.\textsuperscript{5} For these reasons, in vitro temperature increase within pulp chamber of extracted teeth has been investigated.\textsuperscript{6,7} Over the last decade, heat generated during tooth exposure to light emitted by LED LCUs has become an area of concern for clinicians and researchers. These concerns are based on availability of new, powerful light-curing devices that are capable of emitting light with radiant emittance values exceeding 2,000 mW/cm\textsuperscript{2}.\textsuperscript{7}

Several studies evaluated the thermal stimulus caused by LED LCUs. Most of those studies relied on in vitro techniques using extracted teeth to evaluate temperature rise within the pulp chamber of extracted teeth while external heat sources were applied.\textsuperscript{6,8,9} The most common methodology uses thermocouples inserted inside pulp chambers of extracted teeth to measure temperature changes in this location during exposure to various LCUs.\textsuperscript{6,8,9,10,11} In an attempt to simulate the same physiological conditions observed in vivo, some authors developed specific devices in which the roots of extracted teeth were connected to a pump to provide a water fluid flow inside the pulp chamber so blood flow could be simulated, while the temperature inside the pulp chamber was initially stabilized at an average value close to the body core temperature (approximately 37°C)\textsuperscript{6,8,12} or lower.\textsuperscript{10,11} Conversely, other studies focused only on measuring temperature changes during exposure to LCUs, without simulating tooth physiological conditions.\textsuperscript{9,13} Because of differences between these approaches, along with variance in LCU types, radiant emittances, and characteristics of teeth,\textsuperscript{6,8,9,11,13-18} a wide range in temperature value increases inside the pulp chamber, ranging from 1.5 to 23.2°C, is found in the literature.\textsuperscript{6,8,9,11,13-18} However, despite such differences in results and methodologies, many in vitro studies concluded that the use of some LED LCUs can cause an increase in temperature values within the pulp chamber higher than the threshold temperature increase considered harmful for the pulp (5.5°C).\textsuperscript{5}

Despite the important impact of these conclusions based on in vitro methods on the attention of researchers and manufacturers to this possible issue, it is reasonable to assume that in vitro conditions do not fully reproduce the complex physiological mechanism involved in the real in vivo condition. As a consequence, in vitro analysis is expected not to be capable of precisely reproducing in vivo PT when intact teeth are exposed to a LED light using varying exposure modes. However, due to the lack of in vivo studies that evaluated PT changes during heat stimulus when most in vitro studies were published in the past, and because of differences between tested teeth and tooth condition among studies, no contemporary data are available to confirm how well an in vitro model can reproduce temperature changes seen in the in vivo model, when under thermal stimuli such as the exposure to light emitted from a powerful LED LCU. Recently, an in vivo methodology was published that measured PT within the pulp tissue of human premolars,\textsuperscript{19-21} In that approach, the temperature probe of a wireless temperature acquisition system, previously calibrated using National Institute of Standards and Technology (NIST-traceable) methods, is inserted within the human pulp tissue through an occlusal access, and real-time PT is monitored during thermal stimuli.

Thus, the purpose of this study was to evaluate how similar an in vitro model is able to reproduce temperature increase (ΔTEMP) values compared to the in vivo model, in anesthetized intact, unrestored, human upper premolars, in order to validate the in vitro methodology. The unique feature of this work was that the same premolar teeth tested for in vivo temperature rise were extracted for orthodontic treatment, and were subsequently tested in a clinically relevant in vitro system. In addition, the same LCU used in the in vivo analysis was also used for in vitro analysis. The tested alternative hypotheses were that (1) there are no significant differences in ΔTEMP values and time for temperature to reach maximum (ΔTIME) measurements between in vitro and in vivo models, and (2) both the in vivo and in vitro models show a direct, positive correlation between applied radiant exposure to intact facial tooth surface and both ΔTEMP and ΔTIME.
Methodology

In vivo measurement of pulp temperature increase

After approval by the local Ethics Committee (protocol #255,945), study participants requiring extraction of upper right and left first premolars for orthodontic reasons were selected from the Orthodontic specialization program in Ponta Grossa, Brazil, and were recruited in February, 2013. The participants were seen between March and April, 2013. Inclusion and exclusion criteria were based on previous in vivo studies, and included (1) treatment plans indicating premolar extractions for orthodontic reasons, (2) the presence of healthy, intact, non-caries, and non-restored, fully erupted treatment teeth, and (3) patients with well-controlled health conditions that allowed all procedures involved in the research to be performed with minimal risk. Exclusion criteria included (1) patients who did not agree to volunteer for the study, (2) patients not meeting all of the inclusion criteria.

The in vivo real-time temperature analysis within the pulp was evaluated following a method previously described in the literature. A single tooth at a time received approximately 1.8 ml of 2% mepivacaine hydrochloride (36 mg) with 1:100,000 epinephrine (18 µg) (Mepliadre, DFL Industria e Comércio, Rio de Janeiro, RJ, Brazil) by infiltrative and intraligamental anesthesia. The tooth was isolated using rubber dam, and a small preparation was made in the center of the occlusal surface, using a round diamond bur (#1015, KG Sorensen, Cotia, São Paulo, Brazil) in a high speed handpiece, under air-water spray, until the preparation pulpal floor was near the buccal pulp horn. Then, minute pulp exposure was obtained using a diamond bur (2134, KG Sorensen), with no pulp bleeding. Two calibrated T-type temperature probes were connected to a wireless, NIST-traceable, temperature acquisition system (Temperature Data Acquisition - Thermes WFI, Physitemple, Clifton, NJ, USA) and were immersed in a room temperature (approximately 22.0°C), 0.9% sterile saline solution. Both thermocouples indicated similar temperature values. After pulp exposure was obtained, one probe was inserted directly into the pulp chamber through the narrow access created occlusally, and was positioned over a small groove created on the buccal cusp tip ridge to remain stable, while PT was measured, and ensure that the 1-cm long probe tip penetrated approximately 4 mm into the pulp chamber. The other probe was kept in saline solution and acted as internal reference, confirming that any PT change could be attributed exclusively to exposure from the curing light. The room temperature probe reading remained stable, as the ambient air temperature was controlled by air conditioning set to approximately 22°C. The occlusal preparation was filled with provisional restorative material (Cavitec, CalTHEC Ltda, São José dos Pinhais, PR, Brazil) to minimize heat loss from the tooth through the preparation walls and pulp access, while the probe remained in place. PT reached a stable baseline value (approximately 35°C) after approximately 15 min of real-time analysis, during which data were continuously acquired every 0.2 s. The LCU tip was positioned against and as close as possible to the buccal tooth surface and the tooth was sequentially exposed to the radiant output from a Polywave® LED LCU (Bluephase 20i, Ivoclar Vivadent, Schaan, Principality of Liechtenstein) using the following exposure modes (EMs): 10-s at low intensity (10-s/L); 10-s at high intensity (10-s/H); 5-s at Turbo intensity (5-s/T); and 60-s at high intensity (60-s/H). These exposure modes were selected because they are the most clinically relevant modes used for a wide variety of clinical applications. A 7-min time span between each exposure was allowed for the PT to return to baseline levels. The sequence of EMs was randomly determined and the operator was not aware of which mode was being used. The time of data acquisition when each light mode was applied was recorded using a digital time counter that started recording simultaneously to the beginning of real-time PT analysis, so that time of light activation and correlated temperature measurement could be precisely made. The probe was removed from the tooth at the end of the temperature data acquisition, and the tooth was extracted as planned. Radiographs were taken from the proximal side of the extracted tooth with the probe in position as it was intraorally in order to confirm the proper insertion depth and location of the probe within the pulp chamber during temperature measurement.

A laboratory grade spectroradiometer (USB 2000+, Ocean Optics, Dunedin, FL, USA) connected to a 6-in integrating sphere (Labsphere, North Sutton, NH, USA), previously calibrated using a NIST-traceable light source was used to evaluate the spectral power of the tested EMs. In this regard, the LCU tip end was positioned at the entrance of the integrating sphere,
so that all light emitted from the unit was captured. Wavelength-based, spectral and power emission during each EM were recorded using software (SpectraSuite v2.0.146, Ocean Optics) between 350 to 550 nm, which also provided the total emitted power value for that wavelength range. Radiant emittance values of each EM (mW/cm²) were determined as the total measured power value was divided by the light-emitting area of LCU distal tip end. That analysis was performed before the beginning of both in vivo and in vitro analyses. This value was then multiplied by the light exposure duration in order to derive the value of radiant exposure applied to each tooth surface for each light output mode (J/cm²). The corresponding radiant exposure obtained for each EM was as follows: 10-s/L: 6.56 J/cm²; 10-s/H: 12.44 J/cm²; 5-s/T: 11.02 J/cm²; 60-s/H: 74.64 J/cm².

In vitro analysis of PT increase

The same premolars and LCU used in the in vivo study were tested in the in vitro, so any possible difference between outcomes would be exclusively attributed to the differences between the two models. The extracted teeth were stored in 0.1% thymol (Symrise GmbH, Holzminden, Germany) until the moment they were fixtured to and tested in the in vitro model previously established. In that approach, a test assembly simulated the in vivo environment: controlled intrapulpal physiologic baseline temperature of approximately 35°C and a simulated intrapulpal fluid flow. Figure 1 displays the components of the test setup. A K-type thermocouple (part #TT-K-30-SLE, Omega Engineering Inc., Stamford, CT, USA) was fabricated by joining wire ends with a spot-welder (Model R660, Rocky Mountain/Orthodontics, Denver, CO, USA). Apical portions of the root canals were enlarged and the pulp tissue was removed from the pulp chamber through the enlarged root canals using barbed broaches of various sizes. The thermocouple wire was placed into the pulp chamber through the same occlusal access opening made on the teeth during the in vivo analysis. The thermocouple junction was placed in a similar position to that of the thermocouple used in the in vivo analysis. In order to assure similar placement, x-ray analyses were used to compare thermocouple positioning for each tooth between the in vivo and in vitro conditions. Occlusal access was sealed and stabilized using acid etching and a flowable composite (Aeliteflo, Lot Number 1200001055; Bisco Inc., Schaumburg, IL, USA). Small sections of 16-gage stainless steel tubing were attached to the root ends using acid etching using the same flowable composite. A section of flexible plastic tubing was connected to one tube end and a portion of the remaining end was coiled to increase surface area in contact with the warmed water in the Erlenmeyer flask in which it was immersed. The tubing continued through a hole in the plastic plate placed over the water-filled Erlenmeyer flask (Figure 1). The distal end of the tubing was connected to a water-filled, 20 mL glass syringe. The syringe body was held in a fixed position, while the plunger end was connected to a screw-driven extension of an infusion pump (Model 600-900, Multispeed Transmission Pump, Harvard Apparatus Company, Dover, MA, USA). The rate at which water was circulated in the tooth was calculated from existing literature. The average volume of pulp chamber in a human maxillary first premolar

![Diagram of in vitro test assembly](image-url)
is approximately 18.2 mm³ (0.0182 cc).22 Assuming that the density of human pulp is similar to that of connective tissue (1.027 g/cc),23 the tissue mass in this tooth would be approximately 0.01869 g. The reported pulpal flow rate in dogs is 33.32 ml/min for each 100 g of tissue.24 Applying this rate to the mass of pulpal tissue calculated in a maxillary upper first premolar yields a flow rate of 0.0062 ml/min (6.2 μl/min). The setting used on the infusion pump closest to this value, for the syringe size used (20 ml), was 0.0125 ml/min (12.5 μl/min), which falls well within values others have used for similar test setups.21 The roots of the prepared tooth were placed through an opening in the plastic plate that covered the top of the Erlenmeyer flask. The peripheral area remaining between the coronal root surface and the opening of this tooth would be approximately 0.01869 g. The density of human pulp is similar to that of connective tissue (1.027 g/cc).23 Applying this rate to the mass of pulpal tissue calculated in a maxillary upper first premolar yields a flow rate of 0.0062 ml/min (6.2 μl/min). The setting used on the infusion pump closest to this value, for the syringe size used (20 ml), was 0.0125 ml/min (12.5 μl/min), which falls well within values others have used for similar test setups.21 The roots of the prepared tooth were placed through an opening in the plastic plate that covered the top of the Erlenmeyer flask. The peripheral area remaining between the coronal root surface and the opening of the plastic plate was sealed using the same flowable composite. The flask itself rested in the water of a temperature-controlled bath (Model 1-2000, Thermo-Lift, Bruchler Instruments Inc., Fort Lee, NJ, USA). Water temperature was thermostatically controlled to provide an intrapulpal temperature of approximately 35.5°C (±0.5°C), which is similar to that of the NIST-traceable digital temperature measurement device (model AN6503, Analogic Corporation, Danvers, MA, USA). In this manner, the temperature calibration of the main in vitro temperature measurement system could be achieved by adjusting the water offset temperature to match that of the NIST-traceable device. The real-time profile data of in vitro and in vivo PT increase were plotted into line graphs (Excel 2007, Microsoft), which were used to determine ΔTEMP and ΔTIME.

Time constant (τ) of each thermocouple was determined as previously described.19 For the temperature data acquisition system used in the in vivo model, the τ obtained was 1.46 s, while a τ of 0.05 s was observed for the thermocouple used in the in vitro model. In other words, the time required for the temperature data acquisition system to provide a 1-degree Centigrade temperature change using the in vivo PT analysis was approximately 0.07 s, while 0.0029 s were required for the thermocouple to provide the same 1-degree Centigrade temperature change using the in vitro setup.

Statistical analyses

The ΔTEMP and ΔTIME data obtained in vivo and in vitro were subjected to two-way repeated measures ANOVA, followed by Bonferroni's post-hoc test at a pre-set alpha of 0.05. Linear regression analysis was performed to examine the relationship between applied radiant exposure level and ΔTEMP, as well as between radiant exposure and ΔTIME in both conditions. The comparison between the slopes of both regression lines obtained from the in vivo and in vitro data was performed by performing t-tests as well as by comparing possible overlaps in the 95% confidence intervals (CI) for the mean slope values. No overlap indicated a significant difference, and overlap suggested no significant difference. Post-hoc power analysis was performed for the statistical analysis of ΔTEMP and ΔTIME. All analyses were performed using statistical software on a personal computer (Statistics 19, SPSS Inc, IBM Company, Armonk, NY, USA).
Results

In vivo and in vitro ΔTEMP and ΔTIME during curing light exposures

For the number of evaluated teeth (n=15), the in vivo study was adequately powered for EM and condition (in vitro and in vivo) factors (over 99.0%; α=0.05). Table 1 presents the comparison between in vitro and in vivo ΔTEMP values. Overall, the in vitro model recorded significantly higher ΔTEMP values than the in vivo model, regardless of EM. Although a significant, positive relationship [in vivo: (r²=0.917; p<0.001; in vitro: r²=0.919; p<0.001)] was observed between delivered radiant exposure and ΔTEMP in both models, the slope of the regression line created in vitro was significantly higher than that observed in vivo (p<0.001, no overlap in 95% CI of slope values; Figure 2a). As a result, for the 10-s/H, 5-s/T, and 60-s/H EMs, the in vitro ΔTEMP values were approximately 1.6 times higher than in vivo values, while in vitro ΔTEMP values were approximately 1.8 times higher than in vivo results when the 10-s/L EM was used. However, these differences were only 0.4°C for 10s-L, and 0.6°C for both the 10s-H and 5s-T EMs. Using the 60s-H setting produced 2.9°C greater value in the in vitro model than when testing in vivo.

Use of the 60-s/H EM caused the highest ΔTEMP in both in vivo and in vitro models (p<0.001), whereas the 10-s/L EM produced the lowest values (p<0.001). Despite the in vivo ΔTEMP average values being lower than 5.5°C, some teeth exhibited higher ΔTEMP values than that threshold temperature (Table 1). On the other hand, the in vitro model indicated that, when the same 60-s/H EM was used, all teeth produced ΔTEMP values exceeding 5.5°C. In both in vivo and in vitro models, ΔTEMP values of 10-s/H group were not significantly different from those of 5-s/T EMs, which in turn, produced significantly higher ΔTEMP values than did the 10-s/L mode (p<0.001).

Despite such differences in ΔTEMP values, no significant differences in ΔTIME values were noted when the in vivo model was compared to that of in vivo results, regardless of EM (Table 2). In this regard, for both conditions, the 5-s/T mode generated the shortest ΔTIME, while 60-s/H EM produced to the highest intervals. The 10-s/H and 10-s/L EMs developed higher ΔTIME values than did the 5-s/T mode, and lower values than the 60s-H mode. A significant, similar, positive relationship (in vivo: r²=0.917; p<0.001; in vitro: r²=0.919; p<0.001) was also observed between delivered radiant exposure and ΔTIME for both test conditions (Figure 2b). No significant difference was noted between the slopes of the regression lines from in vivo and in vitro data (overlap in the 95% CI of mean slope values).

For both in vivo and in vitro models, the time/temperature profiles (Figure 3) of PT increase during exposure to the LED LCU showed a rapid increase in PT rise approximately 2 s after light activation, while higher radiant exposure levels caused greater magnitude of the PT increase. For all EMs, the in vitro PT increase profile was also more pronounced during and after exposure to LED light than that observed in vivo. For most EMs, when the LCU shut off, PT continued to increase for a few seconds for both test conditions, except for the 60-s/H mode, in which PT increase continued to a different extent.

Table 1- Mean ΔTEMP (SD) (°C) of in vivo and in vitro results values during exposure using different exposure modes

| Exposure duration - Curing Mode | Test Condition | 10s-L | 10s-H | 5s-T | 60s-H |
|-------------------------------|---------------|-------|-------|------|-------|
| In vivo                       | 0.5 (0.2)ab   | 1.0 (0.3)ab | 1.0 (0.3)ab | 4.8 (1.0)aa |
| In vitro                      | 0.9 (0.3)ac   | 1.6 (0.3)b   | 1.6 (0.4)b   | 7.7 (1.6)ab |

Means followed by similar letter (uppercase letters: within column (between test conditions within an EM); lower case letters: within row (within test condition among EMs) are not significantly different. L = low H = High T = Turbo n = 15 Specimens per condition

Table 2- Mean ΔTIME (SD) (s) from start of exposure to reach maximum intrapulpal temperature (ΔTEMP) for in vivo and in vitro test conditions, using different exposure durations and exposure modes

| Exposure duration - Curing Mode | Test Condition | 10s-L | 10s-H | 5s-T | 60s-H |
|-------------------------------|---------------|-------|-------|------|-------|
| In vivo                       | 18.9 (6.1)ab  | 18.2 (5.9)ab | 13.5 (6.8)ac | 65.7 (5.5)aa |
| In vitro                      | 15.9 (3.4)ab  | 17.2 (4.2)ab | 13.6 (5.0)ac | 62.6 (2.2)aa |

Means followed by similar letters (uppercase letters: within column (between test conditions within an EM); lower case letters: within row (within test condition among EMs) are not significantly different. L = low H = High T = Turbo n = 15 Specimens per condition
values dropped immediately after the LED light shut off (Figures 3g and 3h). In teeth exposed to the 5-s/T EM \textit{in vitro}, approximately half of the total PT increase occurred during exposure to the LED light, while the other half was noted after the light shut off (Figure 3f).

**Discussion**

To the extent of our knowledge, this is the first study comparing \textit{in vitro} results of temperature rise within the pulp chamber during exposure to light emitted from a Polywave LCU with those obtained \textit{in vivo} in intact, human premolars, in an attempt to validate the \textit{in vitro} model. Based on the current findings, the \textit{in vitro} model recorded higher ΔTEMP values than the \textit{in vivo} model, regardless of EM. It is worth noticing, however, that the greatest difference in ΔTEMP between \textit{in vitro} and \textit{in vivo} models was only noted when 60-s/H was delivered, whereas only small differences in ΔTEMP were observed when other, more clinically relevant, EMs were delivered to the teeth. Curiously, despite such differences, no significant difference in ΔTIME was observed between both test conditions, even when \textit{in vitro} ΔTEMP was 1.6 times higher than \textit{in vivo} values. Such a finding infers that the \textit{in vitro} rate of PT increase was higher than that...
observed in the in vivo model, as illustrated by the
time/temperature profiles of PT increase (Figure 3).
Therefore, the first alternative hypothesis, stating
that there are no significant differences in ΔTEMP
values and ΔTIME measurements between in vitro and
in vivo models, was partially rejected. Because the
LED LCU, EMs and teeth were the same for both test
conditions, such divergence between these results may
be attributed to the dynamic regulatory mechanism
of pulp tissue for heat distribution during temperature
Figure 3- Examples of in vivo and in vitro real time temperature increase in the pulp chamber during exposure to light using 10-s/L (a and b), 10s/H (c and d), 5-s/T (e and f), and 60-s/H (g and h) EMs. The blue area represents the time interval when teeth were exposed to the curing light.
changes in this tissue used to dissipate heat transferred by external thermal stimuli throughout the dentine/pulp complex. In other words, when any external thermal stimuli generates more heat, fluid movement, either inwards or outwards from the pulp, will increase in an attempt to reduce the magnitude of PT rise.

For this reason, the actual in vivo pulp regulatory system has shown to be more effective in dissipating external heat than the simulated pulpal fluid flow in the in vitro model.

The importance of the increase in pulp blood flow rate during exposure to an LED light may also be confirmed by the comparison between in vivo and in vitro linear regression analyses. Although both in vivo and in vitro models presented significant, positive relationships (in vivo: $r^2=0.917$; p<0.001; in vitro: $r^2=0.919$; p<0.001) between delivered radiant exposure and ΔTEMP, the significantly lower regression slope obtained from the in vivo results (Figure 2a) infers that an in vivo defense mechanism of pulp blood flow against heat rise became more effective as higher radiant exposure values were delivered to teeth. Therefore, the resulting lack of parallelism between both regression lines for ΔTEMP implies that both models presented a significant, positive, but not similar relationship between ΔTEMP and radiant exposure. For this reason, the second hypothesis was partially rejected. One could state that exposure modes delivering radiant exposure values between 20 and 70 J/cm² should be added to the sequence of EMs used in the current study, so a more reliable regression analysis could be obtained. However, a recent in vivo study evaluating PT increase in human premolars with Class V preparations observed similar relationship between radiant exposure values and PT rise. In that study, another EM delivering a radiant exposure of approximately 37.3 J/cm² was tested. Therefore, it is reasonable to expect that the regression analysis using the current data provides a reliable relationship between radiant exposure values and PT increase.

Another reason for such differences in ΔTEMP and rate of PT increase between in vitro and in vivo models is the difference between the content inside the in vitro pulp chamber, and that inside the in vivo pulp chamber, once the pulp tissue was removed from the pulp chambers before the extracted teeth were used in the in vitro model. In this regard, when the enamel surface is hit by blue light, part of the light energy is either reflected or converted into thermal energy, while the remaining portion passes through to the substrates below. Because the thermal conductivity and thermal diffusivity of pulp tissue and blood (0.63) are close to those of water (0.58), the converted thermal energy released by the inner dentin passes through pulp tissue, blood, or water to reach the thermocouples similarly. On the other hand, the remaining portion of light that passes through enamel and dentin interacts differently between the in vitro and in vivo environments inside the pulp chamber. In the in vivo model, due to blood content, pulp is rich in hemoglobin, a chromophore with a coefficient of absorption within the blue light emission wavelength range, so when blue light reaches the pulp tissue, photons are strongly absorbed by blood chromophores to be partially converted into thermal energy, resulting in a slower PT increase in vivo than that observed in vitro. Furthermore, in this context, because of the constant blood flow, the warmed chromophores from absorbed photons are quickly replaced by cooler ones, so most of the heat generated by irradiance of this tissue is dissipated. However, it should be noted that the intensity of the curing light is severely attenuated by the thick buccal wall of intact premolars, so the influence of residual irradiance on PT rise should be lower than the influence of thermal conduction from the heated dentin substrate. Only further investigation could confirm such an assumption.

In the current study, although the in vitro ΔTEMP values were significantly higher than those observed in vivo, both conditions had no influence on the EM effects on PT rise. This fact seems valid because both in vivo and in vitro 10-s/L exposures showed the lowest PT values, which were significantly lower than when the 10-s/H and 5s-T modes were used, while the 60-s/H mode produced the highest ΔTEMP. Indeed, because of the aforementioned differences between test methods, the in vitro 60-s/H EM caused higher ΔTEMP (7.7±1.6°C) than the well-known threshold temperature of 5.5°C, in all extracted teeth, while the same EM applied in vivo resulted in lower average increase (4.8±1.0°C) than that threshold temperature. Based on these findings, it is evident that, overall, in vitro studies may overestimate the effects of LCU exposure on pulp chamber temperature increase when high radiant exposure values are delivered to the teeth.

Although much of the differences in PT rise between in vivo and in vitro methodologies may be attributed
to the physiological response of the pulp tissue against heat rise, some features in these methodologies may have contributed to the differences in the temperature rise within the pulp chamber. For instance, the Class I preparation in the in vivo method was sealed with provisional restorative material, while the Class I preparation in the in vitro methodology was sealed with flow resin composite to keep the simulated fluid flow within the pulp chamber. Because zinc-based cements, such as the provisional material used in the in vivo model, allow for greater heat transfer than do resin composites, these provisional restorative materials may have allowed more heat to be released through the occlusal cavity than when the Class I preparation was sealed with resin composite.

Despite these differences, the current results demonstrated that the in vitro model is capable of detecting temperature changes as the delivered radiant exposure values increased in a similar pattern as that observed in the in vivo model. Therefore, the only flaw observed in the in vitro model is the overestimation of the temperature rise within the pulp chamber. In this regard, some studies have shown that the increase in the in vitro simulated fluid flow rate resulted in lower temperature rise within the pulp chamber. Based on this evidence, increasing fluid flow rate in the in vitro model could be a valuable alternative to compensate for such difference between in vivo and in vitro models. Further studies are required to determine the settings of in vitro methodologies to provide temperature increase values within the pulp chamber similar to those observed in vivo.

Conclusions

Within the limitations imposed by the methodologies used, the following conclusions may be made:

1- The in vitro model detected higher PT increase than the in vivo model, when the same teeth were exposed to the same exposure modes from the same Polywave® LED LCU;

2- In vitro PT increase values were close to in vivo values when clinically relevant exposure modes were delivered (between 7 and 12 J/cm² for 10- and 5-s exposures), and

3- A significant, positive and non-parallel correlation was observed between delivered radiant exposure and PT increase for both in vivo and in vitro models, so the influence of varying exposure modes on PT increase was similar for both models.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1- Ozturk B, Usumez A, Ozturk AN, Ozer F. In vitro assessment of temperature change in the pulp chamber during cavity preparation. J Prosthet Dent. 2004;91(5):436-40.
2- Hannig M, Bott B. In-vitro pulp chamber temperature rise during composite resin polymerization with various light-curing sources. Dent Mater. 1999;15(4):275-81.
3- Brinenso Bo, Ernst CP, Willershausen-Zonnchen B. Rise in pulp temperature during finishing and polishing of resin composite restorations: an in vitro study. Quintessence Int. 1995;26(5):361-5.
4- Leprince J, Devaux J, Mullier T, Vreven J, Leloup G. Pulp temperature rise and polymerization efficiency of LED curing lights. Oper Dent. 2010;35(2):220-30.
5- Zach L, Cohen G. Pulp response to externally applied heat. Oral Surg Oral Med Oral Pathol. 1965;19:515-30.
6- Kodonas K, Gogos C, Tzifa C. Effect of simulated pulpal microcirculation on intrachamber temperature changes following application of various curing units on tooth surface. J Dent. 2009;37(6):485-90.
7- Rueggeberg FA. State-of-the-art: dental photocuring - a review. Dent Mater. 2011;27(1):39-52.
8- Kodonas K, Gogos C, Tzifas D. Effect of simulated pulpal microcirculation on intrapulpal temperature changes following application of heat on tooth surfaces. Int Endod J. 2009;42(3):247-52.
9- Millen C, Ormond M, Richardson G, Santini A, Miletic V, Kew P. A study of temperature rise in the pulp chamber during composite polymerization with different light-curing units. J Contemp Dent Pract. 2007;8(7):29-37.
10- Daronch M, Rueggeberg FA, Hall G, De Goes MF. Effect of composite temperature on in vitro intrapulpal temperature rise. Dent Mater. 2007;23(10):1283-8.
11- Park SH, Roulet JF, Heintze SD. Parameters influencing increase in pulp chamber temperature with light-curing devices: curing lights and pulpal flow rates. Oper Dent. 2010;35(3):353-61.
12- Jakubinek MB, O'Neill C, Felix C, Price RB, White MA. Temperature excursions at the pulp-dentin junction during the curing of light-activated dental restorations. Dent Mater. 2008;24(11):1468-76.
13- Eldeniz AU, Usumez A, Usumez S, Ozturk N. Pulpal temperature rise during light-activated bleaching. J Biomed Mater Res B Appl Biomater. 2005;72(2):254-9.
14- Baik JW, Rueggeberg FA, Liewehr FR. Effect of light-enhanced bleaching on in vitro surface and intrapulpal temperature rise. J Esthet Restor Dent. 2001;13(6):370-8.
15- Baroudi K, Silikas N, Watts DC. In vitro pulp chamber temperature rise from irradiation and exotherm of flowable composites. Int J Paediatr Dent. 2009;19(1):48-54.
16- Oberholzer TG, Makofane ME, du Preez IC, George R. Modern high powered led curing lights and their effect on pulp chamber temperature of bulk and incrementally cured composite resin. Eur J Prosthodont Restor Dent. 2012;20(2):50-5.
17- Rajesh Ebenezar AV, Anilkumar R, Ramachandran S, Srinivasan MR. Comparison of temperature rise in the pulp chamber with different light curing units: an in-vitro study. J Conserv Dent. 2010;13(3):132-5.
18- Yazici AR, Muftu A, Kugel G, Perry RD. Comparison of temperature changes in the pulp chamber induced by various light curing units, in vitro. Oper Dent. 2006;31(2):261-5.
19- Runnacles P, Arrais CA, Pochapski MT, Santos FA, Coelho U, Gomes JC, et al. In vivo temperature rise in anesthetized human pulp during exposure to a polywave LED light curing unit. Dent Mater. 2015;31(5):505-13.
20- Runnacles P, Arrais CA, Pochapski MT, Santos FA, Coelho U, Gomes JC, et al. Direct measurement of time-dependent anesthetized in vivo human pulp temperature. Dent Mater. 2015;31(1):53-9.
21- Zarpellon DC, Runnacles P, Maucoski C, Gross DJ, Coelho U, Rueggeberg FA, et al. Influence of Class V preparation on in vivo temperature rise in anesthetized human pulp during exposure to a Polywave(R) LED light curing unit. Dent Mater. 2018;34(6):901-09.
22- Fanibunda KB. A method of measuring the volume of human dental pulp cavities. Int Endod J. 1986;19(4):194-7.
23- Tissue properties [Internet]. 2018 [cited 2018 July 30]. Available from: https://itis.swiss/virtual-population/tissue-properties/database/density/.
24- Kim S, Dorscher-Kim JE, Liu M. Microcirculation of the dental pulp and its autonomic control. Proc Finn Dent Soc. 1989;85(4-5):279-87.
25- Brannstrom M, Johnson G. Movements of the dentine and pulp liquids on application of thermal stimuli. an in vitro study. Acta Odontol Scand. 1970;28(1):59-70.
26- Raab WH. Temperature related changes in pulpal microcirculation. Proc Finn Dent Soc. 1992;88 Suppl 1:469-79.
27- Dederich DN. Laser/tissue interaction: what happens to laser light when it strikes tissue? J Am Dent Assoc. 1993;124(2):57-61.
28- de Vree JH, Sjoberg LA, Plasschaert AJ. A simulation model for transient thermal analysis of restored teeth. J Dent Res. 1983;62(6):756-9.
29- Ponder E. The coefficient of thermal conductivity of blood and of various tissues. J Gen Physiol. 1962;45:545-51.
30- Jacques SL. Optical properties of biological tissues: a review. Phys Med Biol. 2013;58(11):R37-61.
31- Fodor L, Ullman Y, Elman M. Aesthetic Applications of Intense Pulsed Light: Springer; 2011.
32- Nakajima M, Arimoto A, Prasansuttiporn T, Thanatvarakorn O, Foxton RM, Tagami J. Light transmission characteristics of dentine and resin composites with different thickness. J Dent. 2012;40 Suppl 2:e77-82.
33- Price RB, Murphy DG, Derand T. Light energy transmission through cured resin composite and human dentin. Quintessence Int. 2000;31(9):659-67.
34- Drummond JL, Robledo J, Garcia L, Toepeke TR. Thermal conductivity of cement base materials. Dent Mater. 1993;9(1):68-71.
35- Farah RI. Effect of simulated pulpal blood flow rate on the rise in pulp chamber temperature during direct fabrication of exothermic provisional restorations. Int Endod J. 2017;50(11):1097-103.