**THE IMPORTANCE OF SIMULATED PULPAL PRESSURE IN “*IN VITRO”* STUDIES**

**Abstract**

**Objective:** To evaluate the effects of simulated pulpal pressure (SPP) on the variation of intrapulpal temperature (****T) during light-curing of the adhesive system. **Material e methods:** One hundred sound human molars were sectioned from the highest pulp horn to obtain a 2-mm of thickness dentin. The specimens were randomly divided into two groups: (n=50), according to the presence or absence of SPP (15 cm H2O). The specimens were sequentially treated as follows: 37% phosphoric acid (Scotchbond Universal; 3M/ESPE), adhesive system (Scotchbond Universal/3M ESPE) and light-curing (10 seconds). Next, composite resin block (Filtek Z350 XT; 3M/ESPE) was built up onto the prepared area before ligh-curing with a LED curing unit (Demi Plus, Kerr Corporation, Middleton, WI, USA), operating at power density of 1200 mW/cm2. ****T was evaluated during adhesive light-curing with a K-type thermometer put inside the pulp chamber. Data were analysed by using Mann-Whitney’s test (at 5%). **Results:** According to Mann-Whitney’s test, the absence group presented a ****T of 2°C, whereas the presence group 1°C. The mean values of ****T were 0.82±0.56°C for the presence group and 2.30±0.73°C for the absence group. **Conclusions:** Simulated pulpal pressure significantly reduced the temperature rise in the pulp chamber during light-curing of the adhesive system, showing the importance of inserting this protocol of simulated pulpal pressure in the laboratory procedures.

**Keywords**: Dentin. Light-curing. Pulpal pressure. Temperature.

**INTRODUCTION**

Light-curing has become an indispensable tool in the clinical practice during the polymerization process of adhesive systems, resin luting cements and composite resins [1]. The clinical success of resinous materials, as well as their longevity, depends directly on an adequate light-curing process as it is related to the effectiveness of the photoactivation units, intensity and spectrum of light emission.

Nowadays, the light source most used in the light-curing of resinous materials is the light-emitting diode (LED) [2], which through the use of gaseous semiconductors (usually gallium nitrate) generate the blue light necessary for the polymerization. The light emitted by LEDs has a wavelength between 450 nm and 470 nm, coinciding with the maximum absorption spectrum of the most used photoinitiator, the camphorquinone (468 nm) [3]. These devices have the following advantages: do not emit infrared radiation, produce low heat and have longer durability [2-4].

The first LED curing devices launched in the market were the so-called first-generation LEDs. They had low power density (≤300 mW/cm2) which resulted in lower curing efficiency efficiency of composite resins [5, 6]. With the technological evolution, there was the emergence of the second generation of LED curing devices, with power density between 300 and 650 mW/cm2, and nowadays there are LED devices with high intensity of light (around de 1200 mW/cm2), the so-called third-generation [1, 4].

Although the third-generation devices have a higher efficiency of light-curing compared to previous generations, with a greater depth of polymerization and degree of conversion due to high light intensity, previous studies have shown that the association of these factors can raise the temperature within the pulp chamber [4, 5]. According to Asmussen and Peutzfeldt [7], another factor that may be associated with this temperature rise is the power density of LED lights. Therefore, the light source to be used may have a direct influence on the temperature variation inside the pulp chamber, since the intensity and light density of the LED curing device are linked to the production of heat.

Most clinical procedures using LED light curing units are performed on the dentin surface. Dentin presents structural variability with a dense network of tubules whose density, diameter and orientation patterns vary in depth, increasing in quantity and diameter towards the pulp. These tubules are filled with interstitial fluid that moves through capillaries driven by intrapulpal hydrostatic pressure, favoring wetness on the surface due to direct communication with the pulp [8].

The literature reports that there is a correlation between increase in intrapulpal temperature and occurrence of damage to pulp tissues, showing that increases above 5.5ºC can irreversibly affect the pulp [9]. Considering that the use of third generation LEDs promotes temperature elevation in the pulp chamber, it is important to evaluate this factor during clinical procedure.

Simulated intrapulpal pressure has been a reliable methodology in *in vitro* studies as it simulates clinical conditions analogous to those *in vivo*, such as dentin wetness [10-13]. Recent studies have shown that the fluid flow generated by the pressure gradient makes it possible to dissipate the heat through the renewal of the liquid inside the pulp chamber [12].

Considering the relevance of the topic to clinical practice and the lack of studies on the thermal effects of high intensity light-curing during restorative procedure on dentin surface, the aim of this study was to evaluate the effect of simulated pulpal pressure (SPP) on the variation of temperature (****T) during light-curing of the adhesive system. The null hypothesis was that simulated pulpal pressure would not result in a significant influence on the variation of temperature during light-curing of adhesive system.

**MATERIAL AND METHODS**

*Specimen preparation*

This study was approved by the local institutional review board according to protocol number 283.030. One hundred sound human molars that were extracted for therapeutic reasons were used in this study. The teeth were cleaned by using periodontal curettes and stored in deionized water at 4ºC, for a maximum period of six months [14, 15].

The teeth were attached to an acrylic holder (2.5 cm diameter and 2.0 cm height) with dental wax and then sectioned using a laboratory cutting machine (Labcut 1010, Extec Technologies Inc., Enfield, CT, USA), at low-speed and under water cooling. Two sections were made: one parallel to the occlusal surface to expose dentin and other 1 mm below the enamel-cementum junction to separate the crown from the roots. The pulpal soft tissues were removed by using curettes.

The dentin specimens were standardized at 2-mm thickness from the highest pulp horn [16], after measurement with a caliper (Otto-Arminger & Cia Ltda., RS, Brazil). Dentin surface was polished by using a polishing device (DP-10, Panambra, São Paulo, SP, Brazil) with 600-grit aluminum oxide abrasive discs (Extec Corp., Enfield, CT, USA), under water cooling.

The one hundred specimens were randomly divided into two groups (n = 50), according to the treatment to be submitted: presence and absence of simulated pulpal pressure.

*Simulated pulpal pressure*

 In the group submitted to simulated pulpal pressure, the self-cured acrylic resin holders (Jet, Artigos Odontológico Clássico, Campo Limpo Paulista, SP, Brazil) were constructed measuring 1.5 cm x 1.5 cm x 0.5 cm. In these holders, two holes were done to simulate water fluid flow inside the pulp chamber. The pressure device was adapted from a model proposed elsewhere [12].

 The device for simulated pulpal pressure had a reservoir filled with deionized water at 37ºC [17-19], placed 15 cm above the level of the pulp chamber [20]. Before applying tge simulated pulpal pressure, the deionized water was injected into the pulp chamber to avoid bubbles inside the chamber and to assure full filling.

*Temperature measurement*

To measure the change in temperature, a digital thermometer with a K-type thermocouple sensor (MT-507, Minipa Indústria e Comércio Ltda., São Paulo, Brazil) was used. The thermocouple sensor monitored the temperature inside the pulp chamber during the light-curing of the adhesive system on the occlusal surface. The maximum peaks of temperature (ºC) were recorded for 10 seconds.

In the group ABSENCE (no pulpal pressure simulation), the pulp chamber was filled with thermal paste (Implastec, Tietê, SP, Brazil) [19, 21]. This enabled the determination of temperature changes within the pulp chamber during light-curing without the interference of the external medium.

In the group PRESENCE, the specimens were attached to the holders to simulate pulpal pressure. The thermocouple sensor was placed below the roof of the pulp chamber (in the highest pulp horn) and kept in contact with the dentin and in the presence of water.

*Adhesive and restorative procedures*

 The dentin surface was etched with 37% phosphoric acid (Scotchbond Universal Etchant Etching Gel, 3M ESPE Dental Products, Saint Paul, MN, USA), for 15 seconds and rinsed. To remove excess water, the surface was gently dried with jets of air. An adhesive system (Scotchbond Universal, 3M ESPE Dental Products, Saint Paul, MN, USA) was applied according to the manufacturer’s instructions. Next, the adhesive system was light-cured with LED light-curing device (Demi Plus, Kerr Corporation, Middleton, WI, USA), at power density of 1200 mW/cm2 for 10 seconds.

 Composite resin blocks (4 mm in diameter and 2 mm in height) were build up on the dentin surfaces. Composite resin (Filtek Z350 XT, 3M/ESPE, St Paul, MN, USA) was inserted in increments of about 2 mm each before being light-cured for 20 seconds.

*Statistical analysis*

With regard to the change in temperature (ºC) occurring during the light-curing of adhesive system*,* the difference between the maximum value and the initial temperature (before curing) was calculated, according to the formula ∆T = Tmaximum- Tinitial. The resulting data were submitted to Mann-Whitney’s test at significance level of 5% (*p*<0.05).

**RESULTS**

According to the Mann-Whitney’s test, the group Absence presented a significant temperature difference of 2°C, whereas the group Presence showed a difference of 1°C (Table 1). The mean temperature differences in the group Absence (2.30 ± 0.73°C) were statistically significant compared to those in group Presence (0.82 ± 0.56°C).

Table 1. Mann-Whitney’s test (5%).

|  |  |  |  |
| --- | --- | --- | --- |
| **Groups** | **Mean ± SD** | **CoefVar** | **Median** |
| Absence | 2.30 ± 0.73 | 31.97 | 2.00 |
| Presence | 0.82 ± 0.56 | 68.32 | 1.00 |

With regard to the percentage of temperature variation during light-curing, it was observed that the group Presence showed 66% of 1-degree variation and 26% without variation (0°C). The group Absence showed predominantly a temperature variation around 2°C (50%) and 3°C (34%), as shown in Figure 1.

**DISCUSSION**

This *in vitro* study has evaluated the variation of temperature inside the pulp chamber, with and without simulated pulpal pressure, during light-curing of adhesive system. In view of the experimental conditions, the highest temperature variation was observed in the group with absence of SPP (coefficient of variation = 68.32). The increased heat generated by the lack of fluid inside the pulp chamber resulted in an increase in intrapulpal temperature. Thus, the null hypothesis was rejected.

When light-curing is used during adhesive procedures, the increase of temperature inside the pulp chamber is directly proportional to the intensity and density of light applied [4, 7]. In our study, we have observed that the variation of temperature in the internal portion of the dentin had a mean variation of 2 to 3ºC in the absence group, thus corroborating with the findings of Mahant et. al. (2016) [1], who observed lower intrapulpal temperature with third generation LEDs. According to previous studies, temperature higher than 5.5ºC causes pulp necrosis, despite producing reversible pulp injuries from 3.3ºC [9]. However, the absence of pulp fluid represents a non-vital condition. Then, there would be no harm to pulp tissues with the increase in temperature.

In a previous study [22], there was a direct correlation between light intensity and pulp temperature increase. Godoy et. al. (2007) [4] showed that LED-based devices with higher light intensity showed higher temperature rise. However, the present study has evaluated only one type of light-curing (1200 mW/cm2), making it difficult to compare intensities and types of light-curing used in other studies.

Third generation LEDs require a shorter polymerization time when compared to other LED generations or halogen light-curing [1, 23], which may reduce the variation of intrapulpal temperature, although the high-light intensity of the device might justify the results obtained in this study. The group Absence, light-cured for 10 seconds at high intensity showed a significant difference in temperature variation. In the studies by Mouhant et al. (2016) [24], increases in activation time would lead to a higher temperature and, consequently, to damage to pulp tissues. However, the thermal effect of a longer light-curing time according to type of photo-polymerization was not the aim of this study and is still unknown.

According to Mahant et al. (2016) [1], the thinner the dentin, the higher the heat inside the pulp chambre, which require caution in the use of light-curing for deep cavities, mainly regarding devices with high intensity and light density. Instead, in the studies by Santis et. al. (2017) [13], it was shown that dentin thickness did not interfere with the intrapulpal temperature evaluated. However, simulated pulpal pressure had a significant influence on the results. Corroborating the studies by Santis et. al. (2017) [13] and Silva et. al. (2016) [12], our study presented a difference in the variation of temperature between the groups with and without SPP groups for the same dentin thickness (2 mm), indicating that temperature is more influenced by the presence of pulpal pressure than the dentin thickness.

As for the presence of SPP, it was observed that there was a low variation of temperature inside the pulp chamber (66% of 1-degree variation and 26% with no variation), thereby demonstrating that the presence of fluid during light-curing interfered significantly with the temperature evaluated. The literature reports that the presence of liquids inside the pulp cavity may alter the absorption of heat [25, 26], allowing dissipation of the fluid through the renewal of the liquid inside the pulp chamber [12]. In addition, dentin thermal conductivity and pulp blood circulation may also contribute to the reduction of intrapulpal temperature [4, 27].

Although the variation of temperature found in this study did not negatively interfere with the pulp tissues, the mean values obtained elucidated the effect of pulp fluid flow simulation by showing the interference of liquids inside the pulp chamber with temperature evaluation, similar to that observed by Silva et. al.(2016) [12] and Santis et. al. (2017) [13].

Thus, our results highlight the importance of simulated pulpal pressure for *in vitro* studies on intrapulpal temperature due to its closeness to *in vivo* conditions, which allows a more reliable analysis. It is also important to point out the importance of this technique for light sources with higher intensities and energy densities, such as high-intensity LED light-curing (3200 mW/cm2) and the different types of laser energy, which could produce a greater amount of heat on dentin surface depending on the exposure time.

Therefore, further laboratory research is necessary to verify the thermal effects of a longer activation time using high-intensity LED light-curing as well as of different dentin thickness according to the simulated pulpal pressure technique.

**CONCLUSIONS**

Within the limitations of the study, it can be concluded that the simulated pulpal pressure has significantly reduced the variation of temperature during light-curing of adhesive system.

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**Figure Legend**

Figure 1. Graph of percentage of temperature variation ****T during light-curing.