RESEARCH ARTICLE

The effect of halogen bulb and light-emitting diode light curing units on temperature increase and fibroblast viability [version 1; peer review: 1 approved, 1 approved with reservations]

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Abstract

Background: This study aimed to compare the temperature increase produced by halogen bulb (HAL) and light-emitting diode (LED) light curing units (LCUs) by irradiating dentin discs (0.5 mm and 1 mm thickness), and to evaluate their cytotoxic effects on fibroblast culture in the presence of dentin discs due to the increasing demand on resin composite restorations and teeth bleaching for esthetic purposes.

Methods: A total of 20 bovine incisors were used to obtain dentin discs and divided into four experimental groups (n=10): HAL0.5: irradiation with halogen-tungsten bulb Curing Light XL 3000 at an intensity of 470 mW/cm² over a dentin disc of 0.5 mm; LED0.5: irradiation with LED Optilight Max (GNATUS- Ribeirão Preto, SP, Brazil) at an intensity of 1200 mW/cm² over a dentin disc of 0.5 mm; HAL1: irradiation as in HAL0.5 but over a dentin disc of 1 mm; LED1: irradiation as in LED0.5 but over a dentin disc of 1 mm. The temperature increase was measured using a digital thermometer and the cytotoxicity was evaluated using an MTT assay with a mouse fibroblast cell line (L929). Parametric Data were analyzed by ANOVA and Tukey and non-parametric data were analyzed by Kruskal Wallis with Conover-Iman for non-parametric data (all with $\alpha=0.05$).

Open Peer Review

Approval Status

	1	2
version 1		
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Any reports and responses or comments on the article can be found at the end of the article.

Results: A significant statistical difference was found between the groups HAL0.5 and HAL1 and both were different of LED0.5 and LED1 which presented higher temperature. All the experimental groups were different of the control group (without irradiation), and promoted reduction of cellular viability.

Conclusions: HAL LCU promoted a lower temperature change in the dentin compared to LED, regardless of the dentin thickness (0.5-1 mm). Both HAL and LED LCUs decreased fibroblast viability; however, LED promoted more significant cytotoxic effects.

Keywords

Halogen light, Light-emitting-diode, mouse fibroblasts, temperature increase

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Author roles: **Memari Trava G:** Data Curation, Investigation, Resources, Writing – Original Draft Preparation; **Almeida Santos J:** Data Curation, Investigation, Resources, Writing – Original Draft Preparation; **Paula Ramos L:** Methodology, Resources, Writing – Original Draft Preparation; **Rosário Estevam dos Santos PB:** Data Curation, Methodology, Writing – Original Draft Preparation; **Abu Hasna A:** Formal Analysis, Writing – Original Draft Preparation, Writing – Review & Editing; **Yui KC:** Conceptualization, Writing – Review & Editing; **Bressane A:** Data Curation, Formal Analysis, Writing – Original Draft Preparation; **Dias de Oliveira L:** Conceptualization, Project Administration, Writing – Original Draft Preparation, Writing – Review & Editing; **Spalding M:** Conceptualization, Formal Analysis, Methodology, Project Administration, Writing – Review & Editing

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Introduction

Dentistry patients are looking for esthetically pleasing smiles and increasingly demanding resin composite restorations and teeth bleaching (Al Otaibi *et al.*, 2020; Sebold *et al.*, 2020). These processes require photopolymerization and photoactivation by halogen bulb (HAL) and light-emitting diode (LED) light curing units (LCUs) (Gallinari *et al.*, 2020; Pieniak *et al.*, 2014). However, these LCUs can increase the temperature and induce thermal transfer, depending on the light source intensity and type (Armellin *et al.*, 2016; Kim *et al.*, 2017).

The elevated temperature resulting from daily clinical procedures can cause an increase in pulp temperature, with the subsequent development of symptoms such as hyperalgesia, dentin hypersensitivity, and spontaneous typical pain of acute pulpitis (Vinagre *et al.*, 2019). The thermal change causes heat-induced bone tissue injury (Eriksson & Albrektsson, 1983) and pulp tissue necrosis, pathology or alteration (Matalon *et al.*, 2010; Nyborg & Brännström, 1968).

Additionally, some thermal injuries may affect the surrounding tissue cells (Baldissara *et al.*, 1997) and form lesions in the odontoblastic layer, which leads to its degeneration, protein coagulation and fluid expansion in the dentinal tubules (Vinagre *et al.*, 2019). Some contributing factors affect the extent of injury, such as the remaining dentin thickness, the type of the LCU, the type of ultrasonic device, or the type of water spray used (Kwon *et al.*, 2013).

Therefore, controlling the temperature increase during the emission of LCUs is an important factor in the use of photopolymerizers. The objective of this study was to compare the temperature increase produced by HAL and LED LCUs by irradiating dentin discs (0.5 mm and 1 mm thickness), and to evaluate their cytotoxic effects on fibroblast culture in the presence of dentin discs.

Methods

Specimen preparation

A total of 20 bovine incisors were used in this study, the crowns were separated from the roots 2 mm below the level of cemento-enamel junction and then embedded in self-curing acrylic resin (TDV, Santa Catarina, Brazil) in a prefabricated PVC mold. Later, longitudinal dentin discs (without enamel) were obtained (10 discs of 0.5 mm and 10 discs of 1 mm) by sectioning the crowns using diamond disc (0.3 mm thickness) and an EXTEC cutting machine (Labpol 8-12, Extec Corp®, Enfield, Connecticut, USA) (Figure 1).

Light irradiation and temperature measuring

A digital thermometer (MT-507 Minipa, São Paulo) was used to measure the temperature variation during light irradiation with HAL and LED LCUs (Table 1). The base of the specimen was covered with an insulating thermal paste (Implastec, Votorantim Ind. Brasileira, São Paulo, SP, Brazil), and the tip of the thermocouple surrounded by paste was placed in contact with the lower wall of each dentin disc. The specimens

Figure 1. Schematic illustration of dentin discs. (A) the bovine tooth was cross-cut on the level of cemento-enamel junction of the lateral surface and the enamel was removed totally with sand paper. (B) the crown was fixed in self-curing acrylic resin JET (fabrication) in a PVC prefabricated mold. (C) Longitudinal discs were obtained (10 discs of 0.5 mm and 10 discs of 1 mm).

Table 1. the protocol of light irradiation of each experimental group.

Groups	Protocol (n=10 per group)
HAL0.5	Irradiation with Curing Light XL 3000 (3M) halogen-tungsten bulb at an intensity of 470 mW/cm ² using active fiber optic tip (7 mm diameter) emitting light wavelength of 400 to 500 nm for 20 s over a dentin disc of 0.5 mm.
LED0.5	Irradiation with Optilight Max (GNATUS- Ribeirão Preto, SP, Brazil) LED at an intensity of 1200 mW/cm ² , emitting a light wavelength of 420 to 480 nm for 20 s over a dentin disc of 0.5 mm.
HAL1	Irradiation with aforementioned halogen-tungsten bulb at an intensity of 470 mW/cm ² using active fiber optic tip (7 mm diameter) emitting light wavelength of 400 to 500 nm for 20s over a dentin disc of 1 mm
LED1	Irradiation with Optilight Max LED at an intensity of 1200 mW/cm ² with emitting light wavelength of 420 to 480 nm for 20 s over a dentin disc of 1 mm.

were irradiated for 20 s and the temperature was measured one time for each specimen obtaining 10 measurements for each experimental group (n= 10).

MTT analysis

Mouse fibroblast cells (L929) (Rio de Janeiro Cell Bank, APABCAM, RJ, Brazil) were grown in cell culture flasks (TPP, Switzerland) containing Dulbecco's modified Eagle medium (DMEM) (LGC Biotecnologia, Cotia, Brazil) and supplemented with 10% fetal bovine serum (Invitrogen, New York, USA) at 37°C and 5% CO₂ with atmospheric humidity. Next, 2×10⁴ cells/mL were cultivated in 96-well microplates (TPP, Trasadingen, Switzerland) in the same medium for 24 hours for cell adhesion. DMEM was used as a control group (0 mg/mL).

The treatment was carried-out for the groups (n= 10), in which each dentin disc was positioned over a well containing 100 µL of cell suspension in DMEM and light irradiation performed (Table 1). This positioning was to simulate the clinical situation when the LCU irradiates the dentin and this irradiation may affect the fibroblast in the adjacent soft tissues (Figure 2).

Next, MTT solution (100 µL/well) was added to the 96-well plate and the plates were incubated at 37°C with 5% CO₂ for 1 h. Then, the MTT solution was discarded and 100 µL/well of dimethylsulfoxide (DMSO; Sigma, Missouri, USA) was added and the plates were incubated again for 10 min and shaken for 10 min. The absorbance of the wells was measured using a spectrophotometer at 570 nm and data generated were converted to cell viability percentage using the formula: = OD of each group × 100 / OD of control group (OD = optical density).

Statistical analysis

After normality testing, data were analyzed by one-way ANOVA with Tukey's post hoc test for parametric data or Kruskal-Wallis with post hoc Conover-Iman test for non-parametric data ($\alpha=0.05$) using GraphPad Prism 6 (La Jolla, CA, USA).

Results

Temperature measurement

A significant statistical difference was found between the groups HAL0.5 and HAL1 and both were significantly different

to LED0.5 and LED1, which presented higher temperatures. However, no significant difference was observed between the two LED groups (Figure 3).

Cytotoxicity analysis

All groups were significantly different to the control group, and promoted reduction of cellular viability. There was no significant difference between the groups HAL0.5 (cell viability 43.5%) and HAL1 (cell viability 41.1%), and between the groups LED0.5 (cell viability 17.1%) and LED1 (cell viability 17.3%). However, both HAL0.5 and LED0.5 were significantly different to HAL1 and LED1 (Figure 4).

Raw cytotoxicity and temperature results are available as *Underlying data* (Paula Ramos, 2020).

Discussion

This paper investigated the heating generated by HAL and LED when irradiating dentin discs of thickness 0.5 mm and 1 mm of for 20 seconds. Hannig & Bott (1999) obtained different readings were obtained (2.9 to 7.9°C) when they evaluated six LCUs, including HAL, for 40, 10 and 5 s, finding that significantly higher pulp chamber temperatures were obtained when compared to conventional LCUs like Heliolux II. Uhl *et al.* (2003) evaluated the heating generated after resin composite photopolymerization and founded that LED LCUs represent a viable alternative to HAL LCUs for dental composite photopolymerization due lower temperature increases within the composite. Different results were obtained in the present study, as HAL generated lower temperature increases than LED in dentin. Conversely, another study showed no difference between HAL and LED LCUs in generating heat (Drost *et al.*, 2019). These different results in the literature may be related to the kind of temperature sensor and the methodology used (Jiang *et al.*, 2019).

Both LED and HAL LCUs negatively influence cellular viability (Passarelli *et al.*, 2020); however, there is insufficient evidence that they cause pulp inflammation/cytotoxicity (Benetti *et al.*, 2018). In the present study, it was verified that LED was more cytotoxic than HAL LCUs; however,

Figure 2. Schematic illustration of dentin discs positioning over 96-well plates containing fibroblasts with DMEM.

Figure 3. Average of the maximum temperatures reached by the 0.5 mm and 1.0 mm thick dentin discs after application of halogen light (HAL) or LED for 20 seconds. Different letters indicate statistically significant differences among the experimental groups with ($P \leq 0.05$).

Gonçalves *et al.* (2016) found that LED had minimal cytotoxicity. This result may be influenced by the dentin thickness, as Daronch *et al.* (2007) found that the increase in pulp temperature was directly related to the remaining dentin thickness. In the present study, the application of LED light to the thickest dentin disc (1.0 mm) was less cytotoxic than the thinnest dentin disc (0.5 mm).

The divergence observed in the present work in relation to the dentin thickness, light source and the possible greater protection that it can confer to the pulp could be related to the wavelength that the devices emit. The HAL LCU used emits a wavelength of 400–500 nm, and the dental structure is capable of absorbing light in a spectrum from 350–400 nm, meaning it can thus exhibit fluorescence at 410–500 nm. Therefore, the

Figure 4. Percentage of cell viability obtained after treatments with halogen light (HAL0.5 and HAL1) and LED (LED0.5 and LED1) for 20 seconds over dentin discs. Different letters indicate statistically significant differences among the experimental groups with ($P \leq 0.05$).

HAL LCU employed herein emits light at an absorbable wavelength for the dentin disc. Thus, the greater the dentin thickness, the greater the absorbance of light and the higher the concentration of photons, thus explaining the increase in the temperature of the disc (Neumann *et al.*, 2005).

This study found that HAL LCU promoted a lower temperature change in the dentin compared to LED, regardless of the dentin thickness (0.5–1 mm). HAL and LED LCUs decreased fibroblast viability; however, LED resulted in greater cytotoxicity.

Data availability

Underlying data

Harvard Dataverse: Replication Data for: Dataset. <https://doi.org/10.7910/DVN/M4FYVV> (Paula Ramos, 2020).

File 'Dataset.tab' contains raw data for cell viability and temperature generated in the present study.

Data are available under the terms of the [Creative Commons Zero "No rights reserved" data waiver](#) (CC0 1.0 Public domain dedication).

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Reviewer Report 22 March 2021

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Afnan O Al-Zain

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Dalea M. Bukhary

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The authors investigated the effect of heat generated from a quartz-tungsten-halogen and a light-emitting-diode curing unit on the temperature increase and fibroblast cell viability by irradiating 0.5-mm and 1-mm dentin slices. The article topic is important and interesting. A few points need to be justified, and sections of the methods need to be clarified.

Title

- Authors should use scientific terminology and abbreviations. According to the literature, the scientific term for "Halogen bulb" light is "quartz-tungsten-halogen" and the correct abbreviation is "QTH". Authors need to kindly correct this by using the scientific term and abbreviation throughout the article.

Introduction

- In the last sentence of the first paragraph, the authors mentioned that "... LCUs can increase temperature and induce thermal transfer depending on the light source and intensity and type"; nevertheless, the LCUs used in this study had approximately a 60% difference in the light irradiance. Therefore, it was not clear why authors used units with such vast differences in irradiance values. This point will be further addressed in the methods.
- The authors used the term "intensity". However, this term should no longer be used. The updated term is "irradiance". A Glossary of Terms for Light Curing was published in 2014. Authors may kindly find these terms in several publications, and the following research article is one of them: Jeffrey A Platt, Richard B Price; Light Curing Explored in Halifax. *Oper Dent* November 1 2014; 39 (6): 561–563. DOI: <https://doi.org/10.2341/1559-2863-39.6.561>¹

Materials and Methods

- I appreciate the authors' novelty in the methods, but it does not simulate in vivo or clinical situations. The authors placed a dentin slice over the well-plate, which resulted in a distance between the slice and cells. It seems that this design would test the amount of light transmission through the dentin slices rather than simulating clinical situations. Did the authors consider using a larger well-plate and placing the dentin slice inside the well? This may better simulate the clinical situation where the dentin is closer to the cells since the soft tissue surrounds the tooth structure. Therefore, when light curing, the emitted light hits the tooth and the surrounding tissue simultaneously. So, it is more clinically relevant if a larger well-plate is used and the dentin slice is placed inside the well. Furthermore, authors could use different slice thicknesses as they did in this study. Authors may consider this for future research. More details, justification, or the thinking process behind the methodology would be appropriate for the readers to relate to clinical settings.
- Authors need to kindly clarify a few things to the readers based on the methodology; how does dentin's thickness relate to temperature increase? Does 0.5- and 1-mm slice represent curing? i.e., which class does it represent curing? a class II or a class III resin-based composite restoration or another clinical situation? What is the degree of insulating vs. presence of composite layer on top of dentin? The authors mentioned a few of these points. However, it is suggested for authors to mention more details and address these points.
- It is not clear why the authors obtained one section per tooth? Since teeth have variations among them, it would have been more relevant for authors to consider collecting both slices (0.5 and 1 mm) from the same tooth or collect more slices per tooth and account for within-sample variation during the statistical analysis.
- It is not clear why authors obtained vertical sections instead of horizontal if the sections were placed flat on top of the well-plate. Also, the dentin histology would differ from the top to the bottom of the specimens. Did the authors consider that during the study design? How would the authors justify their sections? Authors need to add justifications in the discussion section.
- It is not clear how the specimens were stored and tested. The authors need to add details. In what medium were the dentin slices stored? For how long were they stored? were the dentin slices hydrated or dehydrated before testing? These details may impact the light transmission, and it is important to mention them in the methods.
- The authors had a control group without irradiation. However, it would be relevant to include an additional control group with irradiated cells without the dentin slice's presence.
- It is not clear why the authors selected 20 seconds to light cure the dentin slices. The authors needed to justify this point.
- QTH Irradiance values are approximately half that of the LED. Therefore, it would be more relevant to double or triple the curing time when light curing with the QTH or use different units with relatively similar irradiance values. Although the literature is conflicting regarding applying the law of reciprocity on light-curing resin-based composites, the concept is relatively valid, according to some publications. The radiant exposure is the amount of

energy the restoration receives over time [radiant exposure (J/cm^2) = irradiance x time]; therefore, we would expect the amount of radiant exposure the dentin slices received using the LED is double or more than the QTH unit.

- In the cytotoxicity analysis, the authors needed to consider having an additional control group without the disks, as mentioned previously.
- The authors did not mention the average dimensions of the dentin slices. What was the diameter of the slices relative to the diameter of each well in the 96-well plate?

Results

- Figure 3: the control group is missing. Also, the authors need to consider adding another control group with irradiated cells with no dentin slice over the well. However, it may be challenging at this point but may be considered in future studies.
- Figure 4: there is a space without a bar present between the control bar and the LED 1-mm bar. This space is best to be removed. The control here is cells without light irradiation; as mentioned in Figure 3 comment, adding a control group of irradiated cells without dentin slices would be relevant.
- Figures 3 and 4: it is best to place the letter "A" on the bar with the highest significant bar, followed by "B", and then "C" on the lowest significant bar. It would be easier for the reader to follow.
- It would have been nice for authors to show cell morphology images for the different groups. The cell morphology images may be considered in future studies.
- The authors would expect significant differences between the QTH and LED groups due to the significant differences in irradiance values between units.

Discussion

- Authors should discuss the results of their study first before discussing other research articles. Readers would want to know the justification of their study first.
- Justifications for the mentioned comments would best be added in the discussion section.

Conclusion

- The conclusion is accurate to the results and aligned with the aim.

Overall, the research is valuable, and considering the suggested comments would be beneficial for future research.

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Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Dental light-curing units, resin-based composites, dental adhesives.

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Reviewer Report 07 December 2020

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Talal Al-Nahlawi

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This study aimed to evaluate a relevant topic in operative dentistry nowadays because of the increased demand and need for light curing units in many procedures including photo-polymerization of resin composite restorations and for teeth bleaching despite the heavy discussion about the light curing efficacy on improving the bleaching.

Introduction:

- The authors were able to successfully approach these topics in the introduction section. The objective defined at the end of this section was clearly reported.

Material and methods:

- This study protocol is well described and standardized. The light irradiation and

temperature measuring method were described in the literature in other studies. I think it would be more appropriate to cite the original studies, however, it is not of great relevance as a number of these studies were cited in the discussion section. The same should be followed in the MTT assay, it is not a unique test of this study, I think in the future, all the original studies of these tests should be cited.

- I should congratulate the authors for the schematic illustration of MTT assay, this part of the test of applying the dentin discs over the 96-well plate is innovative, or at least, to the best of my knowledge, it was not used in any other study.

Results and Discussion:

- Even I don't agree with the results. However, they were well described and illustrated. And the fact the LED light curing was more cytotoxic and generated more thermal changes than the Halogen light curing unit may be explained by the LED unit intensity used in the study (1200 mW/cm²) which relatively 3 times the intensity of the halogen light curing unit used in this study 470 mW/cm²). Why did the authors use light curing units with great intensity difference?
- Why did the authors not evaluate the laser as well? What about laser-induced photopolymerization?
- There are divergent results in the literature about the thermal changes caused by LED and halogen light curing units, however, the common concept is that the LED generates less thermal changes (Mahant RH *et al.* 2016).¹ Still, the results of this study answered its objective, and the conclusion is supported by the findings.

I think this study has the potential to be indexed justly to alert the importance of more studies about this topic of great relevance.

References

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Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Endodontics and Operative Dentistry

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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