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Effect of the curing light intensity on the biocompatibility and flexural strength of a composite resin

Efecto de la intensidad de las unidades de fotopolimerización sobre la biocompatibilidad y resistencia a la flexión de una resina compuesta

Efeito da intensidade de fotopolimerização na biocompatibilidade e resistência à flexão de uma resina composta

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Abstract

Objective: To determine the effect of the intensity of two light curing units on the biocompatibility, flexural strength and elastic modulus of a composite resin.

Methodology: Two groups of Filtek Z250XT (3M ESPE) composite resin were created, each one photopolymerized using different intensities (<400 mW/cm² for 40s and > 800 mW/cm² for 20s). Cell viability was analyzed by MTT assay at 24 and 48 hours following the ISO 10993-5 standard. The flexural strength and elastic modulus were analyzed following the ISO 4049 standard.

Results: In the group photopolymerized with an intensity <400 mW/cm², cytotoxicity was statistically higher both at 24 and 48 hours and flexural strength and elastic modulus were statistically lower.

Conclusion: A polymerization intensity <400 mW/cm² increases the levels of cytotoxicity and decreases the mechanical properties of composite resins. The importance of the periodic control of the light curing units is emphasized.

Keywords: materials testing, composite resins, cell culture

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Resumen

Objetivo: Determinar el efecto de la intensidad de dos unidades de fotopolimerización sobre la biocompatibilidad, resistencia flexural y módulo elástico de una resina compuesta.

Metodología: Se crearon dos grupos de resina compuesta Filtek Z250XT cada uno fotopolimerizado con intensidades diferentes ($<400 \text{ mW/cm}^2$ por 40s y $>800 \text{ mW/cm}^2$ por 20s). La viabilidad celular fue analizada mediante ensayo de MTT a las 24 y 48 horas siguiendo la normativa ISO 10993-5. La resistencia flexural y módulo elástico fueron analizadas siguiendo la normativa ISO 4049.

Resultados: En el grupo fotopolimerizado con una intensidad $<400 \text{ mW/cm}^2$, la citotoxicidad fue estadísticamente mayor tanto a las 24 como a las 48 horas y la resistencia flexural y módulo elástico fueron estadísticamente menores.

Conclusión: Una intensidad de polimerización $<400 \text{ mW/cm}^2$, aumenta los niveles de citotoxicidad y disminuye las propiedades mecánicas de las resinas compuestas. Se destaca la importancia del control periódico de las unidades de fotopolimerización.

Palabras clave: Ensayo de Materiales, Resinas Compuestas, Cultivo de Células.

Resumo

Objetivo: Determinar o efeito da intensidade de duas unidades de fotopolimerização na biocompatibilidade, resistência à flexão e módulo de elasticidade de uma resina composta.

Metodologia: Foram fabricados dois grupos de resina composta Filtek Z250XT, cada um deles foi fotopolimerizado com intensidades diferentes ($<400 \text{ mW/cm}^2$ por 40s e $>800 \text{ mW/cm}^2$ por 20s). A viabilidade celular foi analisada por ensaio de MTT em 24 e 48 horas seguindo a norma ISO 10993-5. A resistência à flexão e o módulo de elasticidade foram analisados seguindo a norma ISO 4049.

Resultados: No grupo fotopolimerizado com intensidade $<400 \text{ mW/cm}^2$, a citotoxicidade foi estatisticamente maior nas 24 e 48 horas e a resistência à flexão e o módulo de elasticidade foram estatisticamente menores.

Conclusão: Uma intensidade de polimerização $<400 \text{ mW/cm}^2$ aumenta os níveis de citotoxicidade e diminui as propriedades mecânicas das resinas compostas. Destaca-se a importância do controle periódico das unidades de fotopolimerização.

Palavras-chave: Teste de Materiais, Resinas Compuestas, Cultura de Células.

Introduction

Composite resins (CR) are polymeric materials widely used in dental practice. They are indicated for direct and indirect filling, cementing restorations, and orthodontic brackets, among other applications. Their clinical performance is highly dependent on essential characteristics, such as good mechanical properties, easy handling, and non-toxic and non-irritating to dental tissues.⁽¹⁾ Biocompatibility is the materials' ability to coexist harmoniously with the surrounding biological environment.⁽²⁾ This implies that the restorative material must not harm the pulp or soft

tissues, must not contain toxic substances that can be diffused, released and/or absorbed by the surrounding environment, nor cause allergic reactions or have carcinogenic potential.^(2,3) Multiple assays that measure the viability and proliferation status of cells exposed to test materials in vitro can be used to assess the relative toxicity of materials.⁽²⁾ According to the current international standard (ISO 10993-5),⁽⁴⁾ this assessment can be performed using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide). This is the most common test for assessing the cytotoxicity of dental materials as it is quick and

inexpensive. It is based on cells reducing MTT to formazan crystals. Therefore, its concentration measured via spectrophotometry allows us to quantify the viable cells indirectly.⁽²⁾

Furthermore, (tensile, compressive, or flexural) mechanical strength values are used as parameters to evaluate the performance of these polymeric materials.⁽⁵⁾ Flexural strength (FS) is the mechanical property recommended by the International Organisation for Standardisation to study materials based on CR (ISO 4049:2009).

⁽⁶⁾ This value is obtained with the three-point bending test, where the material bends until it fractures. In turn, this type of test can analyse the elastic modulus (EM) of the material, i.e., how stiff or flexible the material is. FS has been shown to be more selective and sensitive than compressive strength when there are subtle changes in a polymer substructure.⁽⁷⁾

RC hardens through polymerization: monomers are converted to polymers. This process occurs through photo-activation, which requires light-curing units (LCUs) at a specific wavelength and intensity. Currently, halogen and LED units are the most widely used, with a wavelength between 360 and 520 nm. They are effective for material polymerization using camphorquinone as a photoinitiator.⁽⁸⁾

It is expected that all the carbon double bonds ($C = C$) of the CR matrix monomer will convert to single bonds ($C - C$), which form the polymeric network.⁽¹⁾ However, the conversion degree (CD) of monomers to polymers ranges from 55 to 75%,⁽³⁾ depending on the chemical structure of the monomers, filler type, material translucency, the thickness of the material to be polymerized, the time, intensity, density of the light and the distance between the LCUs and the material.⁽⁹⁾ The relationship between the wavelength and the photoinitiator system has a significant effect on the CD of the monomers. The literature shows that the right CD requires radiation exposure (RE) between 16-20 J/cm².⁽⁸⁾ The RE is establi-

shed by the power of the light (mW/cm²) multiplied by the exposure time (in seconds). Therefore, to achieve the right CD, we must establish a light exposure time according to the intensity of our LCU.⁽¹⁰⁾ A total energy of 16 J is necessary to achieve the right excitation of the photoinitiator. This RE is achieved, for example, by having a minimum light intensity of 400 mW/cm² for an exposure time of 40 seconds.⁽²⁾

However, LCU intensity might decrease with lamp and filter deterioration, improper maintenance, battery wear, resin residues on the tip of the LCU, improper use of the LCU by placing the light source far away from the material to be polymerized, etc.⁽⁸⁾ This results in a decrease in the CD of the organic matrix, negatively affecting the physicommechanical properties due to reduced cross-linking of molecules.^(3,9) Therefore, it would be expected that the increase in residual monomers would lead to decreased biocompatibility, increasing their cytotoxic capacity after these monomers are released.

In clinical practice, it is essential to monitor LCUs periodically by evaluating the intensity of the light emitted with radiometers⁽¹¹⁾ because it is impossible to determine LCUs lacking optimum intensity clinically, as even if the CRs are light-cured below 16 J, they feel rigid to the touch.⁽²⁾

Therefore, this study aimed to determine the effect of LCU intensity on CR biocompatibility and flexural strength. It also seeks to raise practitioners' awareness of the importance of monitoring their LCUs periodically.

We posit the following hypothesis: CRs polymerized with LCU with an intensity <400 mW/cm² would be more cytotoxic and have lower flexural strength and elastic modulus values than those cured with higher intensities.

Methodology

This study was approved by the Research Ethics Committee of the School of Dentistry, Universidad de la República, File No. 091900000154-18. Two groups were created for this study according to LCU intensity:

- Group 1: GNATUS OPTILIGHT 600 (Gnatus; Ribeiro Preto, Brazil) with an intensity $<400 \text{ mW/cm}^2$ (exactly 340 mW/cm^2).
- Group 2: GNATUS OPTILIGHT MAX LED unit (Gnatus; Ribeiro Preto, Brazil) with an intensity $>800 \text{ mW/cm}^2$ (exactly 860 mW/cm^2).

LCU intensity previously measured using a Bluephase meter II radiometer (Ivoclar Vivadent; Schaan, Liechtenstein, Germany). The LCUs were in operation and showed different degrees

of wear and tear at the time of the study.

A commercial RC Filtek Z250XT (3M ESPE, ST PAUL, MN, USA) was used to conduct the tests. It was polymerized with the LCU according to the relevant group, following the manufacturer's instructions: 40 seconds with the light-curing unit with an intensity $<400 \text{ mW/cm}^2$ and 20 seconds with the light-curing unit with an intensity $>800 \text{ mW/cm}^2$. The manufacturer's composition and instructions can be found in Table 1. A single trained operator manipulated all the material.

Table 1: Features of the Filtek Z250XT composite resin

Commercial Name	Manufacturer	Composition	Manufacturer's instructions
Filtek Z250XT (A2 color)	3M ESPE	Matrix: Bis-GMA, Bis-EMA, TEGDMA, PEGDMA and UDMA. Filler: Nanohybrid (silica, zirconia, zirconia/silica cluster). 82% by weight. Silane	For 2-mm material thickness: – lamps with a maximum intensity of 800 mW/cm^2 and polymerization for 40 seconds. – lamps with an intensity higher than 800 mW/cm^2 and polymerization for 20 seconds.

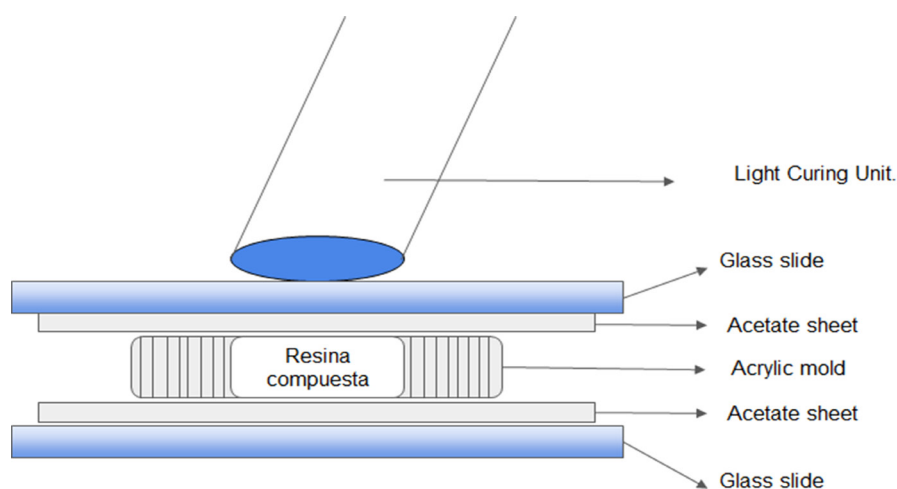
Cytotoxicity analysis

Sample preparation

Five specimens were prepared for each group. Acrylic molds measuring 5 mm in diameter and 1 mm in depth were placed on an acetate sheet. The CR was inserted into the mold and an acetate

te sheet was pressed with a glass slide on top to ensure sample uniformity (Figure 1). Each specimen was polymerized according to the relevant group. Finally, they were removed from the molds and the edges were sanded with 400 grit sandpaper to remove any excess.

Figure 1: Specimen preparation.



Cell culture

All culture procedures were carried out under strict biosafety standards in a FORMA 1300 A2 laminar flow chamber, Model 1386 (Thermo Scientific, Waltham, Massachusetts, Estados Unidos). NIH 3T3, an immortalized mouse fibroblast cell line, was used and previously cultured using clonogenic culture medium (DMEM + 10% Fetal Bovine Serum + 1% Penicillin/Streptomycin) (GIBCO, Denmark) in t25 culture flasks in an incubator (FORMA 311, Thermo Scientific), Waltham, Massachusetts, Estados Unidos) at 37°C with 5% CO₂ under relative humidity.

The material was transferred to a t75 culture flask when 80% confluence was reached. For this, the culture medium was removed, the cells were washed with phosphate buffer saline (PBS) (GIBCO, Denmark), then 1 ml trypsin/EDTA (TrypLE®, Thermofisher) was added for 5 minutes at 37°C. Finally, trypsin was neutralized with 2 ml of clonogenic medium, and the entire cell suspension was placed in a t75 culture flask, adding a clonogenic medium to continue cell expansion. The cell viability assay was started when 80% confluence was reached.

Cell viability assay

The cell viability assay was performed following a previously established protocol,⁽¹²⁾ complying with ISO 10993-5⁽⁴⁾ (Figure 2). Each previously prepared specimen was placed in a 2 ml Eppendorf® type container, and 1 ml clonogenic medium was added and left to incubate for 24 hours. In this way, any toxic components contain-

ed in the sample were released into the culture medium by leaching (eluate).

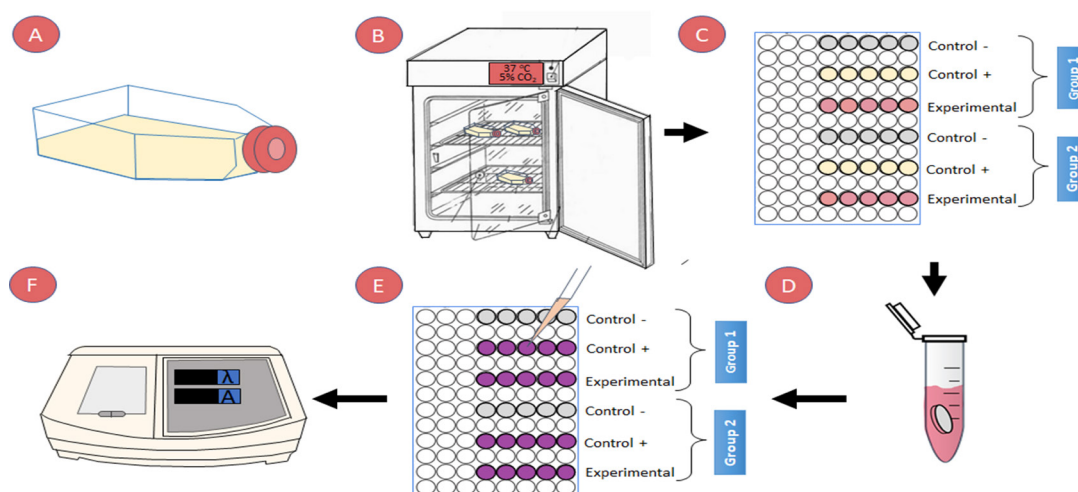
The previously collected cells were streaked in 96-well plates at a density of 3x10⁴ cells per well and incubated for 24 hours with 100 µl clonogenic medium. Thus we obtained six columns of five wells for each group: negative control without cells, positive control with cells and clonogenic medium, and experimental with the medium incubated in the specimens. After 24 hours, the DMEM was changed in the control wells, and 100 µl of the previously incubated medium (eluate) with the specimens was placed in the experimental wells.

Viability was measured at 24 and 48 hours. First, the culture medium was removed, and the wells were washed with PBS. Then, 100 µl of clonogenic medium supplemented with 5% MTT (GIBCO, Denmark) was added and incubated for 5 hours at 37°C and 5% CO₂. Finally, the medium was removed, and 100 µl of dimethyl sulfoxide (DMSO, Sigma-Aldrich, Missouri, United States) was added for 5 minutes to reveal the precipitates under gentle agitation.

Cell viability was quantified with an ELISA ultraviolet visible spectrophotometer at 570 nm (Leytamed LTCM06 Elisa Microplate Reader, Guangzhou, China). This device was used to quantify the precipitates. These precipitates produce a purplish staining, which allows us to indirectly measure MTT metabolism, indicating the viability of the cultured cells. Once the values were obtained, they were analyzed using the following formula

$$\text{Cellular viability\%} = \frac{\text{Experimental optical density}}{\text{Control optical density}} \times 100$$

Figure 2: Cell viability assay



A) NIH 3T3 + clonogenic culture medium (DMEM + 10% FBS + 1% P/S), B) incubated to 80% confluence, C) Cell streaking in 96-well plates at a density of 3×10^4 with 100 μ l clonogenic medium for 24 hours. D) Creation of eluate by incubating each specimen in 1 ml of clonogenic medium. E) Cell viability assay measured at 24 and 48 hrs: – add MTT solution for 4 hours. – aspirate MTT. – dilute the precipitate in DMSO and shake for 5 min to observe the precipitate. F) Quantification of cell viability with ELISA ultraviolet visible spectrophotometer at 570 nm.

Three-point bending test

A three-point bending test was performed according to ISO 4049 to analyze the flexural strength and elastic modulus (Figure 3).⁽⁶⁾

Sample preparation

Ten rod-shaped specimens (25 x 2 x 2 x 2 mm) per group were made by placing the uncured resin in an acrylic mold covered with a strip of celluloid and pressing with a glass slide. The rods were polymerized following the overlapping method described in ISO 4049.⁽⁶⁾

After polymerization, they were removed from the mold, and the excess was removed using 400-grit sandpaper. Each specimen was measured using a digital caliper (Mitutoyo, Japan) with a 0.01 mm accuracy. Before mechanical testing, the samples were stored in distilled water at 37°C for 24 hours.

Mechanical test

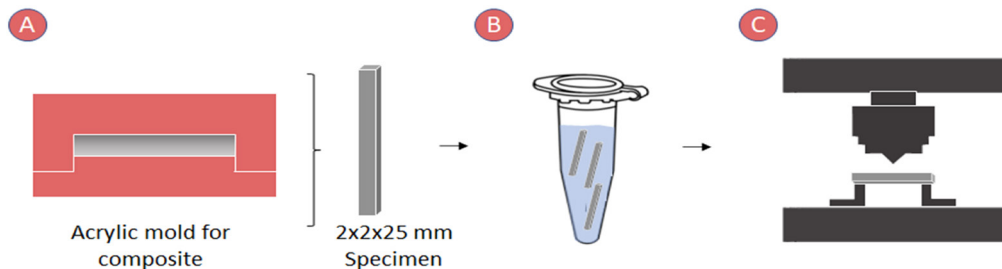
The mechanical test was performed using an MTS SANS CMT2000 universal testing machine (Sans Testing Machine, Shen Zhen, China) with a load cell of 5 kN and a crosshead speed of 0.75 mm/s until fracture. The following equations were used to determine the flexural strength (σ) and the elastic modulus (E):

$$\sigma = \frac{3Pl}{2bh^2}$$

$$E = \frac{3Pl^3}{4bh^3d}$$

Where P is the load at fracture (N), l is the distance between the supports (20 mm), b is the width (mm), and h is the height of the specimen (mm), P1 is the maximum load in the linear portion (proportional limit) of the stress-strain curve, and d is the deformation of the specimen at P1 load.⁽⁶⁾

Figure 3: Three-point bending test



A) Specimen preparation. B) Storage in distilled water at 37 °C for 24 hs. C) Mechanical test (Universal testing machine MTS SANS CTM 2000 5 kN at 0.75 mm/min).

Statistical analysis

The cell viability results were analyzed with a two-way ANOVA test: a) LCU used and b) analysis time (24 or 48 hours). The results of flexural strength and elastic modulus were analyzed with the Student's test. All the results were previously subjected to a normality test, and the significance level was $\alpha=0.05$ in all the analyses. SigmaStat v3.5 (Systat Software, California, USA) was used for this statistical analysis.

Results

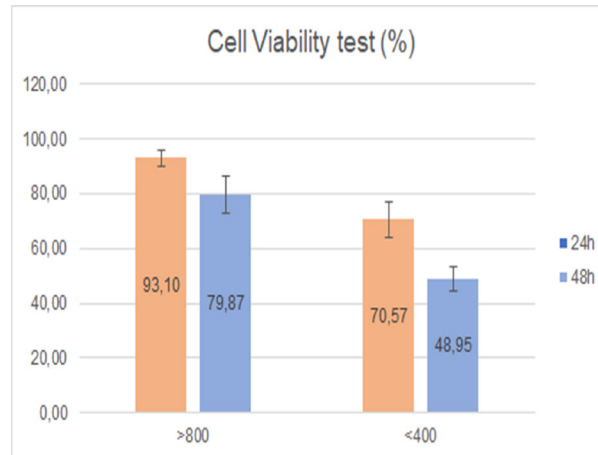
Cell viability

The results of cell viability analysis (Figure 4) show a statistically significant difference ($p<0.001$) between the groups studied, with higher degrees of cytotoxicity when using an intensity $<400 \text{ mW/cm}^2$. A two way analysis revealed a statistical difference when assessing cytotoxicity at 24 and 48 hours ($p<0.001$).

Table 1: Table of two-way ANOVA results

Variation source	Degrees of freedom	Sum of squares	Mean sum of squares	F	P-value
Intensity	1	5000,374	5000,374	150,416	<0,001
Time	1	2163,613	2163,613	65,083	<0,001
Intensity x Time	1	132,134	132,134	3,975	0,057
Residual	25	831,091	33,244		
Total	28	8477,913	302,783		

Figure 4: Cell viability (%) at 24 and 48 hs



Group 1: LED light-curing unit with an intensity $<400 \text{ mW/cm}^2$.

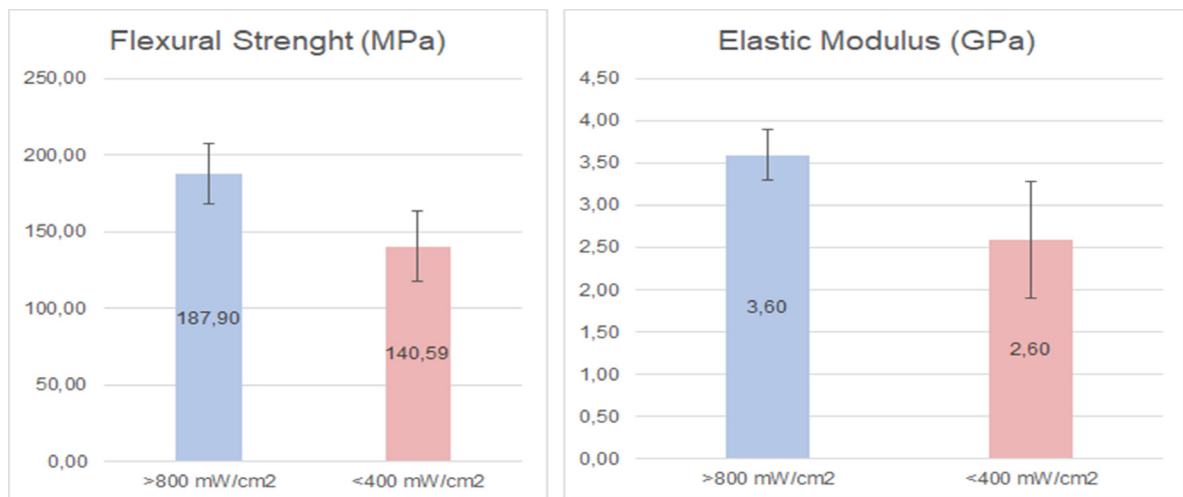
Group 2: LED light-curing unit with an intensity $>800 \text{ mW/cm}^2$.

Three-point bending test

The analysis of the flexural strength (Figure 5) showed a statistically significant difference ($p=0.02$) between <400 and $>800 \text{ mW/cm}^2$.

The analysis of the elastic modulus (Figure 5) showed a statistically significant difference ($p=0.037$) between <400 and $>800 \text{ mW/cm}^2$. Higher flexural strength and elastic modulus values were obtained using a $>800 \text{ mW/cm}^2$ unit.

Figure 5: Results of the three-point bending test



a) Shows flexural strength (MPa). b) Shows the elastic modulus (GPa).

Discussion

This study evaluated the effect of the intensity of two LED LCUs on the mechanical and biological properties of a CR since their proper polymerization cannot be adequately verified. The results showed significant differences in cell viability, flexural strength, and elastic modulus, depending on the intensity used. The group that was light cured with higher intensity showed statistically superior cell viability and mechanical properties than the group light-cured with a lower intensity unit. Therefore, our working hypothesis has been proven.

The biocompatibility of dental materials has been researched with different methods, such as tests to estimate the number of ribonucleic acids and damage to their chains,⁽¹³⁾ studies assessing the severity of proteolytic action,⁽¹⁴⁾ assessment of glutathione level in cells,⁽¹⁵⁾ and metabolic activity evaluation (e.g., MTT assays),⁽⁹⁾ among others. In this study, we selected an MTT assay because, despite the existence of other methods, it is a good indicator of cell viability, and it is the method indicated by current international standards to analyze biomaterials for dental use.⁽⁴⁾ This study showed a significant decrease in cell viability at 24 h (93.1% Group 2, 70.6% Group 1) and 48 h (79.8% Group 2, 48.5% Group 1) regarding cellular control (100%). These data are aligned with those found in the literature.⁽⁹⁾ It is relevant to consider that according to ISO 10993-5, the reduction of cell viability by over 30% is considered a cytotoxic effect.⁽⁴⁾

Cytotoxicity levels in resinous materials can be caused by gradual degradation over time as more toxic components are released into the environment. Therefore, this depends on the time and concentration of the main component of these materials: Bis-GMA.⁽⁹⁾ Several authors have analyzed the cytotoxicity of Bis-GMA in dental materials by exposing fibroblasts to different Bis-GMA concentrations.^(1,16) It has been demonstrated that there is a dose-dependent response over time, where the higher the Bis-GMA concen-

tration, the lower the cell viability.⁽³⁾ Schubert et al. demonstrated that the cytotoxic effect of the material can also be significantly affected by its color shade. They observed higher cytotoxicity in C2-colored resins compared to A2 shades.⁽¹⁵⁾ This could be explained by the fact that the CD is affected by metallic pigments, such as Cu²⁺, Al³⁺, and Fe²⁺, used in the darker resin shades.⁽¹⁷⁾ Some studies have established that Bis-GMA is among the most toxic components due to its liposolubility, followed by UDMA, TEGDMA, and HEMA.^(18,19) In addition, synergistic effects were observed when combinations of TEGDMA with UDMA or particularly with Bis-GMA were tested.⁽⁸⁾ Gonçalves et al. compared the cytotoxic effect of conventional and flowable resins, showing that flowable resins were significantly more toxic than regular consistency resins. This could be because flowable resins contain more monomer and less filler.⁽²⁰⁾ In vitro studies have shown that the polymerization reaction is never complete but ranges between 50 and 70% of the matrix.⁽⁹⁾ As explained above, this could be affected by an improper polymerization protocol, which decreases the CD.^(10,16)

Regarding mechanical behavior, several studies have shown that insufficient intensity and energy significantly decrease the CD of CRs.^(19,21) Furthermore, a significant correlation exists between CD and mechanical properties such as hardness, elastic modulus, and flexural strength of CR.⁽²²⁾ Therefore, we expected the results of this study to show that the group polymerized with an LCU >800 mW/cm² had a superior mechanical behavior, since its CD would be higher compared to the group polymerized with an LCU <400 mW/cm². Although filler size and polymer matrix composition can influence the CD,^(2,11) in this study, only one type of CR (Z250XT) was used. Therefore, the control for this variable shows that the difference in mechanical properties was due to the variation in LCU intensity; hence the CD decreases due to lower RE.^(10,21) On the other hand, some studies have shown that the elastic modulus decreases if linear molecu-

les within the polymer structure are increased.⁽²³⁾ Therefore, we can infer that the samples polymerized with an LCU $<400 \text{ mW/cm}^2$ presented a more linear polymeric structure than those polymerized with an LCU $>800 \text{ mW/cm}^2$, which presented a more branched structure. In addition, there is a relationship between CD, spatial structure, and aqueous sorption of CRs, which favors the diffusion of unreacted monomers into oral tissues increasing their toxicity.⁽²⁾ This could also explain the differences between the cytotoxicity of the two groups.

Regarding the adverse effects of incorrect polymerization, studies have shown a strict connection between the properties of the restorative material and its longevity.⁽²⁴⁾ This would affect not only the mechanical behavior of the material but also its color stability and aesthetics.⁽²⁵⁾ In addition, this would increase the CR solubility,⁽²⁶⁾ causing higher surface roughness and biofilm formation,⁽²⁷⁾ increasing the risk of caries. Therefore, it is not recommended to use LCU with an intensity $<400 \text{ mW/cm}^2$ to reduce the probability of CR damage.

The study's limitations are that it is an in vitro assay, so clinical behavior may vary. Further clinical studies are needed to support these results. In addition, it should be noted that the study used only one CR, so the results are likely to vary in other CR types.

The data obtained in this study suggest that using an LCU PU with an intensity $<400 \text{ mW/cm}^2$ decreases the physico-mechanical and biological

properties of CRs, thus causes a lower CD. This is clinically relevant because there is a relationship between the material's properties and its clinical performance.^(22,24) It is essential to know the intensity of our LCU to establish the irradiation time and thus achieve an adequate CD.^(8,23) It has been reported that the light intensity of the LCU can be affected over time by the deterioration of the unit or dirty or chipped fiber tips,^(11,21,23) so it is essential to monitor LCUs periodically with a digital radiometer. Furthermore, it is important to know the manufacturer's indications, the composition of the CRs, and the LCU's wavelengths, so that its emission profile includes the wavelengths that cover the absorbance profile of the photoinitiators used in the CR.⁽²⁸⁾

Conclusion

Based on the above results, we can conclude that a polymerization intensity $<400 \text{ mW/cm}^2$ leads to an increase in cytotoxicity levels and a significant decrease in the mechanical properties of composite resins.

We believe that this may go unnoticed by dentists in the short term. Therefore, we must emphasize the importance of monitoring the intensity of the light-curing units periodically. The lack of verifications, the use of insufficient intensities, and the application of inadequate protocols either in terms of application technique or application times will result in premature restoration failure and potentially irreversible biological consequences.

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The authors declare that they have no conflict of interest regarding the scientific information provided.

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Authorship contribution:

1. Conception and design of study
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3. Data analysis
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5. Drafting of the manuscript
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BP y APT contributed in 1, 2, 4, 5.

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