

Decalogue of good practices for the use and maintenance of LED Curing Units

Decálogo de buenas prácticas para el uso y mantenimiento de las Unidades de Fotocurado LEDs

Decálogo de boas práticas para uso e manutenção de Unidades de Curado LED

Andrés García Terra¹  0000-0001-6733-4484

Elisa de León Cáceres¹  0000-0002-3780-1034

Romina Tessore¹  0000-0001-6259-042X

Matías Mederos¹  0000-0002-1561-2283

Priscila Vázquez¹  0000-0002-3326-2547

Gonzálo Alonso¹  0009-0000-8063-2726

Betiana Furtado¹  0000-0002-9582-9932

Gonzálo Cruz¹  0000-0001-5718-7759

Daniela D'Angelo¹  0000-0003-4950-3197

Guillermo Grazioli¹,  0000-0001-9969-3780

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Abstract

Currently, several resin-based restorative biomaterials harden through a photopolymerization reaction, for which a light-curing unit (LCU) is necessary.

The objective of this manuscript is to generate a guide based on current scientific evidence for the correct use of LCUs.

A search was made for articles published from 2002 to January 2022 through PubMed and Google Scholar.

The information was organized into 10 relevant topics in the form of a decalogue: wavelength, light intensity, tip diameter, curing time, curing mode, curing distance, use of barriers, battery and charging, cleaning and disinfection, and regular checks.

Health professionals must know and remember the importance of a proper use and maintenance of LCUs, since this can influence the clinical performance of the biomaterial.

Keywords: Photopolymerization, Light Curing Unit, Irradiance.

¹ Dental Materials Academic Unit, School of Dentistry, Universidad de la República.

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Resumen

Actualmente varios biomateriales restauradores resinosos endurecen mediante una reacción de fotopolimerización, para lo cual es necesaria una unidad de polimerización (UP). El objetivo de este manuscrito es generar una guía basada en la evidencia científica actual para contribuir al correcto uso de las UP.

Se realizó una búsqueda de artículos publicados desde el año 2002 hasta enero del 2022 a través de PubMed y Google Scholar. Se organizó la información en 10 tópicos de relevancia en forma de decálogo: longitud de onda, intensidad de la luz, diámetro de la punta, tiempo de curado, modo de curado, distancia de curado, uso de barreras, batería y carga, limpieza y desinfección, finalizando con los controles periódicos.

Los profesionales de la salud deben conocer y recordar la importancia de realizar un adecuado uso y mantenimiento de las UP, ya que esto puede influir en el desempeño clínico de los biomateriales.

Palabras Clave: Fotopolimerización, Unidad de Fotocurado, Irradiancia.

Resumo

Atualmente, diversos biomateriais restauradores resinosos endurecem através de uma reação de fotopolimerização, para a qual é necessária uma unidade de polimerização (UP).

O objetivo deste manuscrito é gerar um guia baseado em evidências científicas atuais para o uso correto de UPs.

Foi feita uma busca por artigos publicados de 2002 a janeiro de 2022 por meio do PubMed e Google Scholar.

As informações foram organizadas em 10 tópicos relevantes na forma de um decálogo: comprimento de onda, intensidade da luz, diâmetro da ponta, tempo de cura, modo de cura, distância de cura, uso de barreiras, bateria e carregamento, limpeza e desinfecção e verificações regulares.

Os profissionais de saúde devem conhecer e lembrar a importância do uso e manutenção adequados das UPs, pois isso pode influenciar no desempenho clínico do biomaterial.

Palavras-chave: Fotopolimerização, Unidade de fotoativação, Irradiância.

Introduction

Many resin-based restorative biomaterials are used daily in clinical practice. Most of them harden with a polymerization reaction, which is triggered by applying blue light emitted by Light Emitting Diode (LED) light-curing units (LCUs). These LCUs are semiconductors; this means that they convert electrical energy into visible light. This process is known as “electroluminescence.”⁽¹⁾

The so-called light particles are photons, which trigger the polymerization reaction. These photons travel at the speed of light as waves. “Wavelength” is defined as the distance between the

peaks of these waves. In turn, wavelengths define the color of visible light.⁽²⁾

Both LED LCUs and restorative biomaterials have been further developed. Various generations of LEDs have been developed over time. The first generation of LED LCUs had a narrow emission spectrum, circa 468 nm. The aim was to activate only camphorquinone—the most common photoinitiator in resin-based materials—and no other photoinitiators.⁽³⁾ At a value of 100-280 mW/cm², the light intensity involved was insufficient. Since achieving adequate photopolymerization under these conditions is impossible, polymerization of 2 mm of composite resin requires exposure times

of about 60 seconds.⁽⁴⁾ This first generation of LEDs was developed between 1999 and 2002. The first LED LCU, UXoMAX LEDs (Akeda Dental A/S, Lystrup, Denmark), was registered in 2000.⁽⁵⁾

The second generation of LED LCUs was launched in 2002. They featured more powerful LEDs, including 1, 5, 10, and 15 W chips. However, LCUs still had a narrow emission spectrum that failed to light-cure all restorative biomaterials properly. These LCUs were usually wireless, and their batteries had a short lifespan and were expensive to replace. They had fragile fiber optic tips, and their temperatures rose due to increased photon emissions. Thus, the longevity of units decreased, and this made it impossible to use them continuously in clinical settings, where several restorations require simultaneous photopolymerization.⁽⁶⁾ For this reason, some units incorporated internal fans or heat sinks to dissipate heat, but they were noisier.⁽²⁾

The third generation of LED LCUs was launched in 2003 in line with the rapid development of aesthetic biomaterials. The most significant steps include the addition of new photoinitiators such as PPD (1-phenyl-1,2-propanedione), Lucirin® TPO (Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide), and Ivocerin®. These photoinitiators require the activation of a broader light spectrum. These third-generation LCUs feature multiple LEDs generating a broader light spectrum ranging from 385 nm to 515 nm. Additionally, in several models, the fiber optic tip is replaced, and LEDs are placed directly at the tip end of the unit. This lowers the chance of breaking the unit tip.^(7,8)

Some factors related to the chemical reaction of polymerization should be considered when photopolymerizing biomaterials. These include factors related to biomaterials (shade, type of photoinitiator included in the composition and thickness of the biomaterial layer applied),⁽⁹⁾ to LCU light sources and their characteristics (wavelength and intensity), and to the tech-

nique used (timing, distance, unit tip angle, etc.). All these variables define the biomaterial's final properties and, therefore, its clinical performance.⁽¹⁰⁾

We must remember that LCUs are essential modern tools that provide many clinical services. LCUs enable clinical procedures such as composite resin (CR) restorations, bonding of indirect aesthetic restorations, hybrid glass ionomers, and resin-based pit and fissure sealants. We should also measure the light intensity emerging from the tip of the unit when evaluating LCUs, among other considerations. This requires using a radiometer. High power intensity values are $>800 \text{ mW/cm}^2$, whereas low power intensity values range from 400 to 800 mW/cm^2 . Power intensity values $<400 \text{ mW/cm}^2$ are insufficient to activate resin-based materials properly. Therefore, we should be cautious at values below 400 mW/cm^2 since some unit component is not in optimal operating conditions.

The lack of consensus over the use of LCUs in dental practice has recently been mentioned.⁽¹¹⁾ Therefore, this paper aims to develop a guide for using LED LCUs correctly. It is based on current scientific evidence and will help health professionals to capitalize on the biomaterials used.

Materials and methods

We performed an electronic search, including articles from 2002 to January 2022. We searched MEDLINE databases, with access through PubMed and Google Scholar.

We used the following major keywords: "light curing unit," "light cure," "intensity," "curing mode," and "wavelength." Moreover, we conducted an additional manual search to determine which articles appearing in references of the initially selected articles were relevant. We also explored the corresponding journals' websites.

In vitro studies, clinical studies, and literature reviews were included as additional resources. The search only included articles published in English.

Decalogue

1. Unit wavelength

All light-curing materials used in dentistry comprise an organic phase (monomers), light initiators, and an inorganic phase (fillers). The photoinitiator system is an essential feature since, under a light beam with a specific wavelength, the system reaches an excited state. When a reducing agent (usually a tertiary amine) is combined, free radicals are decomposed and produced, and the polymerization reaction starts. Therefore, for the process to develop appropriately, both the wavelength emitted by the LCU and the absorption peak of the photoinitiator included in the polymeric material should be equal.^(12,13)

Camphorquinone (CQ) is the most commonly used photoinitiator in CRs, with an absorption peak ranging from 468 to 470 nm. However, CQ is yellow, so it is not as useful for light-colored and translucent resins. Therefore, there are alternative photoinitiators with a lighter yellow shade than CQ and lower absorption peaks, with higher sensitivity to ultraviolet or violet light (380–410 nm).⁽⁸⁾ Figure 1 shows the most commonly used photoinitiators and their absorption spectra.

The best-case scenario for efficient polymerization would be to use LCUs with a wavelength spectrum equal to the absorption curves of all commonly used photoinitiators. Halogen LCUs emit a broad wavelength spectrum (390–520 nm). Therefore, they successfully activate all photoinitiators used in current CRs. However, LED LCUs currently used can only produce a limited spectral range and emit very little light below 420 nm and are thus ineffective on photoinitiators that require violet light. To improve the activation of alternative photoinitiators, some LED LCUs include additional

LED emitters which achieve wavelengths in the violet light range (380–410 nm).^(7,14)

Therefore, to avoid partial polymerization of biomaterials and, consequently, alteration of their properties, operators must be familiar with the composition of the CR to be applied, specifically its photoinitiators, to determine if the LCU is appropriate.⁽¹⁴⁾

2. Light intensity

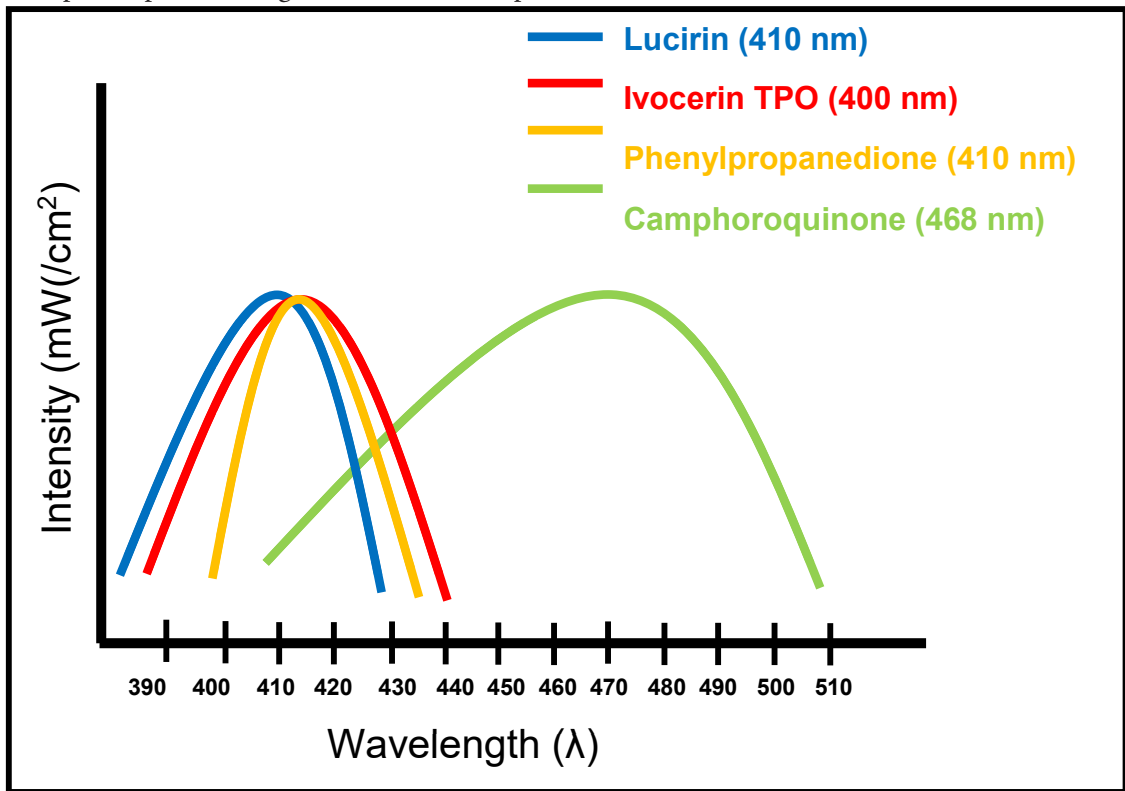
Light wavelengths must be compatible with photoinitiators for correct light-curing, and a specific energy density should be achieved. This is possible with sufficient light intensity (number of photons/surface) and appropriate irradiation time.

Intensity is the parameter manufacturers generally use to describe their LCU and is expressed as the power per unit area (mW/cm^2). Although ISO 10650:2018 International Standard for LCUs does not specify the minimum light intensity required to light-cure biomaterials,⁽¹⁵⁾ some authors suggest that the minimum intensity required is $600 \text{ mW}/\text{cm}^2$.⁽¹⁶⁾ Light intensity is vital since it is one of the parameters defining the polymerization quality of biomaterials. In this sense, biomaterials shall be exposed to more photons at higher light intensities. When more photoinitiator molecules are excited, more free radicals trigger the polymerization reaction.⁽¹⁷⁾

Energy density or irradiance is calculated by multiplying the intensity of the light emitted and the period the material is exposed to the light. It has been discussed that the energy density should be at least $16 \text{ J}/\text{cm}^2$ for every 2 mm increase in CR thickness. At this energy density value, the curing depth and degree of conversion shall be sufficient regardless of the intensity of light emitted. Therefore, with high-intensity LCUs, the light exposure time may be shorter.⁽¹⁰⁾

LCU intensity must be regularly monitored using radiometers (Figure 2). Time may alter LCU intensity. This is because specific LCU components can deteriorate, such as fibers—

Figure 1. Most commonly used photoinitiators in resin-based materials and their wavelength absorption spectra. Image of own authorship.



which may fracture—or polymerized resin residues—which may adhere to the fiber—among others.^(18,19) This becomes particularly relevant upon alterations since exposure may occur at insufficient energy density values. This causes incomplete polymerization, which in turn decreases surface hardness and adhesion and alters mechanical properties, accelerates deterioration, produces marginal degradation, and increases cytotoxicity.^(20,21)

It is worth mentioning that the intensity reported by manufacturers and that monitored with the radiometers refers to the value emitted directly from the tip of the LCU. However, in many clinical situations, placing tips close to the CR or polymerizing the adhesive is impossible (e.g., proximal faces, deep occlusal cavities). According to the inverse-square law, light intensity is inversely proportional to the square of the distance. Therefore, the farther away the

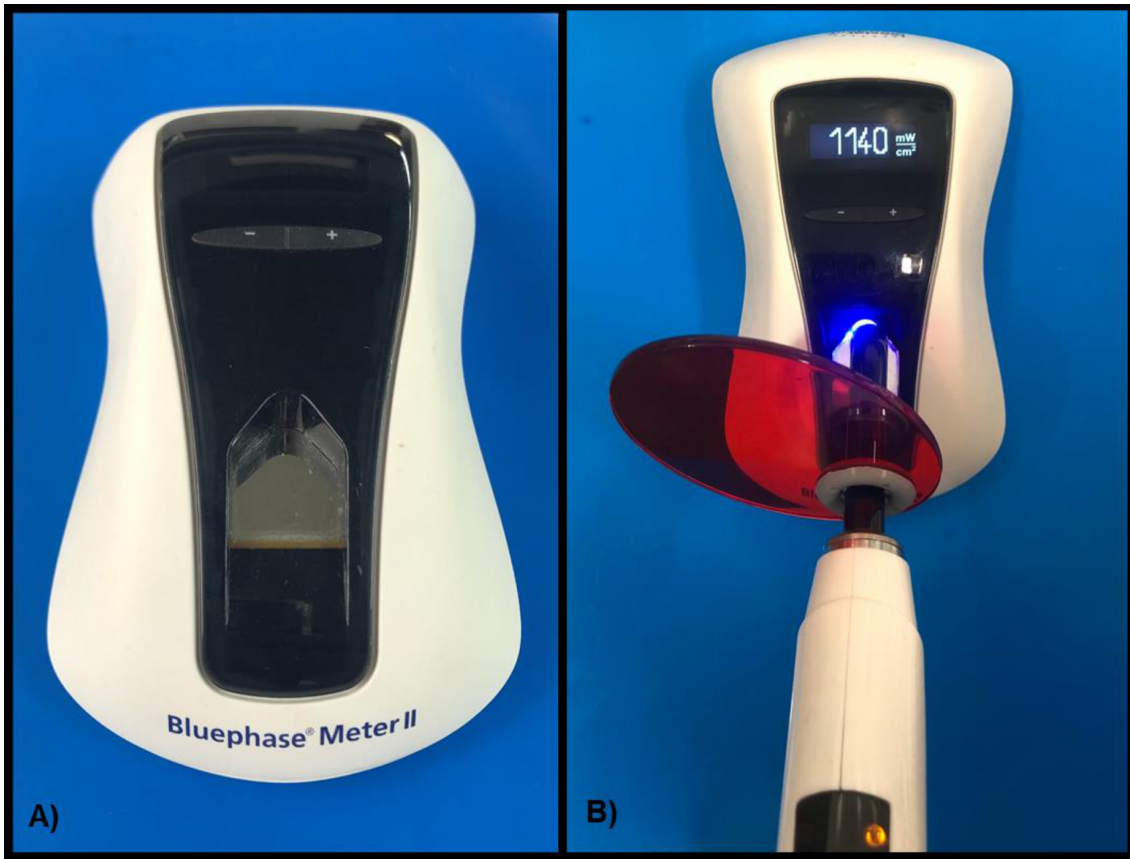
biomaterial to be polymerized is located, the lower the number of photons that its photoinitiators can absorb.⁽²²⁾ Thus, deep and proximal occlusal cavities require an increased exposure time of the light beam. Consequently, an appropriate energy density is achieved at all light intensities (See item 7).

3. Tip diameter and light homogeneity

Different commercial brands and types of LCUs differ in tip diameter. For instance, the effective tip diameter of the Bluephase Style lamp is 9 mm, whereas its outer tip diameter is 9.8 mm. By comparison, the Smartlight Focus unit has an effective diameter of 8 mm and an outer diameter of 12 mm (Fig. 3. A).⁽¹³⁾

Recent studies have shown that the active area of the light beam may be 10% to 20% smaller than the area of the LCU tip.⁽²²⁾ This is significant since it could determine whether biomate-

Figure 2



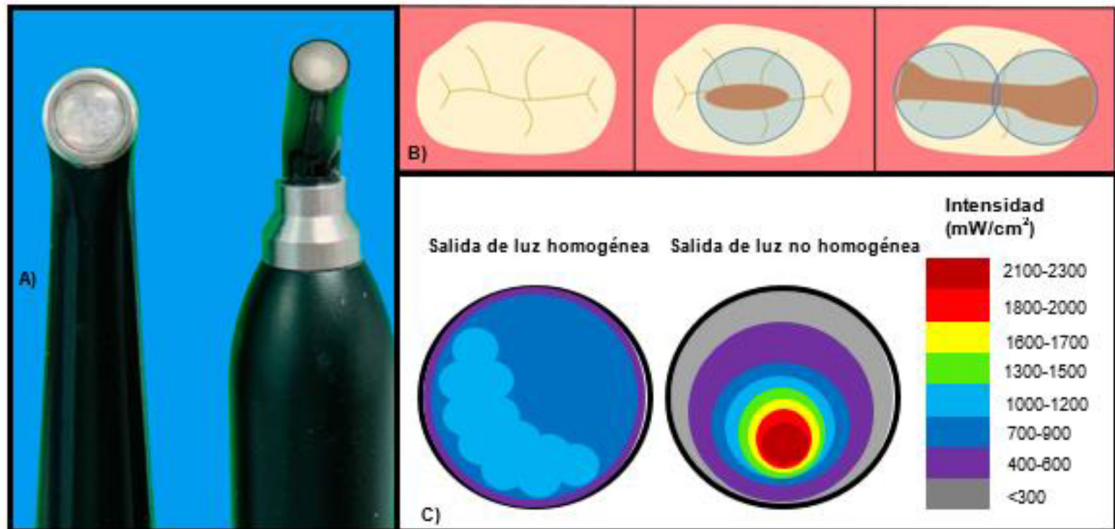
A) Bluephase Meter II digital radiometer. Ivoclar Vivadent. B) Measurement of intensity of light emitted by a LED lamp. The intensity is shown on the digital display with its corresponding measurement unit. Image of own authorship.

rials are appropriately cured. Some cases will require several light applications in different areas of the biomaterial surface to cover 100% of the surface (Figure 3.B). There is a difference between light-curing a smaller cavity (e.g., a small occlusal cavity) and a cavity with a larger surface (mesio-occlusal-distal). In the latter case, curing up to three times in different locations may be required to achieve complete polymerization of resin-based biomaterials.⁽²²⁾

“Light homogeneity” is another essential concept regarding the amount of light per area or surface area emitted by LCU tips.^(22,23) It

relates to the distribution of light emissions from LCU tips, which does not always involve 100% of their tip surface. In some cases, some areas have lower light intensity and even lower than recommended. In these situations, there is a lack of homogeneity or uniformity in light emission/intensity. However, Figure 3.C shows that some LCUs have more homogeneous light emission throughout the active tip surface. This is more convenient since it guarantees a homogeneous light intensity over the entire biomaterial surface, resulting in suitable physical and mechanical properties.^(24,25)

Figure 3



A) Comparison of units with different tip diameters. B) Schematic representation of cavities with different sizes and their relation to the unit tip diameter; in some situations, several curing procedures are required to achieve 100% polymerization of the material surface. C) In some units, the intensity of light emitted is more homogeneous (less variation). In contrast, other units emit light with different intensities that might even have sections where intensities are insufficient. Image of own authorship.

The distribution of light intensity emitted from an LCU depends on the type and shape of the light source and the optical features of the system, such as optical filters and light guides in the unit. Studies have shown that LED units are more homogeneous than previous models, such as quartz halogen or plasma arc units.⁽²⁴⁾ However, traditional intensity measuring with radiometers does not assess outputs of intensity variations through the tip.⁽²³⁾

4. Battery and charging

Batteries in this type of unit generally shut off automatically after three minutes of inactivity. It is a rechargeable lithium battery. According to the product's specifications, it is unnecessary to discharge the unit to recharge it completely. Many units include a battery charging base. It is not recommended to expose batteries to temperatures below 5°C or above 30°C, and to

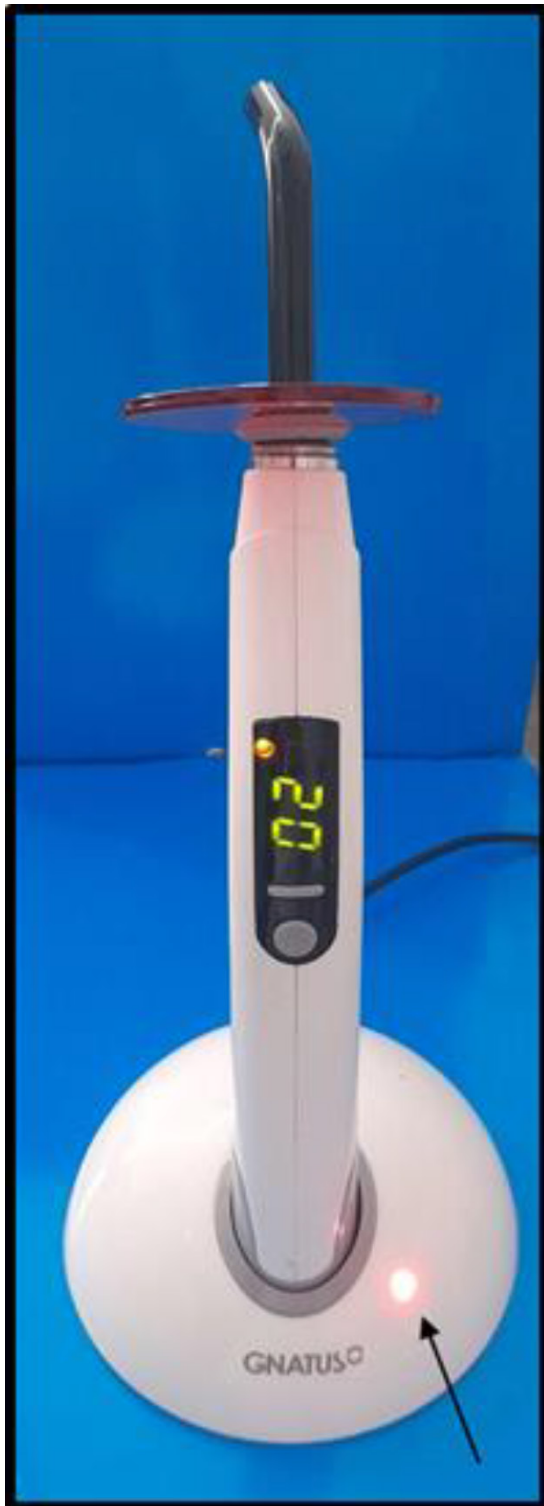
avoid exposure to environments with humidity values above 80%.⁽²⁶⁾

Few studies have focused on the relationship between the battery condition or percentage of charge and the intensity of light emissions. LCUs with batteries that are not fully charged have lower intensity than LCUs that are fully charged. Notably, the number of irradiations or cycles (seconds/use) of LCU emissions as well as intensity, depends on the model of the device. Therefore, in some units, emissions are not altered at different charge states, while in others, light intensity drops by over 50% when the battery is discharged. This can be avoided by keeping the LCUs in the battery charger to have a fully-charged battery available (Figure 4).⁽²⁹⁾

5. Curing time

It is necessary to reach a high degree of polymerization for the biomaterial to have good

Figure 4. Unit placed on its battery charging base.



The arrow points at the light which turns on for fully-charged batteries. Image of own authorship.

properties. This means LCUs should be at the correct wavelength and intensity values (see #1 and #2). We should also know which photopolymerization time is required.

Restoration quality and longevity may be compromised when LCUs are not used for the required photopolymerization times (Figure 5).⁽²⁸⁾

An energy density of 16-24 J/cm² is required for adequate photopolymerization. This entails considering light intensity and exposure time for an adequate energy density.

LCUs are classified based on the light intensity, as follows:

- >800 mW/cm² HIGH power
- 400-800 mW/cm² LOW power
- <400 mW/cm² INSUFFICIENT power

Manufacturers' recommendations for direct restorative resin-based materials:

- 20 seconds - HIGH power
- 40 seconds - LOW power

For adhesive systems:

- 10 seconds - HIGH power
- 20 seconds - LOW power

We recommend reading the user manual of each biomaterial since commercial brands differ.^(29,30)

6. Curing modes

The light-curing process includes the initial polymerization or pre-gel phase and the final or post-gel phase. The polymerization point or gel point sits between both these phases. Biomaterials at gel point cannot leak internally since they lack flow and are rigid. Thus molecules lack mobility.^(31,32)

Different curing modes^(33,34) make it possible to reduce the initial shrinkage stress by delaying the gel point. The aim is to slow the polymer curing reaction to release internal stress.

These curing modes may be classified as follows (see Figure 6):

- Boost: LCUs emit the highest possible power generated during the entire firing interval.

Figure 5. Potential impact of not following the manufacturer’s recommendations during the curing process.

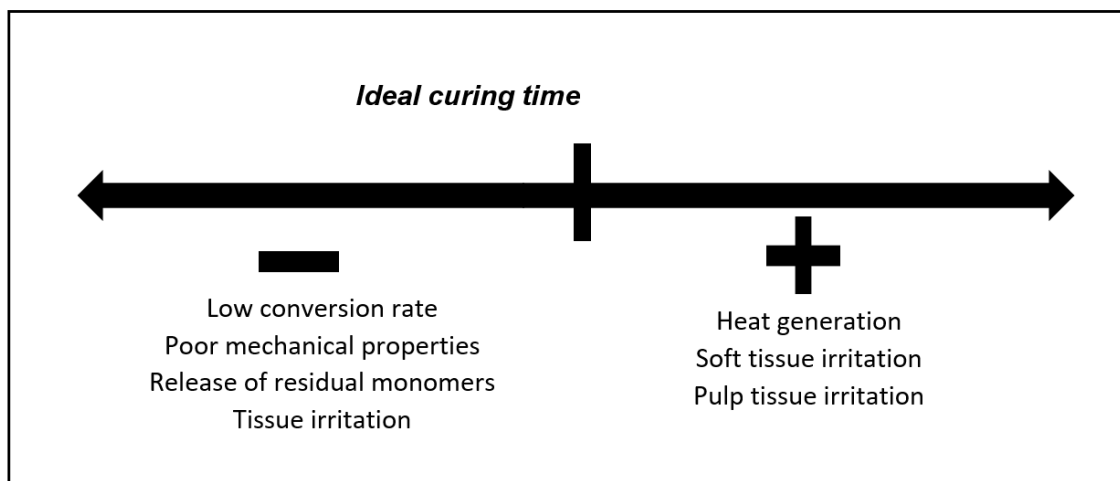


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- Step: LCUs start at low power (circa 150 mW/cm²), which is kept stable for ten seconds and then abruptly increases to a significantly higher power value for the rest of the curing interval.
- Ramp: LCUs start at low power (circa 150 mW/cm²), then power increases steadily until it peaks. This peak value is kept steady until curing ends.
- Pulse: LCUs turn on intermittently or cycle at high and low power every second.

These curing modes have been developed to reduce the shrinkage stress and temperatures reached during polymerization, without altering the physicochemical properties of CRs. (2,30,35,36)

Some procedures involve using instruments at a high rate or biomaterials and CRs undergoing exothermic and polymerization reactions, respectively. These may increase temperatures and result in pulp tissue deterioration. This could lead to postoperative sensitivity, pain, or even pulp necrosis. Temperature increases during photopolymerization are caused by exothermic reactions and the energy related to light absorption. (37,38) The heat produced depends mainly

on biomaterials, irradiance, and polymerization rates. Therefore, the light emissions used on teeth should be gradual to decrease temperatures and reduce contraction stress and eventually improve clinical outcomes. (16,22)

7. Collimation and photopolymerization distance

According to *in vitro* studies, (39,40) irradiance decreases the farther away LCU tips are from the surface of biomaterials during photopolymerization. So for a distance of 0 mm, irradiance of the LED unit measured is 1523 mW/cm² while this value drops to 734 and 521 mW/cm² at distances of 6 and 9 mm, respectively. In other words, there is a reduction of 52% and 66%, respectively. In clinical practice, this could occur when biomaterials are located at the gingival wall of a proximal cavity.

Therefore, we recommend placing LCUs as close as possible to the surface of biomaterials without any contact. In some units, beams of light are collimated, i.e., beams of light are spread minimally because rays are parallel. Consequently, the decrease in intensity is milder along the first millimeters of the distance covered (Figure 7). (41)

Figure 6. Intensities emitted over time for each curing mode.

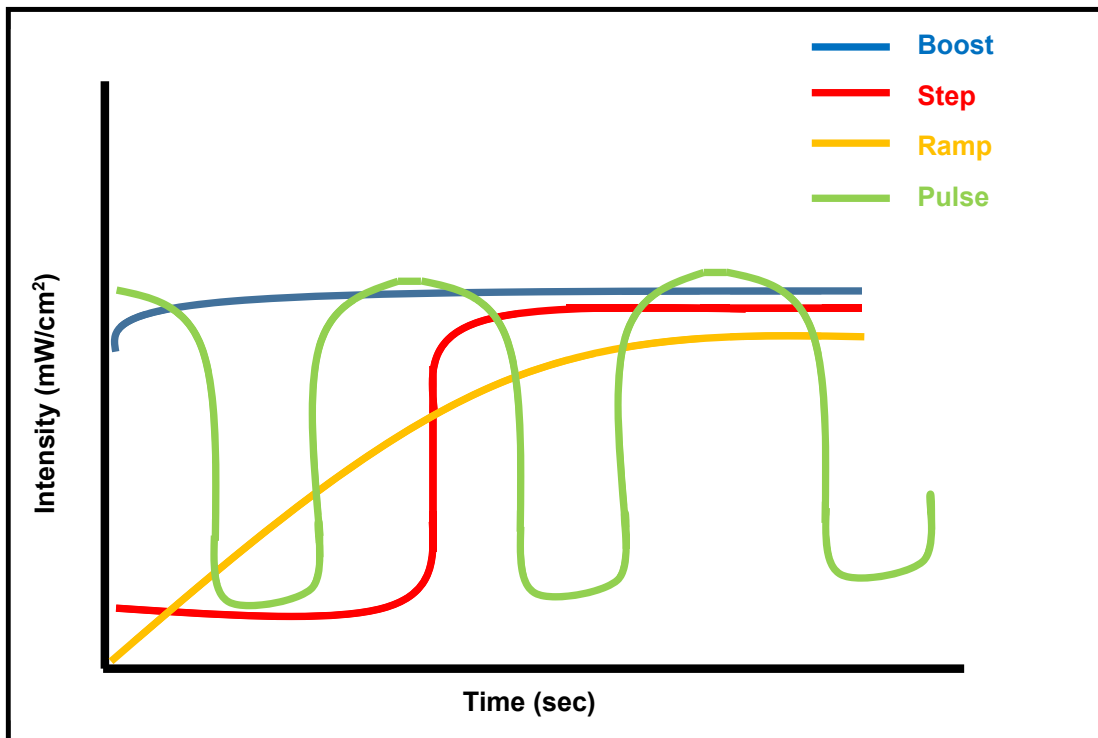


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Figure 7. Three images of a light-curing unit at different distances from the surface. By comparison, we can see that the exposure area increases at longer distances, and intensity and homogeneity decrease.

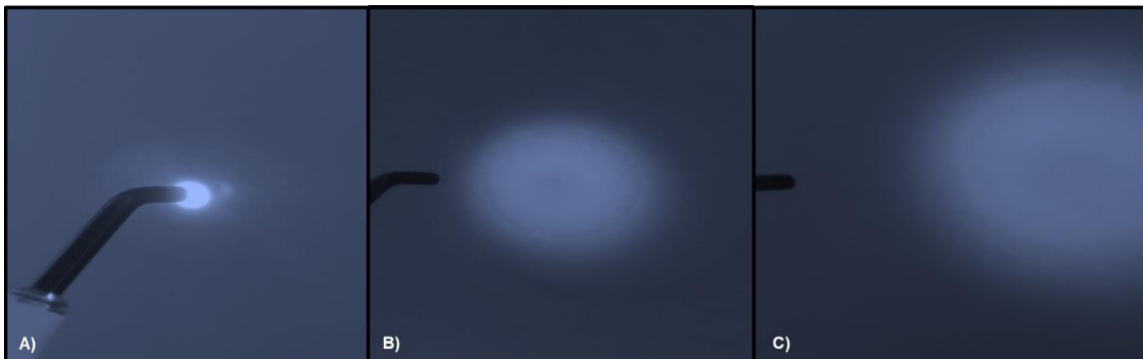


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8. Cleaning and disinfecting the unit

As previously mentioned, LCU tips should be as close as possible to biomaterials for optimal photopolymerization. Given this, it is common for the biomaterial to adhere to the LCU tip, which could interfere with light emission (Figure 8). Therefore, we recommend applying gauze with 70% alcohol regularly. This prevents adhered biomaterial from building up in large volumes. We do not recommend using metallic, sharp, or cutting instruments, which could irreversibly damage the surface of LCUs. Currently, there are four methods to sterilize unit tips.

a) Dry or moist heat sterilization: Some studies have shown that this procedure reduces light emission of the tip by 50% after 3 cycles.⁽⁴²⁾

This could be solved by polishing the tip to restore light transmission. However, this is complex and time-consuming, so it is not recommended.⁽⁴³⁾

b) Using disposable tips: Using previously sterilized single-use (disposable) plastic tips is considered a viable alternative.⁽⁴⁴⁾ However, this method is currently unavailable in our market.

c) Using disinfectants after every patient: 2% glutaraldehyde has effectively eliminated all live bacteria when the unit tip is wrapped in a cloth soaked with the disinfecting solution for 10 minutes. However, it has been reported that glutaraldehyde-based solutions may damage LCU tip fibers and thus diminish light transmission. External polishing may

Figure 8 Polymerized composite resin adhered to the surface of a unit tip.



Image of own authorship.

reverse this effect.⁽⁴⁵⁾ A cloth soaked in 70% ethanol did not remove live bacteria successfully. Cleaning with a disinfectant solution is a quick and convenient option, but we recommend the LCU tip be in contact with the disinfectant for at least 10 minutes to guarantee disinfection.⁽⁴⁶⁾

d) Using disposable translucent barriers is currently one of the most viable alternatives for aseptic chain maintenance. This is discussed in the following chapter.

9. Using barriers and operator protection

LCUs are classified as “semi-critical” instruments since they are in contact with mucosa and skin, and if there are lacerations, there is a risk of infection. Sterilization of LCUs may alter their components. Therefore, “barriers” are helpful in maintaining the aseptic chain and controlling crossed infections.⁽⁴⁷⁾

The market offers specific products differing in composition, such as polyurethane, polyethylene, and polyvinyl chloride, for total or partial coverage (only tips) of LCUs. There are other options, such as food packaging wrappers, nylon bags, latex gloves, etc.^(43,48)

According to the literature, all these barriers decrease the intensity of LCU light emissions. When properly placed, commercial barriers and food packaging wrappers decrease light intensity by 5% to 16%. However, when placement is incorrect, and the product has wrinkles or seams covering the tip, or it has dust inside, the intensity of light emissions can drop by up to 28%. The thicker the barrier, the lower the light intensity.⁽⁴³⁾ (Figure 9. A, B, and C).

So when using barriers, it is critical to measure light intensity to know if light-curing times

need to be increased or whether the device should not be used anymore due to insufficient intensity (see #5).

The use of protective eyewear should also be considered. It is strongly recommended since it avoids exposure to blue light, which can be dangerous. All LCUs emit visible light within the blue and blue/violet spectrum, which can cause eye damage, especially at 440 nm.⁽⁴⁹⁾ Exposure to high levels of blue light causes irreversible retina burning if blue light is absorbed. Even long-term exposure to low levels of blue light accelerates macular degeneration.⁽⁵⁰⁾

Protective eyewear that blocks blue light prevents acute injury and chronic exposure. A suitable blue light filter, such as filtering glasses, reduces light transmission with wavelengths below 500 nm by 99%. Practitioners use orange (amber) lenses to watch the light beam in action and ensure the tip’s correct position, aim and direction, ensuring the correct light-curing procedure. (Figure 9. D).

10. Periodic controls

There is no consensus on the appropriate frequency of LCU monitoring.⁽⁵¹⁾

Irradiance values obtained with radiometers may be good predictors of operating conditions, as they may indicate shortcomings in some parts of the device. We recommend keeping a record of measured intensities over time (Figure 10).

Hygiene and disinfection procedures, as well as battery charging, should be done conscientiously and cautiously daily: an adequate storage site is required.⁽²⁷⁾

Figure 9. A) Barrier correctly placed. B) and C) Barriers incorrectly placed. D) Protective eyewear with a blue light filter.



Image of own authorship.

Figure 10. Images of units in poor condition, most likely due to incorrect use or inadequate maintenance.

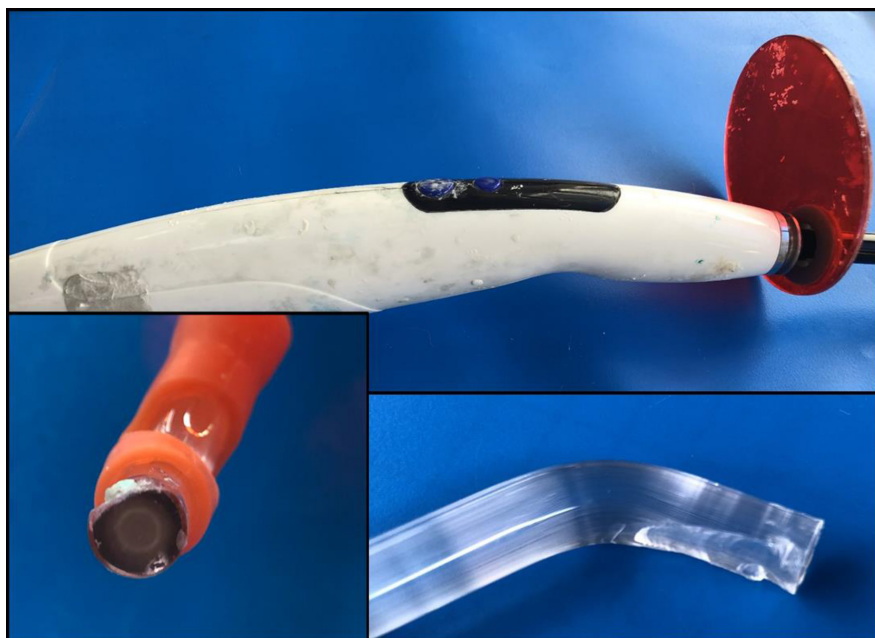


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Concepts developed in this Decalogue and usage recommendations are summarized in Table 1.

| Table 1. Summary of concepts developed in the Decalogue | | |
|---|---------------------------|--|
| FACTORS | CONCEPT | RECOMMENDATIONS |
| Related to UNITS*. | Wavelength | The wavelength emitted by LCUs and the absorption peak of the photoinitiator present in the polymeric material should be equal. ** |
| | Light intensity | We recommend a minimum intensity of 400 mW/cm ² . It is measured with a radiometer. |
| | Diameter of tip | It differs depending on the commercial brand. The size of the surface to be polymerized should be considered, and in some cases, several light applications may be required. |
| Related to OPERATORS | Curing time | LCU intensity and the type of material to be light-cured is considered to determine curing time. ** |
| | Curing modes | Different curing modes allow us to slow down the curing rate of polymers, which decreases shrinkage stress. These are: Conventional, Boost, Step, Ramp, or Pulse. |
| | Light-curing distance | We recommend placing LCUs as close as possible to the biomaterial without any contact. |
| | Using barriers | We recommend using plastic barriers in LCUs, correctly placed, and considering that they may reduce the intensity of LCUs. |
| Related to MAINTENANCE | Battery and charging | We recommend always keeping LCUs in the battery charger to keep it fully charged. |
| | Cleaning and disinfection | We recommend applying a gauze with 70% alcohol regularly and not removing residues of adhered polymeric material with sharp or cutting instruments that might scratch the LCU. |
| | Periodic controls | Light intensity should be monitored regularly with a radiometer. We recommend keeping a record of measured intensities over time. |

* The operator cannot modify these factors. ** Consider the information provided by the manufacturer and usage recommendations.

Conclusions

Health professionals should be informed and reminded about the importance of the proper use and maintenance of LCUs since this can affect the clinical performance of biomaterials, as discussed in this article.

The School of Dentistry of UdelaR (Montevideo, Uruguay) has implemented an annual control of the irradiance of LCUs. Therefore, we comply with most of the points mentioned in this report.

This Decalogue should be distributed and updated when new scientific evidence is reported.

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Corresponding author:

Guillermo Grazioli
Gral Las Heras 1925, CP:11300
ggrazioli@odon.edu.uy
099249098

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2. Acquisition of data
3. Data analysis
4. Discussion of results
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