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Effect of the Sample Preparation and Light-curing Unit on the Microhardness and Degree of Conversion of Bulk-fill Resin-based Composite Restorations

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Clinical Relevance

Resin composite properties are dependent on how the sample is prepared prior to testing. Clinicians should pay attention to the proximal boxes of bulk-fill resin composite restorations, as these areas may be inadequately polymerized.

SUMMARY

Objective: To evaluate the effect of the sample preparation and light-curing units (LCUs) on the Knoop hardness (KH, N/mm2) and degree of conversion (DC, %) of bulk-fill resin-based composite restorations.

Methods: Two molds were made using human molar teeth embedded in acrylic resin. One was a conventional tooth mold where the molar received a mesio-occluso-distal (MOD) preparation. In the other, the tooth was sectioned in three slices (buccal, middle, and lingual). The center slice received

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a MOD preparation similar to the conventional mold. Both tooth molds were placed in the second mandibular molar position in a Dentoform with a 44-mm interincisal opening. Restorations were made using Opus Bulk Fill (FGM) high viscosity bulk-fill resin-based composite (RBC) and light cured using two different lights: VALO Cordless (Ultradent) and Bluephase G2 (Ivoclar Vivadent). The RBC was placed in one increment that was light-cured for a total of 80 seconds (40 seconds at the occluso-mesial and occluso-distal locations). The RBC specimens were then prepared as follows: EmbPol – tooth mold specimen was embedded in polystyrene resin and polished before testing; Pol – tooth mold specimen was not embedded, but was polished before testing; NotPol – sectioned tooth mold, specimen not embedded nor polished before testing. The KH was measured in different depths and regions of the specimens, and the DC was measured using Raman spectroscopy.

Results: The results were analyzed using a 2-way analysis of variance (ANOVA) or repeated measures followed by the Tukey post-hoc test (α=0.05). The preparation method (p<0.001), depth of restoration (p<0.001), and the interaction between method and depth (p=0.003) all influenced the KH values. Preparation method (p<0.001), tooth region (p<0.001), and the interaction between method and tooth region (p=0.002) all influenced DC values. The KH values were reduced significantly from the top to the bottom of the restorations and also at the proximal box when compared with the occlusal region. This outcome was most significant in the proximal boxes. The NotPol method was the most effective method to detect the effect of differences in KH or DC within the restoration. A lower DC and KH were found at the gingival regions of the proximal boxes of the restorations. When the KH and DC values were compared, there were no significant differences between the LCUs (KH p=0.4 and DC p=0.317).

Conclusion: Preparation methods that embedded the samples in polystyrene resin and polished the specimens reduced the differences between the KH and DC values obtained by different preparation techniques. The NotPol method was better able to detect differences produced by light activation in deeper areas.

INTRODUCTION

Ideally, a resin-based composite (RBC) should require a relatively short exposure time, exhibit low shrinkage stress, and have a uniform conversion of monomers in all parts of the restoration.[1,2] The use of incrementally filled resin-based composites (RBC) can produce restorations that are harder, have a higher degree of conversion and lower shrinkage stress.[1] However, the use of the incremental layering and photo-curing technique is both time-consuming and more likely to incorporate voids or contamination between each increment of RBC.[2] The drive for faster strategies to restore deep cavities has led to the development of new materials that have an increased depth of cure and that can be light-cured in increments that are 4- to 5-mm thick.[3-5]

RBC restorations require appropriate polymerization of the material,[4] and the light-curing step is a critical procedure that is often overlooked when providing these RBC restorations.[7-10] When the light-curing process is incorrectly performed, this may lead to debonding, post-operative pain, discoloration, or premature failure of the RBC restoration.[11] Instead, the dentist may look towards using new bulk-fill RBCs that claim the RBC can be placed and adequately light-cured in 4- to 5-mm thick increments, and yet still achieve mechanical properties comparable to restorations made using the incremental filling and incremental light-curing technique.[6] However, the RBC, the light-curing unit (LCU), the restorative protocol, the size and location of the restoration, the emission spectrum from the LCU, and the radiant exposure received by the RBC will all affect the final polymerization of the RBC.[13-15]

Restorations in the posterior regions of the mouth are challenging for clinicians to light cure. Dental structures often get in the way, it is difficult to position the LCU tip perpendicular over the restorations in the mouth, and the type and opacity of restorative material can affect the ability to photo-cure the RBC.[9] For example, the greater the interincisal opening, the easier it is to position the tip of the LCU over the posterior teeth.[11,16] Children,[17] and patients with temporomandibular joint issues often have a limited mouth opening,[18] that will prevent adequate access of the LCU to the teeth. The design of the LCUs, its shape, and the angulation of the light tip, can also affect the ideal positioning of the LCU tip perpendicular to the surface of the restoration.[19] Limited mouth opening, the presence of matrix bands, or a poor design of the LCU may also lead to an increased distance between the restoration and LCU tips. This may introduce regions of the cavity that are in shadow and where less light is delivered. This will negatively influence the mechanical properties, color stability, solubility, dimensional stability, and biocompatibility of the RBC.[11,20]

Microhardness tests (Knoop or Vickers) and Raman spectroscopy are often used to measure directly or indirectly the polymerization of RBCs.[21-23] However, the surface must be flat for hardness or degree of conversion (DC) measurements. Therefore, the restorations are frequently embedded in resin and then cut or polished using copious liquid coolant.[21,22] Unconverted monomers on the surface of the RBCs can be washed away and lost during the cutting, finishing, and polishing processes.[24,25] Also, the exothermic heat produced during polymerization of the embedding material and any heat produced during polishing may increase the polymerization of the RBC.

Few studies have analyzed the effect of the preparation method on the microhardness and DC analyses, or the effect of the LCU design when used in a posterior RBC restoration in a clinical simulation. Therefore, this study aimed to evaluate the effect of the sample preparation of bulk-fill posterior RBC restorations made in a dentoform that had a clinically relevant interincisal mouth opening.[17,26] The null hypotheses were: 1) The method of sample preparation would not affect the KH or the DC of the bulk-fill restorations, and 2) The choice of LC (pen-style vs. angled light guide) will have no influence on the KH and DC of two bulk-fill RBCs.

METHODS AND MATERIALS

Cavity Preparation

This study was approved by the local ethics committee (protocol number 2.985.056). Two extracted intact caries-free human mandibular molar teeth with an average dimension of 10-mm from mesial to distal and a 4.7-mm occluso-pulpal distance were used to make two different molds.[27,28] To prevent extraneous light exposure, for the conventional sample preparation method (Figure 1A), the molar tooth was embedded in red acrylic resin (Dencrilay, Dencril, Pirassununga, SP, Brazil) to a depth of 2.0 mm below the cementoenamel junction.[29] This allowed some light-curing below the cementoenamel junction (Figure 1B). Using a cavity preparation machine,[30] a standard class II mesial-occlusal-distal (MOD) cavity was prepared using a cylindrical round diamond bur #3146 (KG Sorensen, Barueri, SP, Brazil) in a high-speed handpiece (Kavo do Brasil, Joinville, SC, Brazil) with copious air and water irrigation. The preparations had a 6-degree divergence, approximately 4/5 of the intercuspal width, 4.0-mm deep in the occlusal-pulpal dimension, a proximal box that was 2.0-mm wide, and a further 1.0-mm deep, making the proximal boxes 5-mm deep (Figure 1B-D).

For the new sample preparation method, a three-part matrix was developed.[28] The molar tooth was fully embedded, leaving only the occlusal region exposed (Figure 1E) in chemically activated red acrylic resin (Dencrilay). The mold was then sectioned into three parts (buccal, middle, and lingual) (Figure 1F). An impression of the middle part was taken with addition vinyl polysiloxane (Scan Putty Regular, Yller, Pelotas, RS, Brazil) to record its mesial and distal contours (Figure 1G). The MOD cavity preparation was then prepared using the same parameters described for the conventional method (Figure 1F). The transverse surfaces of the buccal and lingual parts of the matrix were polished using silicon carbide abrasive paper of decreasing grit size (#1200, #1500, #2000 and #2500, Norton, Campinas, SP, Brazil) followed by polishing with diamond pastes (6 μm; 3 μm; 1 μm; 0.25 μm; Arotec, São Paulo, SP, Brazil) on felt discs. All three

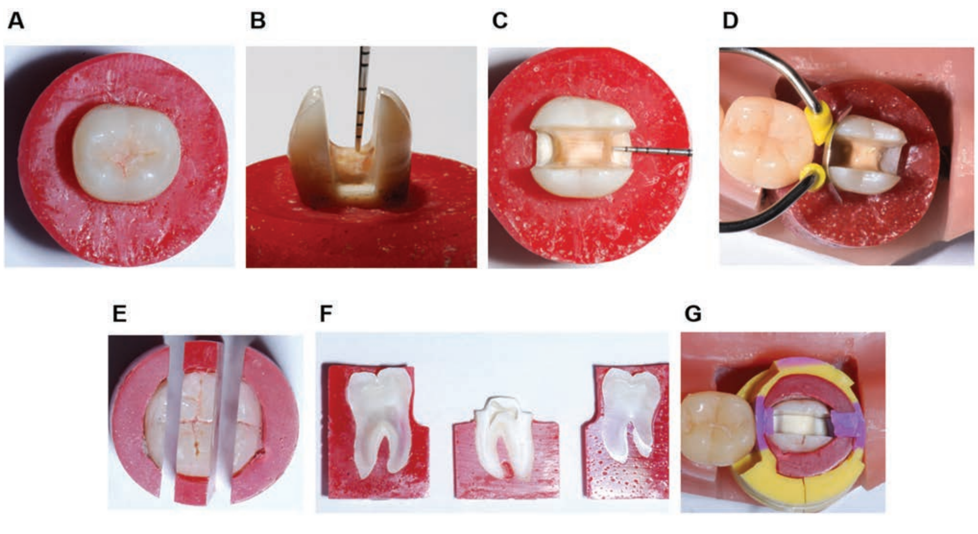

Figure 1. *Conventional matrix: (A) Molar tooth embedded in chemically activated acrylic resin in red colour. (B) Cavity preparation with 5 mm deep proximal boxes. (C) Occlusal view of the sample. (D) Sample showing the sectional matrix on one side. Three-part matrix: (E) Molar tooth totally embedded in chemically activated red acrylic resin, and sectioned in 3 parts (buccal, middle, and lingual). (F) View of the buccal, middle, and lingual parts. Middle part with no resin in the coronal third. (G) Sample showing the assembled mold.*

parts were then clamped together to make a MOD cavity (Figure 1F). This mold enabled the restoration to be easily removed by separating the three parts of the matrix after light-curing (Figure 1).

Development of a Buccal Opening Simulation Device

To better simulate the clinical environment, all restorations were light-cured with the tooth positioned in the second mandibular molar region. A dentoform (MOM, Marília, São Paulo, Brazil) was modified so that a cylinder of red acrylic resin (Dencrilay) containing the human molar tooth could be inserted. The prepared teeth were positioned to allow the proximal contact with its adjacent tooth (Figure 2A). Screws were used to fix and stabilize the resin cylinders in the correct position (Figure 2A). The dentoform was positioned in a dental patient simulator (MOM), and the interincisal mouth opening was fixed at 44 mm (Figure 2B).[17,26] This better simulated clinical reality compared to previous studies that used a tooth mold, but the tooth was not placed in a dentoform.[27, 28]

Restorative Procedure

A high viscosity bulk-fill RBC (Opus Bulk fill APS, FGM, Joinvile, SC, Brazil) and two multiple peak LCUs were used: VALO Cordless used on Standard power (Ultradent, South Jordan, UT, USA) and Bluephase G2 used on High power (Ivoclar Vivadent, Schaan, Liechtenstein). The irradiance (mW/cm^2), emission spectrum (mW/cm^2/nm), and radiant exposure (irradiance x time = energy/area = J/cm^2) emitted from the LCUs was measured five times using the MARC Resin Calibrator (BlueLight Analytics, Halifax, NS, Canada). The LCU's internal tip diameter was measured using a digital caliper (Mitutoyo, Mississauga, ON, Canada). A sectional matrix band (Unimatrix, TDV Dental Ltda, Pomerode, Santa Catarina, Brazil) was positioned and stabilized using an interdental wooden wedge at the mesial contact point in the groups that used the conventional tooth mold. The bulk-fill RBC was placed up to 5-mm thick, and the LCU was positioned 1 mm above the occlusal surface. The RBC was light-cured by hand for 40 seconds over the occluso-mesial and 40 seconds over the occluso-distal regions by a well-trained operator, following the manufacturer's recommendation to cover the whole of the restoration. The light-curing process was performed in a dark room with yellow light to avoid any possible light interfering with the RBC polymerization process. No adhesive system was applied so that the RBC sample could be removed from the mold. After light curing, the restorations were removed and stored in the dark and a controlled humidity at 37°C for 24h.

Preparation of the Samples for Microhardness Test (n=5)

The restorations were prepared following the three groups:

1. EmbPol – after removing from the mold, the restorations were embedded in polystyrene resin (Cristal, Piracicaba, SP, Brazil). Before testing, the RBC surfaces were finished with silicon-carbide paper (#1200, 1500, 2000, and 2500 grit sizes; Norton) followed by cleaning in an ultrasonic bath in distilled water for 5 minutes, and polished with metallographic diamond pastes (6-, 3-, 1-, and 1/4-μm sizes; Arotec) suspended in isopropyl alcohol.
2. Pol – the RBC restorations were not embedded in polystyrene resin, but were polished as described for the CEmPol Group and fixed on a glass coverslip using cyanoacrylate (Super Bonder Loctite, São Paulo, SP, Brazil).
3. NotPol – restorations made using the three-part matrix[28] were not polished nor embedded in polystyrene resin. Instead, the RBCs were removed from the tooth mold and stabilized with cyanoacrylate on a glass slide. The smooth RBC surface required for testing was obtained by the opposing polished surface of the tooth mold.

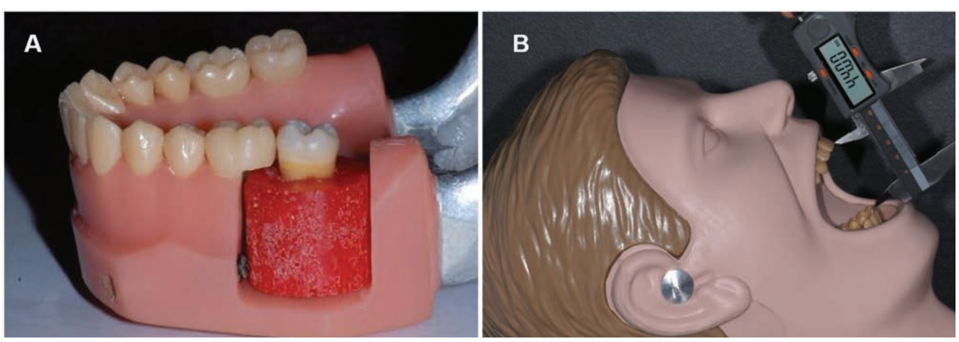

Figure 2. *Dentoform adapted to hold a cylindrical resin block in the second mandibular molar position (A) and sample showing its contact point. (B) The interincisal distance is set at 44 mm.*

Knoop Microhardness Test (KH)

Knoop microhardness indentations (Shimadzu HMV 2000; Shimadzu Corporation; Kyoto, Japan) were made in the transverse surface of the restorations using 50 g for 10 s at every 1.0 mm from the gingival and pulpal walls of the restorations. Ten indentations were made at the proximal box, and eight indentations were made at the occlusal box (A). The recorded KH data were plotted using Origin Pro 2020 (OriginLab, Northampton, MA, USA) software to produce hardness maps of the three groups.

Degree of Conversion (%)

The degree of conversion (DC) in the mesial and distal proximal boxes was evaluated at five locations: M1: occluso-mesial spot; M2: mesial proximal box spot; O: occlusal spot; D1: occluso-distal spot; D2: distal proximal box spot. The occlusal spot and a proximal box spot were at least 2-mm apart from each measurement point, Figure 3B). The DC was measured using a LabRam HR Evolution Raman spectrometer (Horiba LabRam, Villeneuve d'Ascq, France) and an excitation power of 17 mW. Using the radiation emitted by a He-Ne laser (633 nm), a Raman signal was acquired using a 600 line/mm grating centered between 1000 and 2000 cm^{-1} with a 200 μm confocal hole. These settings enabled spectra to be acquired with a resolution of 1.05 cm^{-1}/pixel. The spectra were then adjusted by polynomial function and by manual multiple point baseline correction. From the Raman vibrational modes, the areas of peaks: aliphatic (1638 cm^{-1}) and aromatic (1608 cm^{-1}) were calculated from polymerized (P) and unpolymerized (NP) bulk-fill RBC samples. The formula used to calculate the degree of conversion was: DC (%) = (1 - P / NP) x 100.

Statistical Analysis of Data

The KH and DC values were tested for normal distribution and equality of variances using Shapiro-Wilk and Levene tests. The data were then analyzed using two-way repeated-measures analysis of variance and Tukey post-hoc tests. The study factors were LCU type (2 levels), and sample preparation methods (3 levels), and the repetitions were considered the location of the restorations. All tests used a 0.05 level of statistical significance and were performed using Sigma Plot version 13.1 (Systat Software Inc, San Jose, CA, USA).

RESULTS

LCU Characterization

The mean and standard deviation of the tip irradiance values for the VALO Cordless was 1298 ± 3.3 mW/cm^2, and Bluephase G2 was 1394 ± 4.5 mW/cm^2. The emission spectrum for VALO Cordless ranged between 395-480 and Bluephase G2 between 385-515 nm. In 40 s, the occluso-mesial and at the occluso-distal regions of the restorations received a radiant exposure of 51.9 J/cm^2 from the VALO Cordless, and 55.8 J/cm^2 from the Bluephase G2 at each light-curing location: Thus, both lights delivered similar irradiances and radiant exposures.

Knoop Microhardness – KH (N/mm^2)

Means for KH values obtained in the specimens made using the two LCUs for each sample preparation method at various restoration depths are reported in Figure 4. ANOVA results demonstrated that the sample preparation method had a significant effect (p<0.001), the effects of the restoration depth were significant (p<0.001), and there was an interaction between sample preparation method and restoration depth (p=0.003).

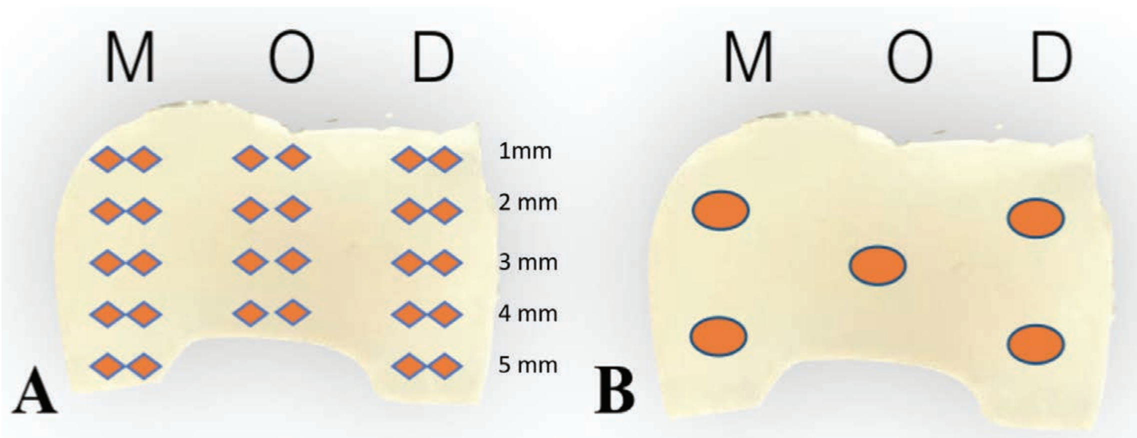

Figure 3. *Locations where the tests were performed: (A) Knoop microhardness indentations. (B) Degree of conversion measurement locations.*

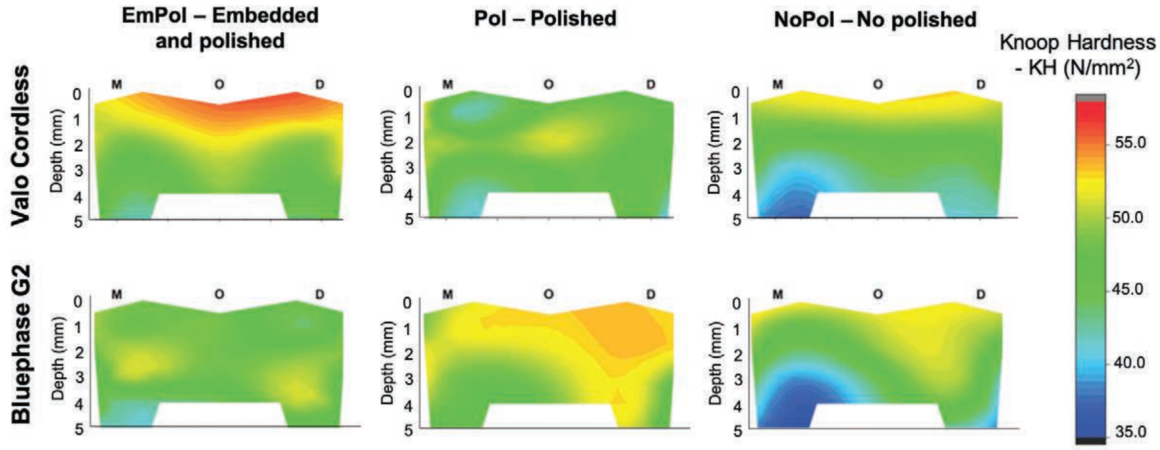

Figure 4. *Knoop hardness maps from the means obtained (n=5) comparing the three different methods used to prepare the samples and the two LCUs used.*

However, the choice of LCU had no significant effect (p=0.475), neither for interaction between sample preparation method and LCU (p=0.734), LCU and depth of restoration (p=0.700); and there was no interaction among sample preparation method, LCU and depth of restoration (p=0.766). The KH values were significantly reduced from the top to the bottom of the restoration. The location of the restoration influenced the KH values only in deeper regions. At 5 mm, the KH values were significantly lower in proximal boxes than at the occlusal region. This occurred mainly at the proximal boxes (p<0.05). The NotPol method was the most sensitive method at detecting the effects of restoration depth and tooth region.

Degree of Conversion - DC (%)

The means and standard deviations for the DC values obtained from the specimens made using the two LCUs for each sample preparation method at various restoration depths are reported in Figure 5. The ANOVA results showed that the sample preparation method (p<0.001), tooth region (p<0.001), and the interaction between sample preparation method and tooth region (p=0.002) were all significant. However, the LCU (p=0.127), sample preparation method and LCU (p=0.104), LCU and tooth region (p=0.114), sample preparation method, LCU and tooth region (p=0.154) all had no effect. The DC values were significantly lower at the gingival region of the proximal boxes compared to the measurements made on the top of the restorations, irrespective of the local, occlusal or distal areas. The DC measured on the M2 spot (gingival region) of the restoration's mesial proximal box for the NotPol method was the lowest compared to all the other methods.

DISCUSSION

The sample preparation method had a significant influence on the KH and DC results. Therefore, the first null hypothesis was rejected. When the RBC was light-cured for 40 seconds over the occluso-mesial and 40 seconds over the occluso-distal regions, the choice of LCU had no significant influence on these parameters. Thus, the second hypothesis was accepted.

In vitro studies usually perform restorations under ideal conditions, without any limitation on mouth opening or any difficulty when positioning the LCU over the restorations. Many studies also polish the RBC before testing.[24,31] Since the bottom or the sides of the restoration in contact with the tooth surface are never polished, and access to the restoration is often challenging in the posterior teeth,[23] these studies do not simulate clinical conditions. For this reason, the experimental design used in the present study was developed to better simulate the clinical condition.

The microhardness test method that is often used to assess the polymerization of RBCs requires a flat smooth surface. For this reason, the samples are frequently embedded and polished before hardness testing.[24] The polystyrene resin, commonly used for the embedment of specimens, has an exothermic polymerization reaction. The concern is that this exothermic reaction may heat the sample and thus increase the polymerization of the RBC.[32,33] The Pol and NotPol specimens made in the present study were not embedded and therefore received no additional heating effect. Since the Pol samples were not embedded in resin, the most likely explanation for the elevated KN and DC results observed in the EmbPol samples was this increase in the temperature of the RBC caused by the exothermic reaction of the polystyrene resin. The

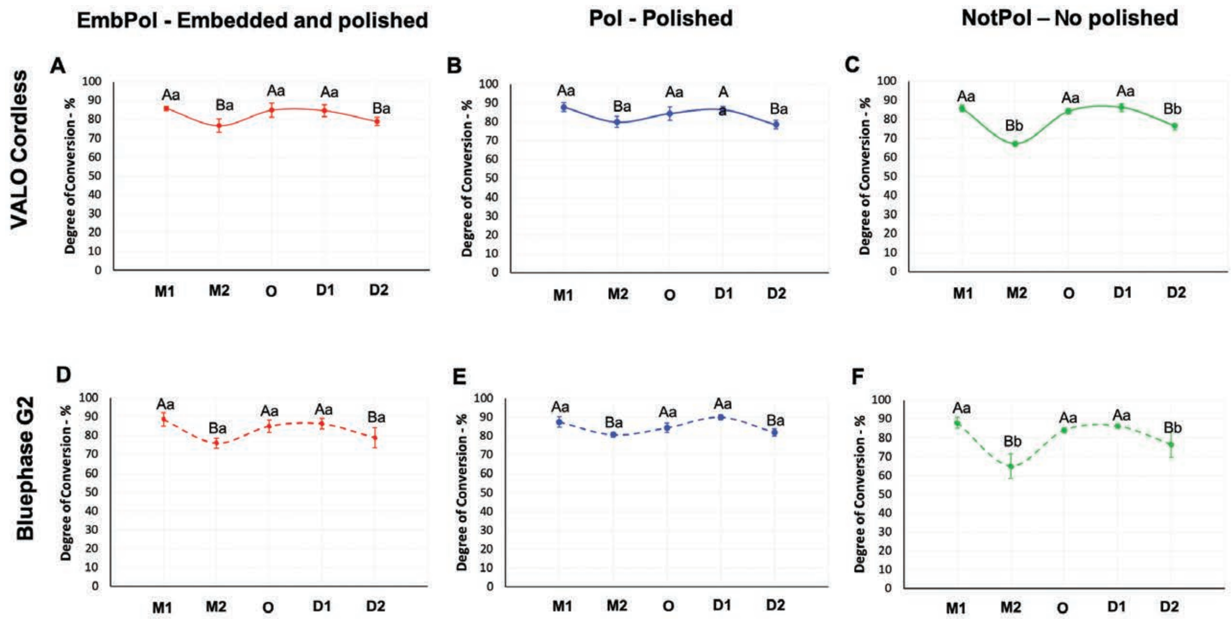

Figure 5. *Means and standard deviation of Degree of Conversion (%) comparing the three different sample preparation methods and the two LCUs (n=5). Different letters indicate a significant difference: uppercase letters are used for comparing the tooth region, and lowercase letters are used for comparing the sample preparation method (p<0.05). No significant difference was observed between the two LCUs. Abbreviations: M1, occluso-mesial spot; M2, mesial-proximal box spot; O, occlusal spot; D1, occluso-distal spot; D2, distal-proximal box spot.*

DC in the gingival region of the proximal boxes of the restorations made using the NotPol method was lower than the DC measured in the restorations that had been polished. This probably occurred because the vinyl polyvinylsiloxane material present surrounding the proximal boxes would have reduced the amount of light received in these regions, but so would an opaque metal matrix. Thus, the three-part matrix method is recommended for future studies.[28]

Regarding the polishing process used before microhardness testing, the use of a copious amount of coolant to prevent local heat generation may also preferentially remove relatively hydrophilic, free monomers, such as residual triethylene glycol dimethacrylate (TEGDMA), or other low molecular weight monomers that have some degree of water solubility,[34-37] from surfaces of cut or polished specimens. In addition, some studies have used an alcohol-based polishing suspension.[38-40] In the present study, this suspension contained isopropyl alcohol. This treatment will likely remove more residual monomer and have an even greater effect on the RBC surface properties than polishing with an aqueous-based solution.[41-43]

The KH and DC tests should be performed at a standardized post-cured time because the restorations do not develop their final mechanical properties immediately after curing.[44,45] Therefore, in the present study, all the specimens were stored for 24 hours before testing. The KH and DC values were significantly reduced from the top to the bottom of the restoration, mainly at proximal boxes, which raises the concern of having a premature failure in those areas due to lack of adequate polymerization. Although the use of bulk-fill RBCs reduces the chair time, a lack of adequate polymerization along the bulk of the restoration may result in lower mechanical properties in some regions, compared to a highly filled nano-hybrid RBC restorations that were placed and light-cured using an incremental technique.[31]

In this study, even at the mesial proximal boxes of the NotPol group, the DC values were greater than 60%. The high DC could be attributed to the long exposure time of 80 seconds delivered to each RBC restoration, which resulted in the VALO Cordless delivering 51.9 J/cm^2, and the Bluephase G2 delivering 55.8 J/cm^2 at each light-curing location (occluso-mesial and occluso-distal regions). The DC values were higher at the surface locations of the RBC restorations (over 80%), which was different from the results of other studies that showed lower DC values.[3,5,6] This exposure time was chosen because the manufacturer (FGM) recommended 40 seconds of exposure time. However, to cover the whole tooth area is also recommended to light cure at more than one spot.[11]

Although it is recognized that specimens can be made in a metal, a Teflon mold,[30] or a silicone mold between two polyester strips[4], these molds do not simulate the difficulties in the positioning of the LCUs over restorations that frequently occur in clinical conditions. In the present study, the specimens were made in a dentoform to simulate clinical conditions. The inter-incisal distance was set to 44 mm.[17,26] This mouth opening is close to the adult (18 to 70 years old) mouth opening that has been reported to range from 56.6 mm to 49.1 mm for men and from 49.8 mm to 44.4 mm for women.[46] Also, an Irish study reported a 43 mm of mouth opening for men and 41 mm for women,[47] which only slightly smaller than the mouth opening of Brazilian children.[17]

The mouth opening, the location of the cavity, and the operator experience all can affect the total energy delivered to RBCs.[16] The use of a patient simulator with an adjustable mouth opening enables *in vitro* studies to better replicate clinical reality. As shown by the results, the interincisal distance of 44 mm did not affect the results produced by either of the quality LCUs tested in this study. The Bluephase G2 has a curved tip, and the Valo Cordless has a straight design, but both showed similar KH and DC results. Therefore, for the mouth opening distance used in this study, both of these LCUs show similar results, which may not be valid for a patient who has a more limited mouth opening, or with LCUs that are not so well ergonomically designed.[11] The hardness maps (Figure 4) showed reduced polymerization at the bottom of the mesial proximal boxes than at the distal proximal boxes. This was photo-cured with the Bluephase G2. This may indicate the difficulty of the Bluephase G2 positioning due to the light tip angle even though the statistical analysis did not indicate any difference between the LCUs. Thus, clinicians should pay attention to the LCU shape and design because of the possibility of reducing the irradiance at the proximal boxes when LCUs with a higher light tip angle are used.[11] Delivering additional light exposure from the buccal and lingual regions of RBC restoration is recommended after removing the matrix band to compensate for the deficient polymerization in this situation. However, this should not be relied upon as the sole method of curing the RBC at the bottom of the proximal box because the significant amount of light attenuation through the tooth structure will reduce the impact of photo-curing through the tooth.[48]

Both LCUs are multi-peak broad-spectrum LED units, and both have been reported to deliver homogeneous beam profiles.[49,50] However, Valo Cordless was a pen style LCU and Bluephase G2 had a light guide. These two different designs were chosen to help elucidate if the tip shape and angulation factors should be considered when choosing the LCU, especially in areas where the position of the tip over the restoration could be affected. In a recent study comparing 22 contemporary light-curing units,[11] it was shown that the tip design can affect the ability to position the light tip at 90° to the posterior occlusal surface. However, further studies are encouraged to evaluate the effect of the mouth opening and different LCU designs on access to restorations in the mouth and irradiance on the beam profile from the LCUs. Further studies could use a mouth opening less than 44 mm, representing a child or a patient with limited mouth opening. Since the properties of the restorations such as DC and KH are dependent on the sample preparation method, the authors suggest that future *in vitro* studies should simulate restorations made under clinical conditions by using unpolished samples made in a dentoform and a three-part matrix.

CONCLUSION

Within the limitations of this *in vitro* study, it was concluded that sample preparation that embedded and polished specimens before testing reduced the differences between the KH and DC values of one bulk-fill RBC. The NotPol method was better able to detect differences produced by light curing in deeper areas of the restorations. When the RBC was light-cured for 40 seconds over the occluso-mesial and 40 seconds over the occluso-distal regions, no significant differences were found between the pen style Valo Cordless and Bluephase G2 that had a light guide when using a 44 mm interincisal mouth opening.

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Conflict of Interest

The authors have no financial interest in any of the companies or products mentioned in this article.

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