Influence of photo-activation source on enamel demineralization around restorative materials

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Abstract: This study evaluated the effects of the photoactivation source and restorative material on the development of caries-like lesions on human enamel after an *in vitro* pH challenge. Enamel cavities were prepared in 36 blocks, which were assigned to two groups according to the restorative material: resin-modified glass ionomer (RMGI) and composite resin (CR). Samples were exposed to quartz-tungsten-halogen lamp, argon-ion laser, or light-emitting diode ($n = 6$). The Knoop microhardness (KHN) values of the top surface of all materials were evaluated. Restored enamel blocks were thermocycled and subjected to 10 demineralization-remineralization cycles at 37°C. KHN analysis of the superficial enamel was performed by four indentations located 100 μm from the restoration margin. The material KHN was not affected by the photoactivation source. No significant difference in KHN was noted between CR and RMGI. The enamel surface around RMGI exhibited a higher KHN (272.8 KHN) than the enamel around CR (93.3 KHN), regardless of the photoactivation source. Enamel demineralization around the dental restoration was not influenced by the photoactivation source. Less enamel demineralization was observed around the RMGI than around the CR restoration.

Descriptors: Glass Ionomer Cement; Composite Resins; Tooth Demineralization.

Introduction

The longevity of dental restorations is related to the durability of the bond and the sealing of the cavity margins.[1] Microspaces or gaps at the restoration/enamel interface may allow the penetration of fluid containing cariogenic microorganisms,[2] resulting in secondary caries progression.[2,3] Secondary caries are frequent in patients with high caries risk and may necessitate the replacement of dental restorations.[4] To prevent restoration failure, researchers developed fluoride-releasing glass-ionomer (GI) restorative materials that inhibit secondary caries formation.[5,6] The subsequently developed resin-modified glass ionomer (RMGI) materials showed some improved mechanical characteristics, such as better flexural strength and surface-wear resistance, compared to conventional GI cements,[7] as well as superior handling properties due to their light-activation.

Vitremer (3M ESPE) is a hybrid material of GI restorative material and composite resin (CR) with acid-base and light-cured reactions. Vit-

remer sets via "triple-cure" mechanisms.[8] The first two setting reactions are based on an acid-base neutralization and free-radical methacrylate. The third setting reaction ensures the continuous polymerization of the remaining monomers that are not excited during light exposure. In the third reaction, microencapsulated potassium persulfate and ascorbic acid form a patented redox catalyst system that provides the methacrylate cure of the glass ionomer in the absence of light. [9]

Exposure of enamel to argon laser (AL) prior to cariogenic challenge has been shown to reduce the depth of caries lesions compared to enamel surfaces without prior AL exposure.[2,3,10-14] This preventive effect has been attributed to the surface coating created by AL, which promotes changes in the mineral structure and organic component of the surface coating. The resulting coating consists of a reactive surface that is less susceptible to caries formation. In addition to the synergistic effect between topical fluoride and argon irradiation,[12-14] increased fluoride retention (400%) has been reported on enamel irradiated with a low energy density of AL (10.72 J/cm²).[15] However, the exact mechanism of caries resistance by AL irradiation remains unknown.[16] Based on the dental literature, it is reasonable to speculate that the activation of restorative materials with AL would prevent secondary caries formation, especially when a fluoride-releasing restorative material is used.

The induction of caries-like lesions around restorations *in vitro* is an experimental approach that provides information about the clinical behavior of the restorative materials under the experimental conditions.[6,17] The objective of this study was to evaluate the cariostatic potential of the light-activation source and restorative materials *in vitro* after a pH challenge. The response variable evaluated was the Knoop microhardness (KHN) profile of human enamel around and in the superficial area of the restorative materials. The following null hypotheses were tested:

1. the superficial enamel KHN values around two restorative materials cured by different light-curing units are not different;
2. the KHN values of the two restorative materials are not different.

Methodology

Ethical aspects

This study protocol was approved by the Guarulhos University Research Ethics Committee (CEP-UnG, process no. 104/2009).

Experimental design

The factors under study were the material (2 types) and photoactivation source (3 types) in a factorial 2 × 3 design for evaluation of the restorative material and the superficial enamel. The response variable was the KHN.

The experimental units consisted of 36 dental blocks (n = 6 per group) obtained from 18 unerupted human third molars stored in 0.1% thymol solution at 4°C. Blocks of 4 × 4 × 2 mm were sectioned from the third molars with double-faced diamond disks (#7020; KG Sorensen, Barueri, Brazil) at low speed (Kavo, Joinville, Brazil) under water irrigation. Cavities of approximately 1.6 mm in diameter and 1.6 mm in depth were prepared with #2292 diamond burs (KG Sorensen, Barueri, Brazil) under water spray. Teeth were distributed into two groups according to the restorative material, and each group was divided into three subgroups (Table 1).

Table 1 - Experimental groups.

Groups	Subgroups		Energy density (J/cm²)	*n* samples
	Activation source/Activation time			
RMGI	1.1	Quartz-tungsten halogen lamp/40 s	28	6
	1.2	Argon laser (200 mW)/20 s	12.8	6
	1.3	Light-emitting diode/20 s	24	6
CR	2.1	Quartz-tungsten halogen lamp/40 s	28	6
	2.2	Argon laser (200 mW)/20 s	12.8	6
	2.3	Light-emitting diode/20 s	24	6

RMGI: resin-modified glass ionomer material; CR: composite resin.

Table 2 - Composition, lot number, and application mode of the selected materials.

Material Manufacturer	Composition	Directions for use
Adper Single Bond 2 (3M-ESPE, Irvine, USA; Lot: 9XL)	HEMA, Bis-GMA, DMAs, functional methacrylate, copolymer of polyacrylic and polyitaconic acids, water, ethanol, nanofillller, photoinitiator	Consecutively apply 2 coats, gently air-dry, and light-cure for 10 s
Conditioner (3M-ESPE, Irvine, USA; Lot: 060808)	**Conditioner:** etchant (37% phosphoric acid)	Etch cavity for 15 s, wash and dry (do not desiccate)
Z350-OA3 (3M-ESPE, Irvine, USA; Lot: 7CN)	Bis-GMA, Bis-EMA, UDMA and camphorquinone. Fillers: Zirconia-sílica	Light-activate each increment for 40 s for QTH source
Vitremer (3M-ESPE; St. Paul, USA; Lot: 8HP; 8HH)	**Powder:** fluoroaluminosilicate glass, microencapsulated potassium, Persulfate, ascorbic acid and pigments **Liquid:** aqueous solution of a polycarboxylic acid modified with pendant methacrylate groups, copolymer, water, HEMA and photoinitiators	Light-activate each increment for 40 s for QTH source with 2-mm maximum thickness
Vitremer Primer (3M-ESPE; St. Paul, USA; Lot: 8CB)	Vitrebond copolymer, HEMA, ethanol and photoinitiators	Apply primer with a brush for 30 s to both dentin and enamel; air-dry and light-cure for 20 s
Vitremer Finishing Gloss (3M-ESPE; St. Paul, USA; Lot: 8FC)	Unfilled resin, TEGMA, Bis-GMA, photoinitiators	Apply and light-activate for 20 s

Bis-GMA = bisphenol glycidyl methacrylate; Bis-EMA = bisphenol A polyethylene glycol diether dimethacrylate; UDMA = urethane dimethacrylate; DMA = dimethacrylate; HEMA = 2-hydroxyethyl methacrylate; TEGMA = triethylene glycol dimethacrylate.

All cavities were prepared and restored by the same calibrated operator. Table 2 shows composition, lot number, and application instructions of the selected materials. A commercial RMGI (Vitremer; 3M ESPE, St. Paul, USA; A3 shade) was tested in this study. Prior to the RMGI application, Vitremer Primer (3M ESPE, St. Paul, USA) was applied for 30 s, dried for 5 s, and light-activated for 20 s. The RMGI was mixed according to the manufacturer's instructions in a 1:1 proportion and inserted in a single increment by syringe (Centrix Inc., Shelton, USA). Groups 1.1, 1.2, and 1.3 were light-activated according to each activation source. After RMGI insertion, Vitremer Finishing Gloss was applied and light-activated for 20 s with a light-emitting diode (LED) (Radii Cal; SDI Limited, Bayswater, Australia).

For all groups, the adhesive was light-activated with a LED curing unit (Radii Cal; irradiance: 1,200 mW/cm²). After the bonding procedure, a nanofilled resin composite (Filtek Z350-OA3, 3M ESPE) was inserted in a single increment and activated by the respective photoactivation source. Restored enamel blocks were stored in 100% relative humidity at 37°C for 24 h and then polished with the Sof-lex (3M ESPE) polishing system for 15 s with each disk.

An 11-mm-diameter light tip was used with a quartz-tungsten-halogen (QTH)-based curing unit (Optilux 501; Demetron/Kerr, Danbury, USA), whereas the LED source (Radii Cal) had 1,200 mW/cm² and an 8-mm-diameter light tip. The power density of both curing units was constantly measured with a radiometer (Demetron/Kerr, Danbury, USA). The power selected for the AL (Accucure 3000; LaserMed, Salt Lake City, USA) was 200 mW for 20 s. A 6.3-mm-diameter spot size was measured with the knife-edge method. The energy density (J/cm²) of the light emitted by all curing units was calculated as the power density multiplied by the exposure time.

Both products were quantitatively evaluated by five central indentations at 100-μm distance on the top surface (Figure 1). A microhardness tester (Pan-Tec; Panambra Ind. e Técnica SA, São Paulo, Brazil) was used with a 25-g load for 20 s, with a dwell time of 15 s. The specimens were thermocycled 1,000 times between distilled water baths held at 5°C and 55°C. The dwell time was 60 s and 5 s of transfer time (MSCT-3e; Elquip, Equipamentos para

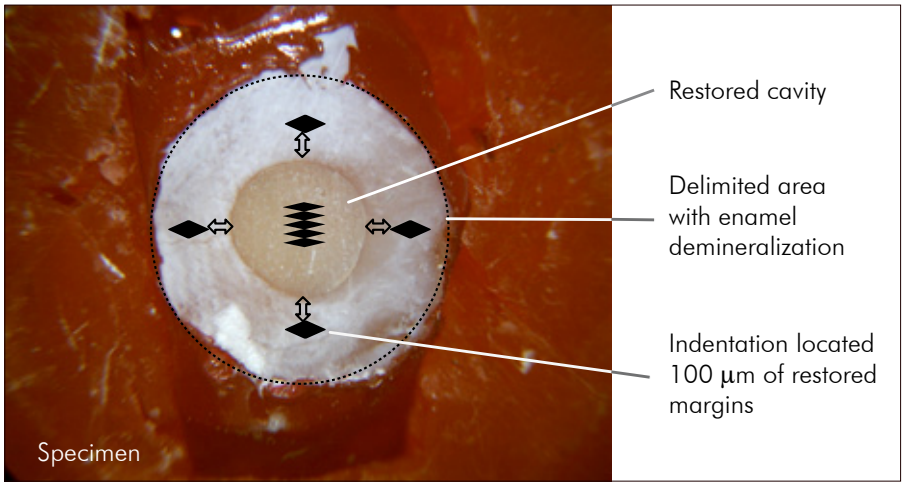

Figure 1 - Schematic representation of the superficial enamel microhardness and indentation location on the restorative materials.

Pesquisa Odontológica, São Carlos, Brazil).

The restored enamel blocks were covered with wax, except for the restoration area and 1 mm around this area, which remained exposed. The restored enamel blocks were submitted to pH challenge to induce caries-like lesions. The *in vitro* demineralization/remineralization dynamic model produced caries-like enamel lesions through a modified Featherstone model[18] to simulate the condition of high caries risk. Each restored block was placed in 15 mL of demineralization solution at 37°C (2.0 mmol/L of calcium, 2.0 mmol/L of phosphate in a buffer solution of 74 mM of acetate, pH 4.3). The pH-cycling regimen was performed over 14 days, with 10 daily cycles of 6 h in demineralizing solution at 37°C. The remineralizing solution contained calcium and phosphate at a known degree of saturation (50 mmol/L KCl, 1.5 mmol/L Ca, 0.9 mmol/L PO_4, 20 mmol/L tri-hydroxymethil-aminomethan, pH 7.0) that was changed daily. The enamel blocks were washed with distilled water before an 18-h immersion in remineralizing solution. On the 6th, 7th, 13th, and 14th days of the cycle, the restored enamel blocks were kept only in the remineralizing solution.[6]

The quantitative KHN evaluation of the enamel caries-like lesions was performed by the PanTec microhardness tester, with a 25-g load for 5 s. Four indentations located 100 µm from the bonded surface were created on the enamel surface in each enamel block in the upper, lower, left, and right sides around the restorations (Figure 1).

Table 3 - Material evaluation of the Knoop microhardness values for the material and photoactivation source factors, mean [standard deviation] and *n* samples.

Restorative material	QTH (28 J/cm^2)	AL (12.8 J/cm^2)	LED (24 J/cm^2)
RMGI (*n* = 6)	43.1 [12.6]	50.8 [11.6]	49.2 [13.3]
CR (*n* = 6)	65.3 [12.6]	55.5 [15.1]	52.5 [24.6]

RMGI: resin-modified glass ionomer; CR: composite resin; QTH: quartz-tungsten-halogen; AL: argon laser; LED: light-emitting diode.

Statistical analysis

Two-way ANOVA was performed to evaluate the influence of the two variables tested: photoactivation source and material for superficial enamel and material evaluations. The means of the KHN values were compared by a Tukey *post-hoc* test ($\alpha = 0.05$). The software used for statistical analysis in both evaluations was SANEST (EPAMIG, Belo Horizonte, Brazil). Power was calculated with the G power 3.1.2 software package (Heine, Universität Dusseldorf, Germany) ($p = 1$) for both material and enamel evaluation.

Results

ANOVA did not show differences for the "material" factor ($p = 0.05$), "photoactivation source" factor ($p = 0.86$), or interactions between factors ($p = 0.25$). Table 3 shows the KHN values and Tukey results of both materials exposed to the evaluated photoactivation sources for material evaluation. In the evaluation of the superficial enamel

Table 4 - Superficial enamel evaluation of the enamel Knoop microhardness values for the material and photoactivation source factors, mean [standard deviation] *n* samples and the results of Tukey's test.

Superficial enamel	QTH (28 J/cm²)	AL (12.8 J/cm²)	LED (24 J/cm²)	Material factor
RMGI (*n* = 6)	275.8 [8.5]	272.5 [17.4]	267.6 [29.0]	272.8 [19.9]A
CR (*n* = 6)	115.4 [74.7]	82.7 [52.9]	80.7 [63.3]	93.3 [62.4]B

Means followed by different uppercase letters indicate significant differences ($p < 0.05$). RMGI: resin-modified glass ionomer; CR: composite resin; QTH, quartz-tungsten-halogen; AL, argon laser; LED, light-emitting diode.

demineralization, ANOVA showed differences for the "material" factor ($p < 0.05$) but no differences for the "photoactivation source" factor or interactions between factors (both $p = 0.7$). Table 4 shows the KHN and Tukey results for the "material" and "photoactivation source" factors for the superficial enamel evaluation.

Discussion

According to the results of the present study, the first null hypothesis (the superficial enamel KHN values around two restorative materials cured by different light-curing devices are not different) was rejected. Null hypothesis no. 2 (the KHN values of the two restorative materials are not different) was accepted. Although several studies have shown that AL irradiation provides reduced depth of caries-like lesions,[10-14] and increased enamel microhardness[12] compared with control groups, in the current study, the AL irradiation promoted no significant changes in enamel KHN values.

A previous study showed that the use of halogen or AL activation with 200 mW for 20 s resulted in similar degrees of conversion for the same resin composite, whereas decreased superficial KHN values were obtained for AL activation with an exposure time of 10 s.[19] Therefore, this protocol was used in the present study. The exposure time of 20 s allowed an energy density of 12.8 J/cm². This lower energy density of AL was sufficient to promote higher fluoride retention for enamel, as demonstrated by Nammour *et al.*[15] Turbino *et al.*[20] recommended against the use of thickness increments of more than 1 mm for the AL with a power setting of 250 mW and an exposure time of 30 s.

The first AL was cleared for marketing and clinical use in 1991 by the U.S. Food and Drug Administration (FDA).[21] Since that time, AL has been used clinically to light-activate dental materials[17] and has been shown to be capable of polymerizing compomers[2] or CR.[20,22] The use of AL in dentistry reduces the chair time in dental offices. The adhesion of orthodontic brackets previously showed favorable results in terms of the time required for the bonding procedure.[23-25] There is evidence that topical fluoride application decreases the depth of enamel primary caries lesions.[10-14] The enamel resistance to caries was also demonstrated *in vitro*[24,25] and *in vivo*[23] after AL irradiation. However, although AL was effective at activating the restorative materials in the present study, laser technology is more expensive than QTH or LED.

The dynamic pH-cycling model has been shown to be adequate for studies of enamel caries-like lesions.[6,18] Various protocols (e.g., with 5 pH cycles[17] or 14 days in demineralization solution[16]) have been used to simulate demineralization/remineralization phenomena in the oral environment with different restorative materials[2] and activation sources.[16] Nevertheless, other important clinical variables that are not addressed in the *in vitro* setting should also be considered, such as the cariogenicity and frequency of the patient's diet or presence of saliva.[6]

Roberts *et al.*[26] demonstrated that increments above 3 mm are not recommended for the same RMGI material evaluated in the present study. A 1.6-mm-deep standardized cavity preparation was used to ensure maximal setting from light activation. Importantly, in this study, the *P*-value for the "material" factor was 0.05 when the microhardness of the CR or RMGI was evaluated (Table 3). This borderline significant result may be due to the small sample size of our study ($n = 6$), which is a limitation of this paper.

The preventive effect of the evaluated RMGI[5-6] was evident once higher enamel KHN values were

recorded around this restorative material compared to enamel KHN values around the resin composite (Table 4). This result was related to the fluoride-release benefit of RMGI material.[27] Fluoride release reduces demineralization on enamel around the restoration and along the cavity wall. The evaluated resin composite contains organic pigments. Because of its dark shade, the manufacturer recommends an exposure time of 40 s, which is similar to the recommended exposure time for the RMGI when a halogen-based curing unit is used. The dark shade (OA3) of the CR promotes light attenuation.

Conclusion

The photoactivation source did not influence enamel demineralization around dental restorations superficially. There was less development of enamel demineralization around RMGI restorations than around CR restorations.

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References

1. Pereira PN, Inokoshi S, Tagami J. In vitro secondary caries inhibition around fluoride releasing materials. J Dent. 1998 Jul-Aug;26(5-6):505-10.
2. Hicks MJ, Ellis R, Flaitz C, Werstermann G, Powell L. Restoration-enamel interface with argon laser and visible light polymerization of compomer and composite resin restorations: a polarized light and scanning electron microscopic in vitro study. J Clin Pediatr Dent. 2003 Summer;27(4):353-8.
3. Hicks J, Flaitz C, Ellis R, Westerman G, Powell L. Primary tooth enamel surface topography with in vitro argon laser irradiation alone and combined fluoride and argon laser treatment: scanning electron microscopic study. Pediatr Dent 2003 Sep-Oct;25(5):491-6.
4. Sarrett DC. Clinical challenges and the relevance of materials testing for posterior composite restorations. Dent Mater. 2005 Jan;21(1):9-20.
5. Dionysopoulos P, Kotsanos N, Koliniotou-Koubia E, Tolidis K. Inhibition of demineralization in vitro around fluoride releasing materials. J Oral Rehabil. 2003 Dec;30(12):1216-22.
6. Rodrigues JA, Marchi GM, Serra MC, Hara AT. Visual evaluation of in vitro cariostatic effect of restorative materials associated with dentifrices. Braz Dent J. 2005;16(2):112-8.
7. Ellakuria J, Triana R, Mínguez N, Soler I, Ibaseta G, Maza J, et al. Effect of one-year water storage on the surface microhardness of resin-modified versus conventional glass-ionomer cements. Dent Mater. 2003 Jun;19(4):286-9.
8. Swift EJ Jr, Pawlus MA, Vargas MA, Fortin D. Depth of cure of resin-modified glass ionomers. Dent Mater. 1995 May;11(3):196-200.
9. Mount GJ, Patel C, Makinson OF. Resin modified glass-ionomers: strength, cure depth and transucency. Aust Dent J. 2002 Dec;47(4):339-43.
10. Flaitz CM, Hicks MJ, Westerman GH, Berg JH, Blankenau RJ, Powell GL. Argon laser irradiation and acidulated phosphate fluoride treatment in caries-like lesion formation in enamel: an in vitro study. Pediatr Dent. 1995 Jan-Feb;17(1):31-5.
11. Hicks MJ, Flaitz CM, Westerman GH, Blankenau RJ, Powell GL, Berg JH. Enamel caries initiation and progression following low fluence (energy) argon laser and fluoride treatment. J Clin Pediatr Dent. 1995 Fall;20(1):9-13.
12. Westerman GH, Ellis RW, Latta MA, Powell GL. An in vitro study of enamel surface microhardness following argon laser irradiation and acidulated phosphate fluoride treatment. Pediatr Dent. 2003 Sep-Oct;25(5):497-500.
13. Westerman GH, Hicks MJ, Flaitz C, Powell GL. In vitro enamel caries formation: argon laser, light-emitting diode and APF treatment effect. Am J Dent. 2004 Dec;17(6):383-7.
14. Westerman GH, Hicks MJ, Flaitz CM, Ellis RW, Powell GL. Argon laser irradiation and fluoride treatment effects on caries-like enamel lesion formation in primary teeth: an in vitro study. Am J Dent. 2004 Aug;17(4):241-4.
15. Nammour S, Rocca JP, Pireaux JJ, Powell GL, Morciaux Y, Demortier G. Increase of enamel fluoride retention by low fluence argon laser beam: a 6-month follow-up study in vivo. Lasers Surg Med. 2005 Mar;36(3):220-4.
16. Das UM, Prashanth ST. A comparative study to evaluate the effect of fluoride releasing sealant cured by visible light, argon lasers, and light emitting diode curing units: an in vitro study. J Indian Soc Pedod Prev Dent. 2009 Jul-Sep;27(3):139-44.
17. Magalhães CS, Hara AT, Turssi CP, Serra MC, Giannini M. Microhardness evaluation around composite restorations using fluoride-containing adhesive systems. J Appl Oral Sci. 2005 Sep;13(3):259-64.
18. Featherstone JDB, O'Reilly MM, Shariati M, Brugler S. Enhancement of remineralization in vitro e in vivo. In: Leach SA. Factors relating to demineralization and remineralization of the teeth. Oxford: IRL; 1986. p. 23-34.

19. Cassoni A, Ferla JO, Shibli JA, Kawano Y. Knoop microhardness and FT-Raman Spectroscopy evaluation of a resin-based dental material light-cured by an argon íon laser and halogen lamp: an in vitro study. Photomed Laser Surg. 2008 Dec;26(6):531-9.
20. Turbino ML, Belan LC, Soprano V, Rode KM, Ramos PL, Youssef MY. Argon ion laser curing depth effect on a composite resin. Lasers Med Sci. 2011 Jul;26(4):421-5. Epub 2010 May 28.
21. Powell GL, Blankenau RJ. Laser curing of dental materials. Dent Clin North Am. 2000;Oct;44(4):923-30. Review.
22. Cassoni A, Ferla JO, Albino LG, Youssef MN, Shibli JA, Rodrigues JA. Argon ion laser and halogen lamp activation of a dark and light resin composite: microhardness after long-term storage. Lasers Med Sci. 2010 Nov;25(6):829-34. Epub 2009 Jul 22.
23. Anderson AM, Kao E, Gladwin M, Benli O, Ngan P. The effects of argon laser irradiation on enamel decalcification: an in vivo study. Am J Orthod Dentofacial Orthop. 2002 Sep;122(3):251-9.
24. Noel L, Rebellato J, Sheats RD. The effect of argon laser irradiation on demineralization resistance of human enamel adjacent to orthodontic brackets: an in vitro study. Angle Orthod. 2003 Jun;73(3):249-58.
25. Tavares JG, Eduardo CP, Burnett Jr LH, Boff TR, Freitas PM. Argon and Nd:YAG Lasers for Caries Prevention in Enamel. Photomed Laser Surg. 2012 Aug;30(8):433-7. Epub 2012 Jun 29.
26. Roberts HW, Berzins DW, Charlton DG. Hardness of three resin-modified glass-ionomer restorative materials as a function of depth and time. J Esthet Restor Dent. 2009;21(4):262-72.
27. Ilie N, Hickel R. Mechanical behavior of glass ionomer cements as a function of loading condition and mixing procedure. Dent Mater J. 2007 Jul;26(4):526-33.