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ORIGINAL ARTICLE

Effect of different root caries treatments on the sealing ability of conventional glass ionomer cement restorations

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Abstract In this study we compared the microleakage of conventional glass ionomer cement (GIC) restorations following the use of different methods of root caries removal. In vitro root caries were induced in 75 human root dentin samples that were divided in five groups of 15 each according to the method used for caries removal: in group 1 spherical carbide burs at low speed were used, in group 2 a hand-held excavator was used, and in groups 3 to 5 an Er,Cr:YSGG laser was used at 2.25 W, 40.18 J/cm² (group 3), 2.50 W, 44.64 J/cm² (group 4) and 2.75 W, 49.11 J/cm² (group 5). The air/water cooling during irradiation was set to 55%/65% respectively. All cavities were filled with GIC. Five samples from each group were evaluated by scanning electron microscopy (SEM) and the other ten samples were thermocycled and submitted to a microleakage test. The data obtained were compared by ANOVA followed by Fisher's test ($p \le 0.05$). Group 4 showed the lowest microleakage index (56.65 6.30; p < 0.05). There were no significant differences among the other groups. On SEM images samples of groups 1 and 2 showed a more regular interface than the irradiated samples. Demineralized dentin below the restoration was observed, that was probably affected dentin. Group 4 showed the lowest microleakage values compared to the other experimental groups, so under the conditions of the present study the method that provided the lowest microleakage was the Er,Cr:YSGG laser with a power output of 2.5 W yielding an energy density of 44.64 J/cm^2 .

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Introduction

The increase in life-span associated with the development and application of preventive dentistry concepts have contributed to the maintenance of the teeth of the elderly. This dental longevity is accompanied by an increased frequency of exposed root surfaces as a result of periodontal diseases, mechanical injury, surgical treatment, or a combination of these factors, which, combined with some situations common to ageing, such as reduced salivary flow due to diseases or drugs and the inability to perform adequate oral hygiene, leading to biofilm accumulation on tooth surfaces, enhance the risk of root caries occurrence [1]. Carious lesions can be prevented or even inactivated through the adoption of preventive actions such as reduction in carbohydrate intake, biofilm control, oral hygiene instruction and the use of fluoride compounds [2]. However, in some situations, when deep carious lesions and/or pulpal sensitivity are present there is a need for restoration. In these cases, caries removal and restorative treatment are required.

The most used method for removing dental caries is the use of rotary instruments [3]. Recently, the use of laser technology as an alternative to traditional methods for cavity preparation has been introduced. The Er,Cr:YSGG laser is safe for dental application and has been used especially in minimally invasive procedures [4, 5]. More-over, it is considered more comfortable for patients than conventional methods for caries removal because it requires less or no anaesthesia during clinical procedures [6].

Scanning electron microscopy (SEM) images of cavities prepared using the Er,Cr:YSGG laser show a dentin surface without thermal damage, free of a smear layer, with opened dentinal tubules and with protrusion of the peritubular dentin [7]. The ablation process increases the area available for adhesion and does not create a smear layer, but irradiated dentin seems less receptive to adhesive processes than acid-etched dentin. Some studies have shown that even after etching by an acid or self-etching agent, the bond strength of irradiated permanent and deciduous dentin is lower than that of regular acid-etched dentin [8–10]. However, different results have been found in other studies, making this a controversial issue [11–15].

Because root caries removal using the Er,Cr:YSGG laser is more comfortable for the patient, it is important to evaluate the quality of restorations carried out after this caries removal method. Thus, the aim of this study was to compare the sealing ability of conventional glass ionomer cement (GIC) restorations following the use of different methods of root caries removal.

Materials and methods

Tooth selection

This study was approved by the ethics committee of the University of São Paulo Ribeirao Preto School of Dentistry. The study included 38 human molar and premolar teeth extracted due to periodontal disease. The teeth were donated by the Ribeirao Preto School of Dentistry, Sao Paulo University. After cleansing and root planing using a curette until the dentin was exposed, the teeth were stored in distilled water under refrigeration at 4°C.

Tooth preparation

The dental roots were separated from the crowns at the cement-enamel junction using a sectioning machine (Minitom; Struers, Westlake, OH) with a diamond disk (Isomet; 10.2 cm×0.3 mm, arbor size 0.5 inches, series 15HC diamond; Buehler, Lake Bluff, IL) at low speed. The dental roots were sectioned into 75 dentin blocks $(5 \times 5 \text{ mm})$. All blocks were polished to expose the dentin. The samples were then sealed with cosmetic varnish (Colorama Maybelline, São Paulo, Brazil) leaving an exposed area of 9 mm² (3×3 mm) for caries induction. Each fragment was fixed with wax at the bottom of a plastic tube leaving the exposed dentin surface at the top. Caries was induced using a dynamic model of demineralization and remineralization simulating the in vivo highrisk conditions for caries, similar to that presented by Featherstone et al. [16]. The demineralization solution (pH 4.3) consisted of 2.0 mmol/l Ca and 2.0 mmol/l phosphate in a buffer solution of acetate 0.075 mol/l, and the remineralization solution (pH 7.0) consisted of 1.5 mmol/l Ca, 0.9 mmol/l phosphate and 150 mmol/l potassium chloride. Each specimen was cycled in 5.0 ml of each solution for 8 h in the demineralizing solution and 16 h in the remineralizing solution. This procedure was carried out for 25 days at 37°C. At the end of each five consecutive days of cycling, the samples were immersed in remineralizing solution for 2 days. This cariogenic challenge produced lesions with a depth of approximately 1.0 mm. The depth of the carious lesions was known since this methodology had previously been standardized in a pilot study.

Experimental groups

After induction of root caries, the samples were randomly divided into five groups of 15 samples each according to the method used for caries removal. In group 1, the root caries lesions were treated by the conventional method with spherical carbide burs at low speed without water cooling. In group 2 atraumatic restorative treatment (ART) was performed using dentin excavators with their active (cutting) edges in a round form and compatible with the lesion size. The Er,Cr:YSGG laser was used in groups 3, 4 and 5. The parameters used are shown in Table 1.

The Er, Cr: YSGG laser (Waterlase; BioLase Technology, San Clemente, CA) used operates at a wavelength of 2.78 μ m with a pulse duration of 140 μ s and a repetition rate of 20 Hz. The average power output varies from 0.0 to 6.0 W. The laser energy is delivered through a fibre optic system to a sapphire tip that has a terminal diameter of 600 µm and is bathed in air/water from an adjustable spray. In this investigation, the power output was set from 2.25 to 2.75 W yielding an energy density from 40.18 to 49.11 J/cm². The samples of groups 3, 4 and 5 were irradiated under an air/water spray adjusted to 55%/65%, respectively. To simulate a clinical situation, the nail varnish was removed before lesion treatment. For this reason, we attempted to perform caries removal only within the restricted area of 3×3 mm, leaving the border of the cavity in sound dentin. In all groups caries removal was considered finished when by visual and probe examination no carious tissue was noted in the cavity walls and at the bottom of the cavity.

Cavity restoration

Prior to GIC placement, the dentin surfaces were conditioned with a polyacrylic acid (Ketac Conditioner, 3 M ESPE) for 10 s. The cavities were then filled with a conventional restorative GIC (Ketac Fil Plus, 3 M ESPE, St. Paul, MN) according to the manufacturer's instructions.

Table 1 Experimental groups

Group	Method for caries removal	Power output (W)	Energy density (J/cm ²)
1	Spherical carbide burs in a low-speed hand-piece	_	_
2	Atraumatic restorative treatment	_	-
3	Er,Cr:YSGG laser	2.25	40.18
4	Er,Cr:YSGG laser	2.50	44.64
5	Er,Cr:YSGG laser	2.75	49.11

A varnish (Colorama) was applied over the restoration prior to water storage. The samples were then placed in distilled water at $37\pm1^{\circ}$ C and after 24 h the restorations were finished using a Sof-Lex disc system (3 M ESPE) with discs of decreasing grit.

Thermal cycling and microleakage test

Ten samples per group were thermocycled and submitted to a microleakage test. The specimens were subjected to thermal cycling using 500 cycles between water baths at 5±1°C, 37±1°C and 55±1°C with a 1-min dwell time a 3-s transfer time between baths. The samples were isolated again with nail varnish leaving a margin of 1 mm around the restoration. Then, they were immersed in a 50% silver nitrate aqueous solution for 8 h in the dark, washed in running water and immersed in a developer solution (Kodak, São José dos Campos, SP, Brazil) for 8 h to acquire a dark colour which allowed visualization of areas with dye penetration. The teeth were then rinsed thoroughly in tap water and were embedded in chemically activated acrylic resin (JET, Classic, São Paulo, Brazil) and sectioned longitudinally using a water-cooled diamond saw in a sectioning machine (Minitom; Struers, Copenhagen, Denmark), providing three sequential sections of each restoration. No distinction was made among the mesial, distal, buccal and lingual faces. Sections were initially thinned in a polishing machine (Politriz; Struers, Copenhagen, Denmark), and then manually smoothed to obtain a flat surface and a final thickness of approximately 0.25 mm. Cuts were identified and carefully fixed on microscope slides, and the entire dentin/restoration interface was analysed under a 5× magnification optical microscope (Axiostar Plus; Carl Zeiss Vision, München-Hallbergmoos, Germany) connected with a 10× magnification lens to a digital camera (Cyber-shot 3.3 MPEG Movie EX, model no. DSC-S75; Sony, Japan). The digital images obtained were transmitted to a personal computer and analysed using Axion Vision 3.1 software (Carl Zeiss Vision), which performed a standardized assessment of the extent of the tracer agent along the dentin/GIC interface and provided quantitative measurements in millimetres. To calculate the percentage microleakage, the total length of the tooth/ restoration interface was measured. Then the length of the interface infiltrated by dye was calculated. With these data we were able to calculate for each section the infiltration index as the percentage of the interface length showing infiltration by multiplying the infiltrated interface length by 100 and dividing that value by the total interface length. As three sections per sample were analysed, it was possible to calculate an average for each sample. The mean dye penetration in the dentin interfaces were then calculated for each group.

Scanning electron microscopy

Five samples per group were randomly selected for analysis by SEM. Samples were sectioned in the middle, one half being polished and immersed in 2.5% glutaraldehyde (Sigma-Aldrich, St. Louis, MO) in 0.05 M sodium cacodylate buffer at pH 7.4 (Merck, Darmstadt, Germany) for 24 h at 37°C. After fixation, the samples were rinsed with distilled water several times for 1 h. The dentin surfaces were etched with EDTA gel (Scotchbond etchant, 3 M/ESPE) for 15 s and rinsed thoroughly for 15 s. The specimens were then immersed for 10 min in an ultrasonic cleaner (T-1449-D; Odontobra's Ind. e Com, Ribeirao Preto, SP, Brazil) containing distilled water, and sequentially dehydrated in a series of ethanol solutions of ascending concentrations (Labsynth Produtos para Laboratorio, Diadema, SP, Brazil) (25% for 20 min, 50% for 20 min, 75% for 20 min, 90% for 30 min, 100% for 60 min). Next the specimens were immersed in hexamethyldisilazane (HMDS; Merck, Darmstadt, Germany) for 10 min, placed on absorbent paper between glass plates and left to dry in an exhaust system. Specimens were mounted on stubs, sputter-coated with gold and examined in a scanning electron microscope (FEG SEM; Philips Electron Optics, Eindhoven, The Netherlands) operating at 20.0 kV.

Statistical analysis

First, the assumptions of equality of variances (modified Levene equal-variance test) and the normality of the error distributions (Shapiro-Wilk test) were checked for the response variables tested. Since the assumptions were satisfied, ANOVA (α =5%) was applied using OriginPro 8 SR0 software (Origin Lab Corporation, Northampton, MA). The Fisher LSD multiple comparison test was used at the 5% significance level to evaluate the differences between the means.

Results

The measurements and standard deviations of the microleakage found in each group are given in Fig. 1. Among the five groups, group 4, irradiated with an output power of 2.5 W, showed the lowest microleakage index (56.65±6.30; p<0.05). There were no significant differences among the other groups.

Figure 2 shows tooth/restoration interfaces in the five experimental groups. In general, the samples of groups 1 and 2 showed a more regular interface than samples from the Er,Cr:YSGG laser-irradiated groups (groups 3, 4 and 5). Demineralized dentin below the restoration could be observed that was probably affected dentin. Surprisingly, this layer was thinner in samples of group 2, indicating that the ART treatment removed more dental tissue than the other methods of root caries treatment.

Discussion

The achievement of an optimal marginal seal is a key factor in restorative dentistry, since the presence of marginal deficiencies has been reported to be one of the main reasons for restoration failure [17]. The sealing of root surface restorations is influenced by the method of cavity preparation, as well as by the material used to fill the cavity.

In this study, the quality of marginal sealing of GIC restorations was tested after root caries induction using a



Fig. 1 Microleakage index in the different groups. p<0.05

pH-cycling model and subsequent lesion treatment with a carbide bur at low speed, by hand excavator, or using the Er,Cr:YSGG laser. A conventional GIC was used because it has been shown to be superior to other restorative materials in terms of inhibiting secondary carious lesions in cementum and dentin [18] and satisfactory marginal sealing. GICs reduce bacterial penetration, probably by adhesion to the tooth structure, by release of fluoride, and because of their low initial pH [19]. Moreover, this material has lower polymerization shrinkage than composite resins and a coefficient of thermal expansion similar to the tooth structure, suggesting that microleakage at the tooth/restoration interface would be less than with composite resins [20, 21].

Several studies have sought to evaluate the microleakage from GIC restorations in cavities prepared by the methods used in this study, but the previous studies were performed in sound dental tissue, thus not reproducing clinical situations [22–24]. For that reason, we chose to induce caries lesions in human root dentin fragments and to analyse the marginal sealing of restorations placed in cavities resulted from the caries treatment, thus simulating clinical situations. The results obtained in the present study showed that the Er,Cr:YSGG laser is a possible alternative to conventional methods for treating carious tissue because laser preparation using 44.64 J/cm² permitted a better interaction between the restorative material and the cavity walls.

The heat generated in the target tissue during irradiation can change the amount of some tissue components. This was shown by Yu et al. who observed that the amount and ratio of calcium and phosphorus by weight in dentin irradiated with a Er,Cr:YSGG laser at an output power of 5.0 W were increased compared to the control group without irradiation [25]. Similar results were found by Hossain et al. who measured the calcium ions in human dentin after irradiation at an energy density of 25 J/cm² [26]. Recently, Secilmis et al. reported that the calcium/ phosphorus ratio may be affected if dentin is irradiated at a power of 1.0 W [27]. Thus, an increase in calcium content could explain the better performance of the restorations in group 4, as the adhesion of GIC to dentin would be enhanced in tissue rich in calcium ions. The energy densities used in groups 3 and 5 were probably not sufficient to contribute to this effect. When conventional GIC is placed on enamel or dentin, a dissolution smear layer is formed, but demineralization is minimal since the tooth hydroxyapatite buffers the acid, and polyalkenoic acid is quite weak [28]. Phosphate and calcium ions are displaced from the hydroxyapatite and absorbed in the unset cement. This results in an intermediate layer between the GIC and the hydroxyapatite called the ion-exchange layer that consists of calcium and phosphate ions from the Fig. 2 SEM images of each experimental group (G1-G5). A layer of demineralized dentin (*asterisks*) is seen between the GIC restoration (*GIC*) and the sound dentin (*D*)



GIC and aluminium, silicic, fluoride, calcium and/or strontium ions (depending on the glass composition) from the GIC [29].

Regarding the laser energy densities used in the present study, before the execution of the present research, a pilot study was conducted to evaluate some parameters of caries removal found in the literature. The pilot study showed that the parameters used in previous studies were high enough to promote dentin carbonization [6, 26, 30]. In This preliminary study showed the best power outputs, i.e. those that allowed caries removal without visible thermal damage and the lesions to be treated with an acceptable speed, to be in the range 2.25–2.75 W.

A previous study involving SEM analysis of lased dentin surfaces revealed cavity surfaces free of a smear layer with open dentinal tubules, which could influence the permeability and surface humidity [7]. The crater-like irradiated surface is considered to be favourable for micromechanical retention as the viscosity of GIC is such as to allow its penetration into the surface microirregularities [31]. This could explain why additional conditioning improved laserprepared cavities, compared with conditioned bur-treated cavities. Application of a weak acid, such as polyacrylic acid, is strongly indicated as a pretreatment of the dentin surface because it cleans the surface and optimizes contact between the restorative material and the substrate by eliminating only the smear layer without demineralising the dentin or removing smear plugs [32]. Such treatment keeps calcium ions available for chemical reaction with the cement, and also avoids contamination of the restoration by moisture from dentinal fluids [22].

Another factor to emphasize is that according to modern concepts of operative dentistry only the infected dentin is removed, leaving affected dentin in the cavity, which has the potential to remineralize [33]. On this basis, the treatments proposed in this study were effective. SEM images showed the presence of demineralized tissue under restorations, being probably the affected dentin. These findings agree with those of Aoki et al. [34] and Geraldo-Martins and Marques [7], who reported that erbium lasers did not remove all carious tissue, the residual carious tissue being noted only at the microscopic level [7, 34].

Although this was not the objective of the present study. an interesting finding in both the SEM and microleakage analyses was that all samples of group 2, in which manual excavators were used for root caries treatment, showed a lower amount of demineralized tissue under the restoration than other groups. This finding suggests that ART removes more dental tissue than laser or bur treatment. Other have evaluated several methods of dentin caries removal and, unlike the results obtained in the present study, found that hand excavation is more conservative than the conventional bur technique [35, 36]. The manual pressure during excavation and the objective of achieving a cavity with the least amount of infected tissue may have been responsible for this increased removal of dental tissue. The opposite may have occurred when using the conventional method. Considered the most invasive method for caries treatment [35], the operator was aware that this method removes more dental tissue than necessary and, for this reason took more care during the procedure, for example in decreasing the pressure in the target tissue to reduce dentin removal and to avoid over-preparation. In contrast, the Er,Cr:YSGG laser, regardless of the applied energy density, resulted in an uncontrolled, random pattern, with deep over-prepared areas and wide under-prepared zones in the same cavity, in agreement with previous studies [7, 35]. According to Celiberti et al., it is very difficult to give accurate guidelines for sound and hard dentin differentiation [35]. This issue is very prone to subjectivity and very dependent on the operator's perception and experience.

Regarding the SEM images there is an important point to note concerning the orientation of dentinal tubules. The 5×5-mm root dentin fragment used had a square format. According to the methodology used, and to facilitate obtaining human tooth root fragments, no distinction was made among the buccal, lingual, mesial and distal faces. Thus, when the samples were prepared for SEM, the cutting position of the samples was not taken into account, which produced images with the dentinal tubules in different directions. As the SEM images were for illustrative purposes only, we believe that this did not change the results and did not confuse the authors during the analysis and interpretation of the results. As the material used was a conventional GIC, which chemical adheres to the tooth structure, the positioning of the samples would not have affected the results when the object of study was the marginal microleakage. If a composite resin had been used in this study, this methodology could not have been applied, since differences in density and direction of the dentinal tubules can interfere in the hybrid layer formation and, consequently, in the adhesion of restorative materials [37].

However, despite the favourable results obtained with the Er,Cr:YSGG laser in improving marginal sealing, none of the treatments promoted the total sealing of the restoration. This result together with the difficulty of doing restorative procedures with a rubber dam and other factors such as the proximity to the gum tissue and even the extent of carious lesions in subgingival areas, emphasize that the restoration of carious lesions extending to the root surface is still a challenge for dentists. Thus, further studies of other methods of cavity preparation and aesthetic restorative materials that promote better adhesion to dentin than conventional GICs and thus provide better sealing of these cavities are needed.

Conclusion

Under the experimental conditions used in this study, it can be concluded that none of the cavity preparation methods was able to totally eliminate microleakage of restorations carried out after treatment of root caries lesions. Nevertheless, the method that provided the lowest microleakage was the Er,Cr:YSGG laser with an output power of 2.5 W (44.64 J/cm²).

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