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In Vitro Microleakage of class V Composite Restorations prepared by Er,Cr:YSGG Laser and Carbide BUR

SUMMARY

Background: *The aim of this in vitro study was to compare the degree of microleakage on enamel and dentin margins of class V cavities prepared with either a high-speed drill or an Er,Cr:YSGG laser (2780 nm) and to associate their use with a beveling method for the margin.* ***Method and Materials:*** *Sixty bovine incisors were randomly distributed into three groups. Group 1 (G1) cavities were laser prepared and bur beveled, group 2 (G2) cavities were bur prepared and beveled, while cavities of group 3 (G3) were laser prepared and beveled. Cavities were restored with selective enamel etching, using the same bonding agent and nano-hybrid resin composite for all groups. After thermocycling, microleakage was assessed using a methylene blue dye penetration method.* ***Results:*** *Statistical analysis (Mann-Whitney, Kruskal-Wallis and post-hoc Dunn's multiple comparison tests) demonstrated significantly higher microleakage for dentin compared to enamel margins in G1. Enamel margin microleakage was found to be significantly higher at G3 compared to G1 (p=0.032) and G2 (p=0.001), while no significant differences were found between G1 and G2 (p=0.850). Regarding dentin margins, G2 group performed significantly better than G1 and G3 (p<<0.001), while there was no significant difference among G1 and G3 scores (p=1.000).* ***Conclusions:*** *The conventional cavity preparation method seems to perform better in terms of microleakage than the Er,Cr;YSGG laser. Laser-prepared cavities could perform better in terms of microleakage if an additional step of enamel bur-beveling is performed prior to restoration.*

Keywords: Bovine Teeth, Enamel Etching, Er,Cr:YSGG Laser, Microleakage

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Introduction

Current dentistry supports a minimal invasive approach focusing on sound dental tissue preservation and caries removal[1]. Minimal invasive dentistry is directly associated with the wide use of composite resins and adhesive systems[2]. However, despite continuous development of resins and adhesive systems, there are still some problems concerning adhesion.

Although the mechanical, physical and esthetic properties of contemporary composites have improved in comparison to their predecessors, polymerization shrinkage and its related stress remain a major concern which requires improvement[3,4]. Achieving good marginal sealing remains a considerable challenge in composite restorations[5]. The nature of dentin and its complex structure compromise the stability of the dentin-resin bond. The highly organic content of dentinal tubules makes adhesion to dentin more difficult than adhesion to enamel[6,7].

The durability and integrity of the marginal seal plays a crucial role in the longevity of composite resin restorations[8].

Microleakage is defined as the penetration of minute amounts of fluids, debris and microorganisms through the interface space between a dental restoration and the adjacent cavity walls. Microleakage leads to hypersensitivity, secondary caries, possible pulpal pathology and marginal staining[9,10].

Factors that contribute to the formation of interproximate gaps and cause microleakage include: polymerization shrinkage, different thermal expansion coefficients of composite resin and tooth structures, no incremental technique during restoration, roughness of composite margins caused by inadequate finishing and polishing that leads to microgaps/ microfractures on the interface[2].

The removal of decayed tooth structure is routinely performed by high-speed handpieces and burs or even the use of instruments by hand. This conventional method of cavity preparation has disadvantages, such as pain and discomfort for the patient, noise, overheating of the dental structure and vibration[1]. Recently, the use of laser irradiation, as an alternative method of cavity preparation, has been suggested and is increasingly investigated[11,12].

In 1997, the Food and Drug Administration (FDA) approved the clinical use of Er:YAG lasers in cavity preparation. Later, another laser of the erbium family, the Er,Cr:YSGG was also approved for the same application[1]. Despite the initial concerns about the safety of the laser irradiation, several studies showed that a laser is as safe as the conventional high-speed drills[13,14]. The erbium family of lasers can be used on dental hard tissues. The Er:YAG laser emits a wavelength of 2780 nm, which is highly absorbed in water and hydroxyapatite. In contrast, the Er,Cr:YSGG laser's wavelength of 2780 nm has a higher absorbance in hydroxyapatite than in water. Both wavelengths can remove dental hard tissues through a thermomechanical ablation process[10,15].

Cavities prepared with an erbium laser are shown by SEM evaluation to lack a smear layer, with open dentinal tubules, ablation focused mainly on intertubular than on peritubular dentin, exposed enamel rods, extensive surface fissuring, and less homogeneous and regular surface patterns[4,10,16]. This surface's micromorphology is likely to promote adhesion[17]. However, there are controversial results regarding microleakage in laser-prepared cavities. The collagen fibrils' fusion caused by dentin laser ablation could be related to the higher microleakage at dentin walls. Collagen fibrils' fusion and the consequent lack of inter-fibrillar space produce conditions in which resin is obstructed from diffusing into the subsurface of the intertubular dentin[16,18].

The ideal substrate for in vitro research is human teeth. However, collecting an adequate amount of sound and healthy teeth without defects, such as caries or other conditions that lead to extraction is often difficult. Additionally, there are some parameters, such as age and source that are difficult to control. These could affect tissue quality and, consequently, the results of the experiment. Concerns also arise about transmitting diseases and about ethical issues. Bovine teeth are the most widely used substrate substituted for human teeth[19]. Several studies show no statistically significant differences between bovine and human substrates regarding microleakage[20,21].

Several techniques have been described in the literature for microleakage evaluation[22]. One of the most widely accepted techniques is the dye penetration technique[8,23]. The dyes usually used are methylene blue, basic fuchsin, silver nitrate, rhodamine and erythrocin[24]. In the present study, we choose to use the methylene blue 2% solution, because of its low molecular weight and its high penetrability[25].

The aim of this study was to compare the degree of microleakage on both incisal/enamel and cervical/dentin margins of class V cavities prepared with either a high-speed drill or an Er,Cr:YSGG laser and to associate them with the beveling method applied to the margin. The null hypothesis was that there is no difference at microleakage on the enamel and dentin margins for each cavity preparation and beveling method.

Material and Methods

Sixty bovine incisors were cleaned by removing their organic remnants with periodontal curettes and then polished with flour of pumice and a rubber cup in a slow-speed handpiece before being stored in chloramine 0.5% solution at 4°C for 30 days.

Class V cavities were prepared on the buccal surfaces of the bovine incisors. The incisal margins were placed on enamel, while the gingival margins were placed on dentin. Thus, the cementoenamel junction was located at the center of the preparation[26]. The cavity dimensions were standardized with the use of a template to trace an outline at a mesio-distal and inciso-gingival width of 2 mm. The cavity depth was defined as 2 mm with the use of a periodontal probe.

The teeth were divided randomly into 3 groups (n=20) and prepared as follows:

Group 1 (G1): The cavity was prepared with an Er,Cr:YSGG laser (2780 nm; Waterlase MD, Biolase, Irvine, CA, USA). The parameters used for cavity preparation with the laser handpiece 'Gold' and tip MZ8 are: power of 4 W, repetition rate of 20 Hz, air cooling 50%, water spray 90%, pulse width 150 μsec (H mode) and spot size 800 μm. The enamel margin was beveled with a diamond bur (6847KR, 018, Komet, Besigheim, Germany).

Group 2 (G2): The cavity was prepared with a carbide bur No 330 in a high-speed handpiece cooled with air-water spray. The bur was replaced with a new one after every five preparations. The enamel margin was beveled with a diamond bur (6847KR, 018, Komet, Besigheim, Germany).

Group 3 (G3): The cavity was prepared with an Er,Cr:YSGG laser (2780 nm; Waterlase MD, Biolase, Irvine, CA, USA). The parameters used for cavity preparation with the laser handpiece 'Gold' and tip MZ8 are: power of 4 W, repetition rate of 20 Hz, air cooling 50%, water spray 90%, pulse width 150 μsec (H mode) and spot size 800 μm. The enamel margin was ablated with the laser using altered parameters compared with those for the cavity preparation, namely a power of 1.25 W, repetition rate of 50 Hz, air cooling 50%, water spray 90%, pulse width 150 μsec (H mode) and spot size 800 μm.

All cavities were restored with the same nano-hybrid resin composite (Filtek Z550, 3M ESPE, St. Paul, MN, USA, shade A2). Initially, the enamel margins were acid etched (selective etching technique) with orthophosphoric acid 37% (Etching Gel DMP Ltd, Markopoulo, Greece) for 30 sec, then rinsed thoroughly and air-dried. The bonding agent (Single Bond Universal, 3M ESPE, St. Paul, MN, USA) was then applied with the use of a small brush for 20 sec, thoroughly dried to evaporate the solvent and light cured for 10 sec with LED photopolymerization light (Silverlight, Mectron, Carasco, Italy – 3.6 V and light radiation 440-480 nm). The light intensity was checked with the light-intensity sensor on the polymerization unit during the experimental procedure. The cavities were filled with the resin composite in two oblique increments. The first increment was placed at the inciso-axial part of the cavity and the second increment was placed at the cervico-axial part of the cavity. Each increment was light-cured for 20 sec using the same light source. All specimens were stored in distilled water for 24 hours at 37°C. Afterwards, they were polished with abrasive discs (Super-Snap, Shofu Dental, San Marcos, CA, USA). Four abrasive discs with progressively reduced roughness were used for each tooth. To simulate clinical stress, the specimens were thermocycled for 3000 cycles (5°C, 37°C, 55°C and 37°C, respectively) with 15 sec dwell time and 10 sec transfer time.

Following thermocycling, the apices were cut with a diamond bur (5881, 016, Komet, Besigheim, Germany) in a high-speed handpiece cooled with air-water spray and sealed with sticky wax (Yeti dental Thowax, Keystone Industries, Gibbstown, NJ, USA) to prevent dye penetration. The specimens were air-dried and fully covered with two coats of colored nail varnish (Beauty Line Cosmetics, red shade, Cream Team Ltd, Greece, www.creamteam.gr), excluding the restoration and a 1 mm rim zone of tooth structure around it. The samples were then immersed in a 2% methylene blue solution for 24 hours[27]. After storage, the teeth were rinsed thoroughly under running tap water and then sectioned longitudinally through the center of the restoration from the buccal to the lingual surface, using a water-cooled low-speed diamond disc (Isomet low-speed saw, Buehler, Germany). Evaluation was performed at 25X magnification under a light stereomicroscope (Carl Zeiss Stemi/DV4, Oberkochen, Germany). Microleakage scores were based on a 4-grade scale presented in Table 1[28].

Two blinded examiners evaluated both sections of each tooth and scored the highest microleakage for each tooth's section. Incisal (enamel) and gingival (dentin) margins were separately scored for each tooth.

Table 1. Qualitative scale for dye penetration

Score	Content
0	No dye penetration
1	Dye penetration reaching enamel/cementum
2	Dye penetration reaching dentin
3	Dye penetration reaching cavity floor

Samples that demonstrated evidence of dye penetration through the sealed area of the root apex, were not scored and were excluded from statistical analysis. Thus, from the initial number of 20 samples for each group, we analyzed 16, 18 and 15 samples for G1, G2 and G3, respectively. The statistical analysis was performed using the SPSS 17 and SPSS 22 (for the post hoc tests) for Windows.

Results

The Mann-Whitney non-parametric test (level of significance was set at 5%) was conducted for each group separately. The test revealed statistically significant higher microleakage at the dentin margins as compared to the enamel margins in only the G1 ($p<0.05$). In G2 and G3 despite slightly higher microleakage scores at the dentin margins, there were no statistically significant differences ($p=0.251$ and $p=0.326$, respectively). G3 samples had the highest microleakage score at both margins. The dye penetration scores at the enamel and dentin margins for each group separately are presented in Figure 1.

A non-parametric Kruskal-Wallis one-way analysis test of the three groups was performed on enamel and dentin margins separately. This test revealed statistically significant differences among the groups ($p<0.05$). A post-hoc Dunn's multiple comparison test showed that G3 microleakage for enamel margins was statistically significantly higher than G1 ($p=0.032$) and G2 ($p=0.001$). No significant differences were found between G1 and G2 regarding enamel margins ($p=0.850$). For the dentin margins, the G2 had lower microleakage scores than did the G1 and G3 ($p<<0.001$), while there was no significant difference among G1 and G3 scores ($p=1.000$). These results are presented in Figure 2 & 3.

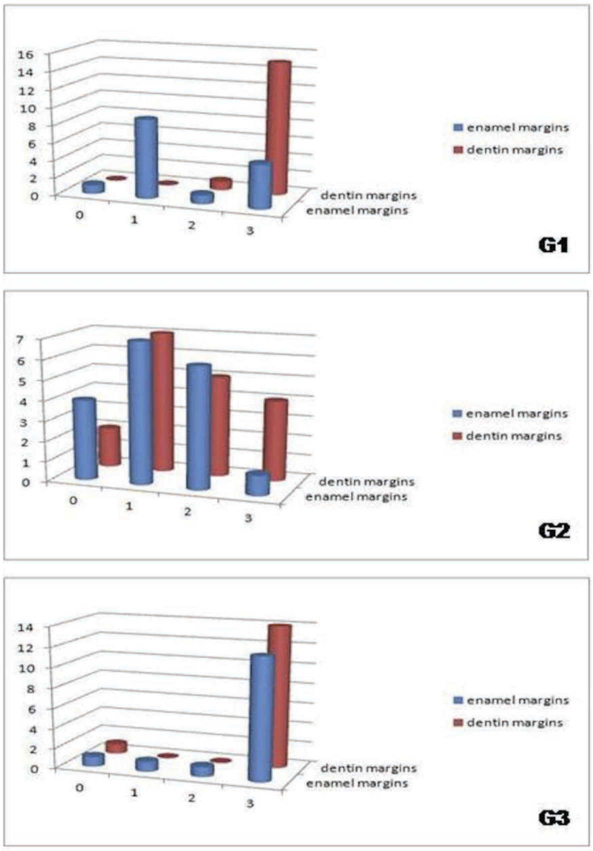

Figure 1. Microleakage scores at enamel and dentin margins for each group separately

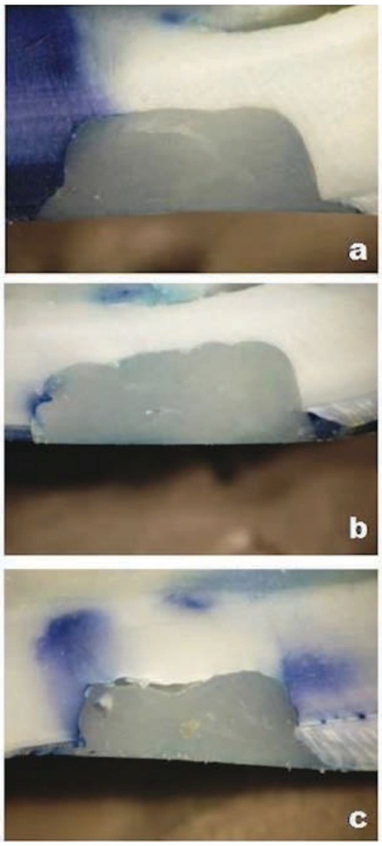

Figure 3. Representative photos of each group (magnification x25)
G1 sample with microleakage score 1 on enamel and 3 on dentin margin (a).
G2 sample with microleakage score 1 on enamel and 2 on dentin margin (b).
G3 sample with microleakage score 3 on enamel and dentin margin (c).

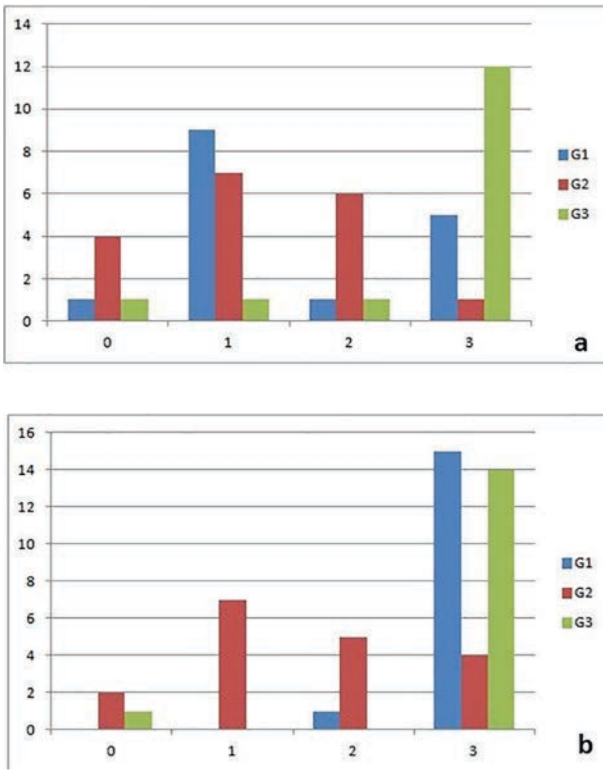

Figure 2. Microleakage scores of the three groups for enamel margins (a) and dentin margins (b)

According to these results, this study showed that for enamel margins there is significantly higher microleakage for the cavities prepared and modified exclusively by laser, while for dentin margins bur prepared cavities demonstrated the lowest microleakage. Therefore, the null hypothesis that there is no difference in microleakage via enamel and dentin margins for each cavity preparation and beveling method has to be rejected.

Discussion

The results of the present study showed that conventional preparation with the use of a high-speed bur led to significantly less microleakage at enamel margins, compared to Er,Cr:YSGG laser preparation. These results are in agreement with those of other studies that have used an Erbium group laser device (Er:YAG laser)[3,12,29-31]. Bahrololoomi et al. reported results that are similar to our study when an etch-and-rinse adhesive system is applied, but for cavities treated with a self-etch adhesive system, they reported no significant difference independently of the preparation method[31]. This could be attributed to self-etch systems being not sufficient to decalcify enamel. An additional selective enamel etching step is required when self-etch systems are applied to such margins. According to several studies, this additional step is important to reduce enamel margin microleakage[23,27,32-35].

Acid etching or laser etching of enamel margins prior to adhesive application has been studied by Fattah et al. who tested microleakage at cavity margins treated either with laser, or acid etching, or with the combination of the two procedures[23]. They reported that the combined method demonstrated the lowest microleakage, followed by the group that had acid etching alone[23]. In addition, Ceballos et al. concluded that cavities prepared with a bur and treated by acid etching before bonding yielded less microleakage at the enamel margins when compared with cavities prepared with a bur and treated by Er:YAG laser irradiation with or without acid etching[32]. They also compared enamel laser etching to acid etching and reported that laser etching alone does not provide adequate marginal sealing and cannot be deemed as a reliable alternative[32]. Muhammed et al. found that conventional preparation led to higher microleakage scores on enamel margins when using a self-etch adhesive (Swiss TEC SL Bond). Acid etching of enamel margins was performed prior to adhesive application, but no beveling was performed[36].

Beveling appears to play an important role in microleakage restriction. Independently of the cavity preparation method, microleakage was reduced when the enamel margins were beveled with a diamond bur. Significantly less microleakage was found for the groups G1 and G2, when beveling was performed with a high-speed diamond bur compared to the G3, in which the margins were modified with an Er,Cr:YSGG laser. Beveling exposes a larger number of enamel rods, which are susceptible to acid decalcification and bonding resin infiltration. According to Armengol et al., the combination of acid etching and diamond bur beveling was better in terms of microleakage than acid etching and laser margin modification[33]. Several studies that did not include beveling and/or acid etching modification of marginal enamel in their methodology found no significant differences in microleakage between the two cavity preparation methods[4,10,26,27,35-38]. Trelles et al. found higher microleakage of conventional preparations than those prepared with a low and a high energy Er,Cr:YSGG laser. Trelles et al. used a single-step self-etch adhesive (Clearfil S3 Bond) and did not perform beveling or/and acid etching[8]. The results of Trelles et al. can be accepted because self-etch adhesives are intermediate in acidity, and despite their adequate adhesion to dentin, they are unable to sufficiently modify and seal enamel margins[39,40].

Regarding cervical margins, which are not protected by enamel, our microleakage scores were significantly higher for cavities prepared with laser ablation (G1, G3) than for those prepared with a carbide bur (G2). A single-step self-etch adhesive system was applied without an additional acid etching step, which would interfere with the morphological characteristics of the residual dentin surface. Conventional bur preparations leave a smear layer, which blocks the dentinal tubules, while laser ablation produces a smear layer free surface with irregular characteristics, which probably favors mechanical resin interlocking and microretention[7,10,41]. Bertrand et al. showed that dentinal margin microleakage is minimized when an etch-and-rinse adhesive system is used[34]. Given this, and the fact that we applied an adhesive system with a pH=2.7 (ScotchBond Universal), which is classified as one of the "mild" self-etch adhesives[7] and does not totally remove the smear layer or penetrate it towards sound dentin, one would expect the results to be the reverse. The possible justification of these results is that the cavosurface margins produced by laser irradiation are quite rough, and the adhesive did not completely penetrate the texture of the residual dentin surface, creating microspacing and increasing microleakage.

Our results are in accordance with those of similar studies by Korkmaz et al. who used an adhesive with pH=2.3 (Clearfil S3 Bond)[12] and Trelles et al. who used a self-etch system with pH=2 (Clearfil SE Bond)[8]. The same results have been acquired by Baghalian et al. for Clearfil S3 Bond; however they reported less microleakage when a two-step self-etch adhesive was used (Clearfil SE Bond)[1]. The more acidic nature (pH=2) and the dual hydrophobic primer-hydrophilic resin nature of the two-step adhesive improve marginal sealing and restrict micropenetration. Despite acidic modification of dentin improves marginal sealing, microleakage remains higher for laser ablation compared to diamond bur preparation even when a separate acid-etching step is added[29].

A contemporary alternative to acid-etching method is laser conditioning (BLc) for marginal modification so that hard tissues can become susceptible to adhesives. There are numerous studies with controversial results regarding marginal sealing that can be achieved with this method and its comparison to the conventional preparation. Three studies reported that acid-etching is superior in terms of microleakage compared to laser conditioning[23,33,37]. Ceballos et al., attribute superiority to acid etching, which makes the orifices of dentinal tubules wider and exposes collagen fibers of dentin, promoting penetration of the adhesive and hybrid zone formation[32]. However, laser ablation does not expose collagen fibers and does not widen tubules, leaving a calcified surface that is not susceptible to adhesive penetration[32]. The micromorphology pattern of the dentin surface is also different according to the method used. Laser irradiation is mostly absorbed by intertubular dentin due to its conciseness in water and organic components, while acid-etching influences the most calcified part of dentin, which is peritubular dentin[42]. According to Esteves-Oliveira et al., even an additional laser modification step following the acid-etching does not improve sealing[37]. Arami et al. did not find any statistically significant differences between the two methods[27], while Shahabi et al. reported larger amounts of microleakage for acid-etched dentin[25]. The last group used a two-step etch-and-rinse adhesive system (Adper Single Bond 2), which is based on water and ethanol. Such systems perform optimally on dry surfaces. The laser ablation function is based on explosive evaporation of dentin water. This leads to a dry surface[32] on which a water-ethanol based adhesive should penetrate better. The hypothesis is that etch-and-rinse adhesive systems perform better at the laser-ablated marginal dentin than do self-etch systems. This is verified also by the study by Moldes et al[10]. According to this study, an equal amount of microleakage occurs when an etch-and-rinse adhesive is used on dentinal margins either on conventional cavity preparation or on a laser-prepared cavity. For the self-etch systems, the same authors are reporting less microleakage for laser preparations[10] and this is in compliance with the findings of two other studies[37,43]. A possible explanation of less microleakage could be that laser alterations on dentin surfaces leave micro-irregularities and no smear layer. Such alterations result in both macro and micro-roughness. Laser induced changes in the surface texture of the dentin could potentially affect the microleakage of adhesive restorative materials[4].

When comparing microleakage between enamel and dentin margins for all groups and each one of the samples individually, dentinal margins demonstrate vastly larger amounts of dye penetration. In agreement with this observation is a summation of the published literature[1,3,8,10,12,23,27-30,32,37,38,44,45]. This agreement can be attributed to the more difficult and technically sensitive procedure involving adhesion to dentin[8], as well as to the more complex and reduced inorganic content of the dentin compared to enamel[1]. An additional factor that could promote microgap formation on the dentinal margin is polymerization contraction that occurs at the strongest bonded surface of the cavity, which is the enamel margin[8].

Conclusions

Based on the findings of our study and within the limitations of an in-vitro study, we can conclude the following:

- The conventional cavity preparation method with a bur performs significantly better in terms of microleakage than preparation with a laser.
- The method of enamel margin beveling seems to influence the sealing ability of a resin composite restoration. Beveling with a diamond bur leads to less microleakage.
- Microleakage at gingival/dentin margins is higher than at incisal/enamel margins.
- Laser cavity preparation in conjunction with laser ablation on enamel margins seems to produce the highest microleakage scores at both incisal and gingival margins.

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