ISSN 1807-5274
Rev. Clín. Pesq. Odontol., Curitiba, v. 6, n. 3, p. 231-237, set./dez. 2010

Effect of load cycling on nanoleakage of butt joint and beveled occlusal enamel margins in Class V composite resin restorations

Efeito do ciclo de carga na nanoinfiltração de margens de esmalte oclusal com e sem bisel em restaurações Classe V

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Abstract

Objectives: This study evaluated the influence of mechanical and thermal cycling on the nanoleakage of Class V composite resin restorations with and without enamel beveling. **Material and method**: Using 60 Class V cavities prepared on the buccal surfaces of human molars, specimens were divided into two groups (n = 30) based on the configurations of the enamel cavosurface margins. (beveled and non-beveled) After restoring the cavity preparations with a flowable composite, half of the specimens were mechanically load-cycled. The specimens were sealed leaving a 1 mm window around the cervical and enamel margins. Specimens were placed in a 50% (W/V) silver nitrate solution then immersed in photodeveloping solution and exposed to fluorescent light for 8h. The teeth were buccolingually sectioned, gold sputter coated and examined with a SEM to evaluate the nanoleakage values. The data were analyzed using two-way ANOVA ($\alpha = 0.05$). **Results**: Enamel margin configuration had no significant effect on nanoleakage ($p > 0.05$). However, the nanoleakage value was significantly higher in the load-cycled group than in the group not load-cycled ($p < 0.05$). **Conclusion**: Since enamel margin configuration does not affect nanoleakage, there is no need to bevel enamel margins of Class V cavity preparations for composite restorations.

Keywords: Class V cavity preparation. Enamel bevel. Nanoleakage. Load cycling.

Resumo

Objetivos: *O presente estudo avaliou a influência dos ciclos de carga mecânico e térmico na nanoinfiltração de restaurações Classe V em resinas compostas, com e sem biselamento do esmalte.* ***Material e método***: *Os espécimes (60 cavidades Classe V preparadas na superfície vestibular de molares humanos) foram divididos em dois grupos (n = 30) baseados nas configurações das margens cavosuperficiais do esmalte (biseladas e não biseladas). Após restauração dos preparos cavitários com um compósito tipo* flow*, metade dos espécimes foi carregada mecanicamente. Os espécimes foram selados mantendo janela de 1 mm em torno das margens cervical e de esmalte. Os espécimes foram colocados em solução de nitrato de prata (50%), imersos na sequência em solução processadora fotográfica e expostos à luz fluorescente por oito horas. Os dentes foram cortados em sentido vestíbulo-lingual, revestidos em ouro e observados em microscopia eletrônica para avaliar os valores de nanoinfiltração. Os dados foram analisados pelo ANOVA (α = 0,05).* ***Resultados***: *A configuração das margens do esmalte não teve efeito significante na nanoinfiltração (p > 0,05). Entretanto, os valores da nanoinfiltração foram significativamente mais altos no grupo de carga que no grupo sem carga (p > 0,05).* ***Conclusão***: *Uma vez que a configuração da margem do esmalte não afeta a nanoinfiltração, não há necessidade de biselar as margens do esmalte nos preparos de cavidades Classe V em restaurações de compósitos.*

Palavras-chave: *Preparo de cavidades Classe V. Biselamento de esmalte. Nanoinfiltração. Ciclo de carga.*

Introduction

Teeth are subjected to stresses during chewing, swallowing and parafunctional habits. Vertical loading introduced by a food bolus between opposing teeth can be evenly distributed over the entire occlusal surface to disseminate stresses (1). Compressive stresses develop on the aspect of the tooth that is being bent while simultaneously tensile stresses occur on the opposite aspect of the tooth (2). The same patterns of stress may be generated on restorations placed in the cervical region of teeth under a load (3).

An assessment of nanoleakage provides important information to evaluate the sealing ability of a restorative material (4). Typical microleakage of a restoration may be the result of defects or porosities while nanoleakage may be due to imperfect penetration of primer and adhesive resin inside of the demineralized dentin collagen layer when using a total-etch bonding system. This phenomenon can occur in the hybrid layer as well as in the adhesive layer (5).

An *in vitro* simulation of masticatory forces at the resin-dentin interface is necessary to study the effect of load cycling on nanoleakage since the constant development of new adhesive system and restorative makes long-term clinical trials impractical (6).

Very few studies have been carried out to examine the effect of load cycling on nanoleakage. However, the effect of load cycling on the nanoleakage of different margin configurations of Class V composite restorations has not been explored. Therefore the purpose of this study was to investigate the effect of load cycling and margin configuration on nanoleakage. This study tested the following hypotheses:

1) Margin configuration has no effect on sealing ability of a restoration.
2) Load cycling may not affect nanoleakage.

Material and method

Specimen preparation

Sixty caries-free, freshly extracted human premolars were selected for this study and stored in physiologic saline solution for less than two months. A standardized Class V cavity, 3.0 mm wide (mesial-distal), 2.0 mm high (occlusal-gingival) and 1.5 mm deep, was prepared on the facial surface of each tooth. The location of occlusal and gingival margins were 1.0 mm on enamel and 1.0 mm on dentin/cementum. The preparations were made using #12 diamond round burs (Drendel Zweiling®, Diamont Gmblt, Georzalee, Germany) using a water cooled, high speed handpiece. Each bur was used for five preparations then replaced. The prepared specimens were randomly divided into two groups (n = 30 each): Group N (non-beveled) and Group B (beveled). In Group B the enamel cavosurface margins were prepared with a 0.5 mm bevel using a #318 flame-shaped diamond bur (KG Sorensen®, São Paulo, Brazil).

A flowable composite (Tetric Flow®, Ivoclar Vivadent-AG, Schann, Lichtenstein) was used to restore the prepared teeth following the application of a total-etch, one-bottle adhesive system Excite® (Ivoclar®, Vivadent-AG, Schann, Lichtenstein) to both the enamel and dentin according to the manufacturer's directions. The adhesive was light cured using an Optilux 500® curing unit (Demetron-Kerr-Orange®, CA, USA) at 500 mw/cm² for 20 seconds. The composite material was inserted using two oblique increments with each increment light-cured for 40 seconds (7).

After finishing and polishing with Sof-Lex® Pop-on discs using sequential grits (3M-ESPETM, St. Paul, MN, USA) the teeth were stored at 37 ºC and 100% humidity.

Load cycling

Half of the specimens in each group were exposed to a cycling loading for 250,000 cycles. Each specimen was loaded on the occlusal surface at a frequency of 3 Hz (8) using a 10 mm static ceramic ball (9) (Hoechst Cerantec®, Wunsiedel, Germany) at a 90 N load value (10). The load direction was almost parallel to the long axis of the tooth. During load cycling all specimens were subjected to continuous thermal cycling between 5-55 ºC for 60 seconds each. The load cycling device was designed and fabricated at the Mashhad University of Medical Science, in Mashhad, Iran (Figure1). The different test groups are shown in Table1.

Figure 1 - Loading machine

Table 1 - Description of the specimens

Group	Subgroup (n = 15)	Description
N	NL+	Non-beveled, Load cycling
	NL−	Non-beveled, without load cycling
B	BL+	Beveled, Load cycling
	BL−	Beveled, without load cycling

Nanoleakage evaluation using SEM analysis

Following the load cycling procedure the tooth surface was coated with two layers of nail varnish except for the bonded interface and the area within 1mm surrounding the margin of the restorations. Further preparation for the evaluation of nanoleakage included immersion of the teeth in a 50% (W/V) silver nitrate solution (pH 3.4) for 24h in total darkness then rinsed in running water for 5 min before being immersed in photodeveloping solution, and exposed to fluorescent light for 8h in order to reduce silver ions to metallic silver.

Upon removal from the developing solution, the specimens were rinsed in running water for 5 min. The roots were then cut off 2 mm beyond the cementoenamel junction and the specimens were then immersed in clear epoxy resin (Arodlite®, Ciba-Geigy, Basel, Switzerland). After 24h, each tooth was sectioned longitudinally in a bucco-lingual direction through the center of each restoration using a low speed diamond saw (Isomat®, Buchler Ltd, Lake Buff, IL, USA) under a water coolant.

The cut surfaces were polished with increasingly fine diamond pastes (6, 3, 1 µm; Buehler Ltd®, Lake Bluff, IL). The specimens were ultrasonically cleaned in distilled water, air dried, mounted on aluminum stubs, gold sputter coated and then examined in a Vega II scanning electron microscope (TESCAN®, s.r.o., Brno, Czech Republic) using the backscattered electron mode.

Specimen evaluation

The length of silver penetration along the cavity walls was recorded under both low (50 X) and high magnification (1000 X) because, in some cases, the loosely distributed silver particles could not be observed under the lower magnification. The specimens were first observed at high magnification to locate the end of silver penetration and marked electronically with a circle on the SEM screen at the lower magnification.

Leakage scores of each restoration were calculated as the percentage of the total length of each interface that was penetrated by silver nitrate. In other words, the ratio of the length of the silver nitrate penetration along the resin-tooth interface to the total length of each interface.

Results

The mean and standard deviations of four experimental groups in terms of nanoleakage values are shown in Table 2

The nonparametric analysis of Kolmogorov-Smirnov did accept the normality of data ($p > 0.05$); therefore a parametric analysis two-way ANOVA was used for evaluation of the interaction between two factors (margin configuration and mechanical load cycling). It was recognized that there was no interaction between them ($p > 0.05$).

Although enamel margin configuration did not influence the nanoleakage value ($p > 0.05$), there was a significant difference between the two groups regarding load cycling ($p < 0.050$). This means the nanoleakage value was significantly higher in the load-cycled group than the group not exposed to load cycling. ($p < 0.05$) (Table 3). Figure 2 (A to D) shows the backscattered SEM images showing nanoleakage of dentin margins in each group.

Table 2 - Nanoleakage results of the experimental groups

Groups	Load Cycling	Mean	SD
BL	Negative	10.647	.21336
	Positive	11.693	.26851
NL	Negative	10.647	.23563
	Positive	11.693	.24339

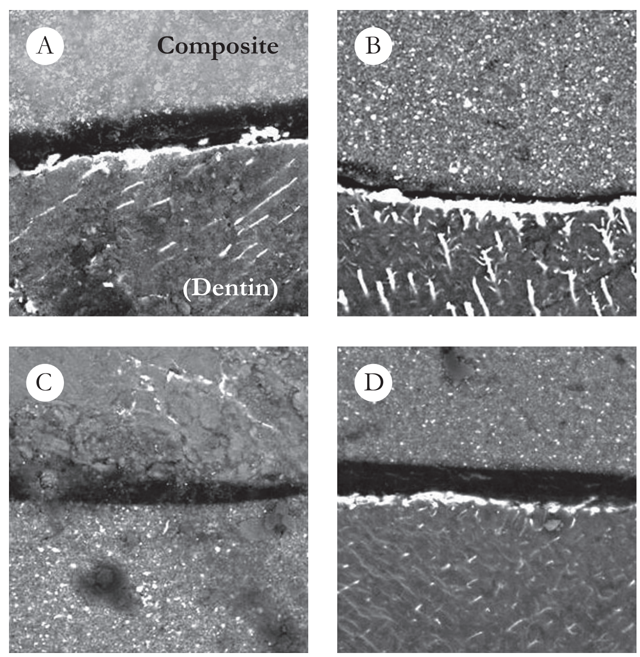

Figure 2 - (A) Beveled unloaded group; (B) Beveled loaded group; (C) Non-beveled unloaded group; (D) Non-beveled loaded group

Discussion and conclusion

This study evaluated the effect of margin configuration and load cycling on nanoleakage in Class V composite restorations. Nanoleakage is defined as a leakage pattern occurring within the nanometer-sized spaces around collagen fibrils within the hybrid layer. The assessment of nanoleakage

Table 3 - Results of the two-way ANOVA

Source	TYPE III sum of squares	df	Mean square	F	Sig.
Corrected Model	16.433[a]	3	5.478	94.286	.000
Intercept	7486.134	1	7486.134	128859.68	.000
BL-NL	.000	1	.000	.000	1.000
Neg-Pos(Load)	16.433	1	16.433	282.857	.000
BL-NL*Neg-Pos	.000	1	.000	.000	1.000
Error	3.253	56	.058		
Total	7505.820	60			
Corrected Total	19.686	59			

Note: R Square = .835(Adjusted R Square-.826)

using SEM technology is a valuable method for use in the investigation of the sealing ability and quality of the hybrid layer (11). As stated previously this study tested the following hypotheses:

1) Margin configuration has no effect on sealing ability of a restoration.
2) Load cycling may not affect nanoleakage.

The first hypothesis of this study was accepted because there were no differences between the two types of margin configurations tested in this study. While there has not been a previous study of the effect of enamel margin beveling on nanoleakage, some previous results have demonstrated the effect of margin configuration on microleakage values. These microleakage studies had conflicting results.

Baratieri et al. (12) found no difference between two groups in terms of post-operative sensitivity, marginal discoloration and secondary caries in a clinical evaluation of the microleakage of composite restorations using beveled and non-beveled Class V non-carious lesions. Satini et al. (13) showed a significant difference in marginal leakage between Class V cavities with and without a margin bevel.

In the present study both enamel and dentin margins in half of the Class V cavities were beveled, in addition the total etch and self-etch dentin adhesives were compared together. It has been recognized that there was no need to perform dentinal margin beveling if the gingival margin of the cavity preparation is to be placed on cementum (14, 15).

Sunders et al. (16) compared marginal leakage associated with enamel beveled and non-beveled cavosurface margins of Class V cavity preparations using a radioactive isotope containing 45 Ca, and an autoradiographic technique. They demonstrated that the beveled cavity margins allowed significantly less leakage than non-beveled margins. They further demonstrated that the use of a second generation of dentin bonding agents did not improve the enamel marginal seal of composite restorations while the beveled enamel margin in the preparation did improve the margin seal.

Owen et al. (14) found that a Class V restoration with a gingival bevel demonstrated greater microleakage. However, Bagheri and Ghavamnasiri (8) compared the microleakage of hybrid and microfilled composites in Class V conventional and beveled conventional cavity preparations. They demonstrated no difference between either two types of composites or the two types of the enamel margins.

In the present study, no nanoleakage was found along enamel margins, which is in agreement with a few previous studies with similar results (5, 17). In a clinical situation, resin tag formation on the enamel surface following phosphoric acid-etching resulted in efficient bonding to enamel to provide a favorable seal of the enamel margins (18). So, it is highly probable that the nanoleakage may be reduced provided that the cavity is completely surrounded by enamel margins.

The second null hypothesis was not accepted, as the load cycling groups showed significantly greater nanoleakage values. Previous nanoleakage studies combined with load cycling have yielded inconsistent results. Yamazaki (19) demonstrated that the application of mechanical load cycling did not increase the amount of silver nitrate deposition. The results of these two studies are in contrast with the results obtained in the present study. However, a previous study by Bedran et al. (6) was similar to the conclusion of the present study regarding the effect of load cycling on nanoleakage. Such variations in reported findings may be due to the differences in materials used, load direction and value, number of cycles and cavity types and sizes, and operator variability (20).

Mandras et al. (20) showed that occlusal loading has an adverse effect on restorations in molar teeth. This may have been due to the increased Class V cavity length in molar teeth. In the present study Class V cavity preparations were made in maxillary canines and mandibular molars, extending 1 mm above and 1 mm below the cementum. The depth of cavities was 1.5 mm for all of the specimens, while the mesio-distal width of the cavity preparations in canine and molar were 4-4.5 and 6-6.5 mm respectively.

The forces and movement during mastication are highly complex and factors such as age, sex, bruxism and bite habits have a significant influence on force measurements (21). Furthermore, a load testing machine only produced axial cyclic loads while the movements of mastication in the oral environment are in a three-dimensional in pattern (22). Thus, the test conditions simulate, but do not duplicate clinical conditions which must be considered when interpreting the findings. For example, the number of load cycles in the laboratory may only represent only several days or months of masticatory forces on the dentition compared to longer periods of time such as a year. Nevertheless, this was the first study that evaluates the effect of load cycling and margin configuration on nanoleakage in Class V composite restorations. After load cycling, nanoleakage increased significantly along the margins, while margin configuration had no effect on nanoleakage.

Within the limitations of the study, it may be concluded that load cycling influences nanoleakage of margins of Class V composite restorations, but enamel margin configuration does not affect nanoleakage. Because enamel margin configuration does not affect nanoleakage, there is no need for enamel beveling of shallow Class V cavity preparations for composite restorations at present.

Conflict of interest

The authors declared no conflict of interest in the present manuscript.

Ethics committee

The research was approved by the Ethical Commitee of the Institution.

Acknowledgement

This study was supported by a grant from the Research Council of Mashhad, University of Medical Sciences, Mashhad, Iran.

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Received: 04/23/2010
Recebido: 23/04/2010

Approved: 06/09/2010
Aprovado: 09/06/2010