Nd:YAG laser influence on the hybridization quality, employing total-etching or self-etching adhesives: sem analysis

Influência do laser de Nd:YAG na qualidade de hibridização empregando-se sistemas adesivos de condicionamento total ou auto-condicionante: análise em MEV.

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ABSTRACT

Background: The application of Nd:YAG laser on dentin with the non-polymerized adhesive can influenced the quality of hybrid layer formed.

Objectives: The aim of this study was to assess through analysis by scanning electronic microscopy (SEM), the Nd:YAG laser influence on the hybridization quality, using total-etching Single Bond (SB) or self-etching Clearfil SE Bond (CSEB) adhesives.

Material and Methods: Nine bovine incisors were sectioned and resulted in 36 specimens and divided into 2 groups (n=18): Group SB – SB total-etching adhesive; Group CSEB - CSEB self-etching adhesive. Before the polymerization of these adhesives, each group was divided in 3 subgroups, according to the laser parameters (n=6): Subgroup Control - polymerization for 10 s (600 mW/cm²); Subgroup Laser 60 mJ: Nd:YAG laser (60 mJ, 10Hz, 74.72 J/cm²) irradiation on dentin impregnated with non polymerized adhesives + polymerization for 10 s (600 mW/cm²); Subgroup Laser 140 mJ - Nd:YAG laser (140 mJ, 10Hz, 174.34 J/cm²) irradiation on dentin impregnated with non polymerized adhesives + polymerization for 10 s (600 mW/cm²). All samples were restored with composite resin (Filtek Z350 - 3M). The thickness, the presence of failures (gap/porosity) and tags/microtags in the hybrid layer were analyzed using SEM. The data were submitted to ANOVA and Tukey statistical tests 5%. Results: the smallest hybrid layer thickness.

RESUMO

A aplicação do laser de Nd:YAG na dentina sobre o sistema adesivo não polimerizado pode influenciar a qualidade da camada híbrida formada. Objetivo: o objetivo deste estudo foi verificar através da análise em microscopia eletrônica de varredura (MEV) a influência do laser de Nd:YAG na qualidade de hibridização, usando-se o sistema adesivo de condicionamento total Adper Single Bond 2 (SB) ou o auto-condicionante Clearfil SE Bond (CSEB). Material e Métodos: nove incisivos foram seccionados resultando em 36 espécimens que foram divididos em 2 grupos (n=18): Grupo SB - adesivo de condicionamento total SB; Grupo CSEB-adesivo auto-condicionante CSEB. Previamente à aplicação dos sistemas adesivos, cada grupo foi dividido em 3 subgrupos de acordo com os parâmetros do laser de Nd:YAG (n=6): Subgrupo controle- polimerização do adesivo por 10s (600 mW/cm²); Subgrupo Laser 60 mJ: laser de Nd:YAG (60 mJ, 10Hz, 74,72 J/cm²) com irradição sobre a dentina impregnada com sistema adesivo não polimerizado + polimerização por 10s (600 mW/cm²). Todos os espécimens foram restaurados com resina composta (Filtek Z350 - 3M). A espessura, a presença de falhas (fendas/porosidades) e características dos tags/microtags na camada híbrida foram analisados em MEV. Os resultados foram submetidos aos testes estatísticos.
was observed in the subgroup control using CSEB (1.36 ± 0.14), and statistically lower than all other groups / subgroups. The multiple comparison test showed that the Subgroup laser 140 mJ using CSEB showed significant less prevalence of failures compared to the same subgroup using the SB; and for the presence of tags and microtags, the Subgroup laser 140 mJ using SB showed significant higher prevalence of tags/microtags compared to the same subgroup using CSEB. **Conclusion:** Independent of the adhesive used, the Nd:YAG laser, according to the parameter applied, had a positive influence on the hybridization quality.

**KEYWORDS**
Adhesive; Dentin-bonding agents; Lasers.

**CLINICAL RELEVANCE:**
The Nd:YAG laser, according to the parameters applied, had a good influence in the thickness and the quality of hybridization, varying according to the intensity of energy employed.

**INTRODUCTION**

The formation of a good quality of hybrid layer in restorative dentistry is important not only for the improvement of bond strength but also for the sealing of dentinal tubules that can prevent the leakage which increases the treatment longevity [1]. Several studies [2,3] state the importance of hybrid layer thickness and formation of resin tags for bond strength.

The quality of hybrid layer formed depends of several factors. These factors are not only related to the characteristics of the dental substrate and kinds of treatment, but also the type of adhesive system employed must be considered. The use of total-etching adhesives suggest that the dentinal etching with the phosphoric acid removes the smear layer, opens dentinal tubules, exposes the collagen fibrils promoting the infiltration of the primer and bond [2,3]. These adhesives with this mechanism of action create a thick hybrid layer, but, with greater probability of failure, due to the hydrolysis of collagen that was not penetrated by the bond [4].

The introduction of self-etching adhesives allowed a new mechanism of action, where a simultaneous demineralization and infiltration of monomer on the dentin is observed [5]. This adhesive increases the probability of bond penetration between the collagen fibrils [6]. The smear layer is not removed; it is incorporated into the hybrid layer, interacting with the dentin subsurface in different ways, depending mainly of the acidic potential of adhesives[7-9]. The hybrid layers are denominated integration layers that are thinner but homogeneous with few, short and narrow tags and microtags [1,2,10].

Many researches have been made to explain the doubts, analyzing the interaction...
of the laser in the hard dental tissues, allowing their use in an efficient and wide way in Dentistry [11-13]. The way that this interaction occurs can influence the adhesion of restorative materials in the dental structures, especially in the formation and the quality of hybrid layer which need more studies.

Wigdor et al. [14], observed alterations on the dentin morphology using the Nd:YAG laser, where the fusion of intertubular dentin was induced. The dentine re-crystallization, with consequent obliteration of some tubules, was also reported by Dederich et al. [11] and Matsumoto et al. [13].

According to this findings about dentin morphology after treatment with Nd:YAG laser, Gonçalves et al. [15] studied the application of Nd:YAG laser on dentin with total-etching adhesive non-polymerized, believing that a fusion between them could form a hybrid layer more resistant, increasing the bond strength and the dentine re-crystallization. The results proved this hypothesis and leaded Matos et al. [16], Matos et al. [17], Arisu et al. [18], Marimoto et al. [19] and Ribeiro et al. [20] to reproduce this methodology developed by Gonçalves et al. [15] to confirm that the Nd:YAG laser applied after the adhesive, but before their polymerization, increases the immediate bond strength of the composite resins.

In spite of the association between the Nd:YAG laser and dentin promoting positive results related to the bond strength, it is necessary to carry out other studies in order to verify if this association can indeed cause alterations on the hybrid layer. In this way, the analysis in Scanning Electron Microscopy (SEM) is an important evaluation method to investigate the surface treated with the adhesive and Nd:YAG laser.

The purpose of this morphological study is to examine, with the use of SEM, the quality of hybrid layer, considering the thickness, presence of failures (gaps/pores) and tags/microtags when the laser Nd:YAG is applied to non-polymerized total-etching and self-etching adhesives. The hypotheses to be tested were:

1. there is difference between the energy parameters of the Nd:YAG laser (60 ou 140 mJ) when considering the quality of the hybrid layer (thickness, presence of failures and tags/microtags); and
2. the total-etching and self-etching adhesives, when associated with Nd:YAG laser, produce different results with respect to the quality of the hybrid layer.

**MATERIAL AND METHOD**

The study sample was composed of 9 freshly extracted intact erupted bovine mandibular incisors. The roots were sectioned at the cervical third, the coronal pulp was removed and the pulp chamber was irrigated with distilled water and gently air-dried. The opening was made on the lingual side using a spherical diamond tip 1012 (KG Sorensen, Rio de Janeiro, Brazil) to allow the amount of remaining dentin to be measured.

The tooth surfaces were worn under water cooling with SiC paper grit 80, adapted in a trimming machine (Kohl Bach Motores Elétricos), for removal of enamel and exposure of medium depth dentin. It was used a caliper thickness gauge (Golgran, São Caetano do Sul, Brazil) to standardize the remaining dentin thickness at 2 mm. The exposed tooth surfaces were sequentially regularized with sandpaper grit 100, 180, 240, 320, 400 and 600 for 30s each in a polishing machine (Dp-10 Panamba Industrial e Tecnica AS, Brazil) at 600 rpm under water cooling.

The specimens were adapted to a cutting machine (Labcut 1010 – Extec Corp., Enfield, CT, USA) to perform a vertical section of the specimens in the occlusal-cervical direction, dividing it into two hemi-crowns. Then, a horizontal section was made, dividing each hemi-crown into a cervical specimen and an incisal specimen.

A test area of the 36 hemi-crowns were identified and marked on the surface, with a Teflon adhesive tape with a 9.8 mm² area orifice, localized on the region of exposed dentin. The 36 specimens were divided in two groups of 18 hemi-crowns (n = 18) according to adhesive used:
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Group SB: surfaces received the application of Single Bond (SB) total-etch adhesive (3M ESPE, St. Paul, MN, USA). Surfaces were etched for 15 s with 37% phosphoric acid gel, rinsed and the excess moisture was removed with absorbent paper. Two layers of SB total-etch adhesive were applied on the surface actively for 15 s and gently air dried for 10 s.

Group CSEB: surfaces received the application of Clearfil SE Bond (CSEB) self-etching adhesive (Kuraray Medical Inc, Tokyo, Japan). One layer of primer agent was applied actively for 20 s and gently air dried for 10 s. One layer of bonding agent was applied actively for 20 s and gently air dried for 10 s.

Then, groups were divided into three subgroups of six specimens (n = 6) each, according to laser irradiation:

• Subgroup Control: The adhesives were light activated for 10 s with a Curing Light XL 3000 (3M Dental Products, St. Paul, USA) with power density of 600 mW/cm². The tip was at a 90º angle, perpendicular and to the dentin surface, and at a distance of 1 mm from it.

• Subgroup Laser 60 mJ: Before light polymerization of the adhesives, the cavities were irradiated with Nd:YAG laser based on protocol used by Marimoto et al. [19] and Ribeiro et al. [20]. The Nd:YAG laser equipment used in this study was the Laser Pulse Master 600 iQ (American Dental Technologies Inc, TX, Corpus Christi, USA) at a wavelength of 1.064µm. The output energy of this laser device was 60 mJ per pulse; with a pulse repetition rate of 10 pulses per second (10 Hz), and was applied freehand by one calibrated operator, in non-contact mode (tip 320µm in diameter) scanning mode for 60 s. The energy density was 74.72 J/cm². During laser application the laser tip was at a 90º angle, perpendicular and to the surface, and at a distance of 1 mm from it. Then, the adhesives were light activated for 10 s following the same protocol utilized for Subgroup Control.

• Subgroup Laser 140 mJ: Nd:YAG laser were applied following the same protocol utilized for Subgroup Laser 60 mJ, however, the output energy of Nd:YAG laser was 140 mJ per pulse; with a pulse repetition rate of 10 pulses per second (10 Hz), yielding an energy density of 174.34 J/cm². Then, the adhesives were light activated for 10 s following the same protocol utilized for Subgroup Control.

Composite resin Filtek Z350 (3M ESPE) restorations were performed in two increments, each increment was polymerized for 20 s, as indicated by the manufacturer. Before being prepared for SEM analysis, the specimens were immersed in distilled water and stored in a bacteriological oven (MA 032-Marconi) at 37 ºC (±2ºC) for 24 h.

The bonding profile of each hemi-crown were sequentially regularized with 600-grit carbide paper disks for 30 s and silicon carbide disks 1200 and 4000 for 60 s each in a polishing machine (Dp-10 Panambr Industrial e Técnica AS, Brazil) at 600 rpm under water cooling in order to be able to visualize by SEM. The surfaces were also polished with Diamond Paste (3 µm – 1 µm) for 60 s.

For SEM analysis, the samples were submitted to the basic-acid challenge (chloridric acid 6 N for 15 s, followed by sodium hypochlorite 1% for 10 min) to reveal the subsurface of bonding interface. The samples were fixed with glutaraldehyde 2.5% sodium cacodylate 0.1M buffer, 4 ºC, pH 7.4, for 12 h. They were rinsed with solution of 0.2 M of cacodylate buffer, pH 7.4 for 1 h, changing the substance 3 times followed by distilled water for 10 min.

The samples were sequentially dehydrated in an ascending ethanol series (30%, 50%, 70%), for 20 min in each solution, 90% ethanol for 30 min and 100% ethanol for 60 min [21]. They were immersed in hexamethyldisiloxane (Fluka Chemika, Switzerland) (HMDS) and stored for 15 min, in a closed container [21]. Specimens
were mounted on stubs with their treated surfaces faced up and sputter-coated with gold.

Finally the samples were gold coated using the Denton Vaccum – Desk II serial n. 19539, NJ, USA) and examined by means of scanning electron microscopy (SEM) (JSM - 5310, JEOL, Instituto de Pesquisas Espaciais – INPE, São José dos Campos, SP, Brazil), using 15 KV of power, with a distance ranging from 7 to 10 mm. The images were collected by a SEM connected to a computer, at 8000X – 10000X magnification.

The central area region of the specimens was chosen for SEM analysis. The bonding interface was analyzed considering two morphological aspects: the hybridization quality and thickness of the hybrid layer. For hybridization quality, were analyzed presence of failures (gap/ pores) and presence of tags/microtags.

The presence of failures gap/pores in the hybrid layer was analyzed according to the scores presented in Table 1. Four numerical score of each sample were obtained. Statistical analysis was performed based on percentage (%) of score 2, which indicate a better quality of hybridization.

In addition, four numerical thickness measures (µm) of the hybrid layer of each sample were obtained using the software Image

### Table 1 - Scores for presence of failures (gap/ pores).

<table>
<thead>
<tr>
<th>Scores</th>
<th>Failure:gap</th>
<th>Failure:Pores</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>1</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

The presence of tag/microtags in the hybrid layer was analyzed according to the scores presented in Table 2, and four numerical score of each sample were obtained. Tags have been considered when presenting the typical funnel shape and microtags when presented as projections from the tags (Figures 1, 2 and 3). Statistical analysis was performed based on
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The data evaluation was done in two parts as follows; the evaluation of hybrid layer quality, estimated using the multiple proportion comparison (5%) statistical test and evaluation of hybrid layer thickness, estimated using the two-way Analysis of Variance ANOVA (Adhesive X Technique) and Tukey (5%) statistical test.

RESULTS

Hybrid layer analysis: thickness

The application of the two-way ANOVA test revealed that for technique factor, Subgroup Laser 140 mJ showed the hybrid layer thickness higher compared to the other subgroups (df = 2; f = 7.06; p = 0.0069*); for adhesive factor, SB showed the hybrid layer thickness higher compared to the CSEB (df = 1; f = 22.18; p = 0.0003*), and the interaction between the factors were statistically significant (df = 2; f = 12.83; p = 0.0006*).

For interaction between the factors, the Tukey’s test (5%) indicates that the experimental condition of smaller hybrid layer thickness (1.36 ± 0.14) was established for Subgroup Control using CSEB that was statistically different from all other subgroups studied (Table 3).

Hybrid layer analysis: thickness

Table 4 showed the results of presence of failures (gaps/pores) for all subgroups (Prevalence in % of score 0).

Table 5 showed the results of multiple proportion comparisons (presence of failure: gap/pores) between the groups of adhesive systems. (Prevalence in % of score 0).

*p < 0.05.
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**Table 6** - Results of presence of tags and microtags for all subgroups (Prevalence in % of score 2)

<table>
<thead>
<tr>
<th>Score</th>
<th>Subgroup Control</th>
<th>Subgroup Laser 60 mJ</th>
<th>Subgroup Laser 140 mJ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SB</td>
<td>CSEB</td>
<td>SB</td>
</tr>
<tr>
<td>0</td>
<td>4.17%</td>
<td>16.6%</td>
<td>25%</td>
</tr>
<tr>
<td>1</td>
<td>45.8%</td>
<td>58.3%</td>
<td>20.8%</td>
</tr>
<tr>
<td>2</td>
<td>50%</td>
<td>25%</td>
<td>54.1%</td>
</tr>
</tbody>
</table>

Through the multiple proportion comparison statistical test (Table 7) was possible to verify that among the adhesive systems employed in Subgroup Laser 140 mJ, statistically significant difference was found, demonstrating that for this technique, the SB had better result than CSEB.

**Table 7** - Results of multiple proportion comparison (tags/microtags) between the groups of adhesive systems. (Prevalence in % of score 2)

<table>
<thead>
<tr>
<th>Comparison</th>
<th>SB</th>
<th>CSEB</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subgroup Control</td>
<td>50%</td>
<td>25%</td>
<td>0.064</td>
</tr>
<tr>
<td>Subgroup Laser 60 mJ</td>
<td>54.1%</td>
<td>50%</td>
<td>0.772</td>
</tr>
<tr>
<td>Subgroup Laser 140 mJ</td>
<td>58.3%</td>
<td>16.6%</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

*p < 0.05.

**DISCUSSION**

The methodology employed in this study consisted in the irradiation with the Nd:YAG laser after the application of the adhesive, because a positive influence of this methodology in the bond strength of composite resins was observed [15-20]. In this way, this research has as its focus the morphological evaluation of the hybridized complex studied in the literature. The Nd:YAG laser is absorbed by the dental and pigmented tissues [22], altering the mineral content of the sound or demineralized dentin [23]. The Nd:YAG laser removes the organic components of dentin, principally the collagen [24], reducing the quantity of microorganism from the dentin [25,26].

It is observed in the literature, that the hybrid layer can vary from 1 to 5 µm [1-3], in agree with our results, which the thickness values of hybridization ranged from 1.04 to 2.71 µm. A higher thickness mean of the hybrid layer was found in the samples that were treated with CSEB, followed by those also irradiated with Nd:YAG laser 140 mJ (1.91±0.16 µm). In contrast, the lowest hybrid layer thickness mean occurred in the subgroup that did not have the irradiation of the Nd:YAG laser on the CSEB (1.36±0.14 µm) (Table 3). The Nd:YAG laser had an important effect on the CSEB altering significantly the thickness of hybrid layer.

The hypothesis that the total or self-etching adhesive irradiated with Nd:YAG laser improves the quality of the hybridized complex was accepted. In our study, without the Nd:YAG laser irradiation, the formation of a thin hybrid layer for the self-etching adhesives when compared to the total-etching adhesives was observed as stated in the literature [3,5,8,10,27]. The thickness of hybridized complex varied not only according to the adhesive system, but also the kind of treatment influenced the results (Table 3). The groups without Nd:YAG laser treatment presented higher variation of the measures of the hybrid layer thickness, in function of the adhesive employed, being more accentuated for SB (1.78±0.12 µm) compared to CSEB (1.36±0.14 µm).

The interaction of Nd:YAG laser and adhesives presented a different behavior according to the energy parameter (60 or 140 mJ) used. However, independently of the energy parameter, the Nd:YAG laser influenced positively the hybridization thickness. Chemical and physical alterations from the interaction of laser light occur on the dentin-adhesive complex [13]. From the findings of this study, it is suggested that different behaviors in relation to the formation of hybrid layer could be caused due to the energy intensity employed.

Independently of the hybrid layer thickness, the most important characteristic is related to its quality [27]. The failure presence in the hybridized complex can interfere in the bonding process. In this way, in this study, it was
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considered that the absence of gaps and pores (score 0) was the best performance expected.

For presence of failures (gaps/pores), subgroup Nd:YAG laser 140 mJ using CSEB presented significantly higher prevalence of score 0 (62.5%) when compared to subgroup Nd:YAG laser 140 mJ using SB (20.8% score 0) (Table 4). The tested hypothesis that according to the Nd:YAG laser energy parameters (60 or 140 mJ) associated with the adhesive systems, different patterns of hybrid layer can be formed, was accepted. The alterations on hybrid layer that occurred according to the variation of Nd:YAG laser energy, was probably due to the difference of intensity of energy transmitted by the laser, where 140 mJ, 10 Hz, 174.34 J/cm² is known that is approximately 133% higher than 60 mJ, 10 Hz, 74.72 J/cm² parameter of energy. The high energy intensity, probably can promote more effect on dental tissues, increasing the physical, chemical and morphological alterations. The Nd:YAG laser causes vaporization of organic components and increases the mineral components of dentin forming an irregular surface with microcavities, probably as result of a fusion and a recrystallization process[28]. The smear layer is consequently evaporated and melted [29], and the dentine tubules partially closed [28]. It is possible that the action of Nd:YAG laser on smear layer is favorable to the CSEB, since in this adhesive it has an interaction with the smear layer, that is not observed in total-etching adhesives that remove all the smear layer to form an effective adhesive interface.

The hybrid layer is the result of the diffusion and impregnation of resin monomers into the subsurface of pretreated dentin substrates that forms a mixed structure composed of a demineralized collagen network surrounded by resin monomers. However, resin tags are formed inside the dentin tubules that in theory can contribute to a good retention of resin, if these structures seal the walls of the dentine tubule [4].

In this study, the tags and microtags in the hybridized complex were evaluated considering, that their presence showed the best formation of hybridization zone (Figures 1 to 3). Thus, subgroup Nd:YAG laser 140 mJ using SB presented significantly higher quantity of tags and microtags (58.3% prevalence of score 2) when compared to subgroup Nd:YAG laser 140 mJ using CSEB (16.6% score 2) (Figure 3). This results were predicted because the total-etching adhesives remove the smear layer, increasing the tubules diameter, giving a conical shape to the tags after the resin polymerization[31] and from these tags form the lateral extensions (microtags). The Nd:YAG laser, independent of the energy parameter used (60 mJ or 140 mJ), had a good influence on hybridization, when the formation of tags and microtags were considered.

The analysis of this treatment on dentin shows that the adhesive used has a critical influence, as its variation promoted the best and the worst results considering the tag and microtag formation (Figure 3), using the same experimental conditions with Nd:YAG laser. An effective sealing and consequently elimination of marginal leakage is based more on the adhesive system's ability to block the dentinal tubules than the resin tags' length [29].

For the aspects evaluated in this study with respect to the thickness and the quality of hybridization, it was observed that the Nd:YAG laser had a good influence varying according to the intensity of energy employed. Additional studies analyzing adequate energy for improving the bonding interface should be conducted, as within the limitations imposed by the experimental conditions it was observed that not only the laser but also the level of the intensity of energy can produce different results.

**CONCLUSION**

Based on the findings of this study it was possible to conclude that there is significant evidence that the irradiation of Nd:YAG laser on dentin impregnated with self-etching and total-etching adhesives non polymerized, can modify the morphological bonding interface, varying according to the intensity of energy employed.
REFERENCES


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