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Original Article

Micromorphology and Analysis of Dentin Surfaces after Preparation with Er:YAG Laser and Application of Self-Etch Adhesive System with 10-MDP. A Confocal Laser Scanning Microscope Study

Nikolay Simeonov[1], Vesela Stefanova[1]

[1] *Department of Operative Dentistry and Endodontics, Faculty of Dental Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria*

Corresponding author: Nikolay M. Simeonov, Department of Operative Dentistry and Endodontics, Faculty of Dental Medicine, Medical University of Plovdiv, 3 Hristo Botev Blvd., 4002 Plovdiv, Bulgaria; Email: n.m.simeonov@gmail.com; Tel.: +359 882 705379

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Abstract

Aim: The purpose of this study was to analyze and compare the micromorphology of the hybrid layer and the dentinal surface when the dentin was prepared with an Er:YAG laser or burs, and to find if there was any difference if the time of application of the self-etching primer on the dentin prepared with an Er:YAG laser was doubled.

Materials and methods: Fifteen freshly extracted human teeth with preserved crowns were selected. Three cavities were prepared on each extracted tooth. Two of the cavities were prepared with an Er:YAG dental laser, and the third with burs. A self-etching adhesive system was applied. The primer of the adhesive was labeled with Rhodamine B, and the bond was labeled with fluorescein prior to application. The teeth were sectioned mesiodistally through the cavities and the cavity of each sample was examined using a confocal laser scanning microscope. The infiltration and micromorphology were determined qualitatively on ninety samples.

Results: The dentinal surface in the samples prepared with burs was smoother and with shorter resin tags than the surface prepared with an Er:YAG laser. When using an Er:YAG laser for preparation, crater-like irregularities of the surface were observed. In five of the samples prepared with burs, no resin tags were found. We could not detect any difference in the hybrid layer when the time of application of the adhesive system was increased.

Conclusions: The clinical significance of the tag length and quality, as well as the infiltration ratio, needs to be further studied.

Keywords

confocal laser scanning microscope, Er:YAG, hybrid layer, MDP, resin tags

INTRODUCTION

Composite materials in restorative dentistry require the use of adhesive systems. These systems create a micromechanical bond between dentin/enamel and the composite material.[1,2] While bonding to enamel has been demonstrated to be easy and reliable, bonding to dentin can be quite challenging[3] because of the great morphological and physical variations of dentin (variable tubular structure, high organic content, and fluid flow).[4] Adhesion to the tooth substrate implies an exchange process which involves two phases: in the first, calcium phosphates are removed, exposing microporosities at both the enamel and dentin surfaces; the second phase involves infiltration and subsequent in situ polymerization of resin within the created surface microporosities.[5] One of the leading manufacturers in the market manages to implement in its adhesive systems the so-called MDP monomer – 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP), which performs both micromechanical and chemical bonding.[6] The 10-MDP functional monomer has a chemical structure that allows better binding, but also forms MDP salts that protect the collagen fibrils.[7]

Lasers are high-tech tools that have been successfully used in dentistry for numerous manipulations.[8-11] Classic rotary instruments remove hard dental tissues mechanically, lasers interact with water and hydroxyapatite and cause them to evaporate.[12-14] The nature of operation of classic rotary instruments and dental lasers is different and, according to many studies, leads to differences in the surface of the prepared dentin. When using burs, smear layer is present and plugged dentinal tubules are detected, and with Er:YAG lasers – rougher surfaces, no smear layer, and many open canals.[8,15-17] Furthermore, treating dentin with Er:YAG lasers leads to an increased acid resistance.[18] The use of adhesive systems on laser treated hard dental tissues has not yet been well studied.[19]

Confocal laser scanning microscope (CLSM) allows in-depth observation of the surface, which makes it a good choice when studying the hybrid layer and its penetration.[20]

AIM

In the present study, we aimed to analyze and compare the micromorphological structure of the hybrid layer and the dentinal surface when the dentin was prepared with an Er:YAG laser or with burs, and to find if there would be any difference if the application time of the self-etching primer on the dentin prepared with an Er:YAG laser increased two times.

MATERIALS AND METHODS

Using visual inspection, we selected 15 freshly extracted human teeth with preserved crowns and no carious and/or non-carious lesions and fillings; these teeth were stored in a 0.2% aqueous-alcoholic solution of thymol at a temperature of 2–6°C. In this study, we used the Clearfil SE Bond 2 adhesive system (Kuraray, Japan) which contained 10-MDP. The primer was mixed with Rhodamine B and the bond – with fluorescein, with both dyes used at a concentration of 0.1%. Their emission peaks are different, which makes it possible to examine them simultaneously with CLSM.

Three cavities were prepared in each extracted tooth until the middle zone of the dentin was reached – one on the occlusal surface, one on the mesial surface, and one on the distal surface. All cavities were box-shaped having five walls and one open surface. Random two cavities were prepared with an Er:YAG dental laser (LiteTouch, Syneron, Israel) with the manufacturer's recommended sapphire tips and settings. We used burs in the preparation of the third cavity.

The prepared surfaces were wiped thoroughly with a cotton pellet soaked in 3% hydrogen peroxide, then with a cotton pellet soaked in 70% alcohol, and finally dried gently with air from the dental unit. The cavities prepared with the Er:YAG dental laser were randomly divided into two groups. The adhesive system was applied to the cavities of group 1 in strict accordance with the manufacturer's instructions (self-etching primer for 20 s, air-drying, bonding, and light-curing); the cavities of group 2 were prepared by applying the adhesive system twice as long as specified by the manufacturer (self-etching primer for 40 s, air-drying, bonding, and light-curing). The same adhesive system was applied to the cavities prepared with burs according to the manufacturer's instructions (group 3 – controls). Using a diamond saw, all teeth were split along the longitudinal axis in the mesiodistal direction simultaneously through the three cavities.

The adhesive system and the prepared dentin in the area of shear were examined with a confocal laser-scanning microscope (Leica TCS SPE DM2500) with optical magnification 10× and the possibility of additional digital magnification using software Leica Microsystems LAS AF – TCS SPE. The size of the observed field at 10× magnification was 1100×1100 μm with the possibility of changing the resolution. The areas of interest were scanned and saved as digital images.

RESULTS

The infiltration and the micromorphology were determined qualitatively on ninety samples. Only the fluorescent dyes added to the adhesive system were visualized on the images obtained by CLSM. The primer was displayed in red and the bond – in green. When a superimposed image of the two dyes was created, the areas where they coincided were colored in yellow.

What are visualized are the primer and the bond. An image of the relevant area is seen as a strip that depicts the adhesive system on the dentinal surface and resin-tags, which are in the form of cones entering the dentinal tubules. In five of the samples prepared with burs, no resin-tags were found.

Analysis of the images in group 1 **(Fig. 1)** revealed well-defined fine stripes of primer and adhesive, respectively, and a high degree of overlap was observed on the superimposed image. The reason for the discontinuation of the strips of primer and/or adhesive in some areas is the unevenness of that surface, which does not allow it to be observed by CLSM in the respective section of slices.

The dentinal surface prepared with burs appears to be smoother than the dentinal surface prepared with the Er:YAG laser. It is noteworthy that the images of the fluorescent primer do not completely match the areas of the fluorescent adhesive. This is very clearly seen in the superimposed image **(Figs 1, 2, 3)**.

We could not find a significant difference in the hybrid layer when the time of application of the adhesive system was increased **(Figs 1, 2)**. They had similar thickness and similar distribution.

In the control group, both the primer and the bond created a dense, uniform layer **(Fig. 3)**. When superimposed, the images visualizing the primer and the bond coincided with each other and a very good matching between them was noticed.

In some of the specimens prepared with an Er:YAG laser (group 1 and group 2), a more uneven surface and deeper areas resembling craters were found, which were filled with the adhesive system **(Fig. 4)**. We noticed very wide tags at the bottom of the craters.

By using digital magnification in the CLSM software, we were able to perform a more detailed study of the micromorphology of the fluorescently labeled objects, which was particularly useful in the control group. **Fig. 5** is a scan of a sample of group 3 with a selected area of high digital magnification.

DISCUSSION

Several studies have examined the specifics of working with Er:YAG lasers both in terms of dentin and enamel.[21-23] Since the adhesive is visualized, we analyze the dentinal surface it covers. The dentinal surface created using burs was smoother than the dentinal surface prepared with an Er:YAG laser, confirming the findings of other authors.[8,10,15-17] According to the literature we reviewed, CLSM is less commonly used than SEM for assessing dentinal surfaces after preparation with an Er:YAG laser. Lack of tactile sensation in the performed preparation with the Er:YAG laser is probably the reason why 'craters' filled with the fluorescently labeled adhesive system formed **(Fig. 4)**. The thin, uniform hybrid layer in the control group was also observed on the smooth dentinal surface and described by other authors.[24]

The craters visualized in our study are also found by Yamada et al. in a preparation with an Er:YAG laser in the dentin without the presence of carbonization of the collagen structure.[25]

The micromorphological structure observed with CLSM in our study in the samples from groups 1 and 2, showing increased roughness and the presence of craters and long resin tags, could be considered as a favorable factor for increasing the strength of the adhesive bond, despite

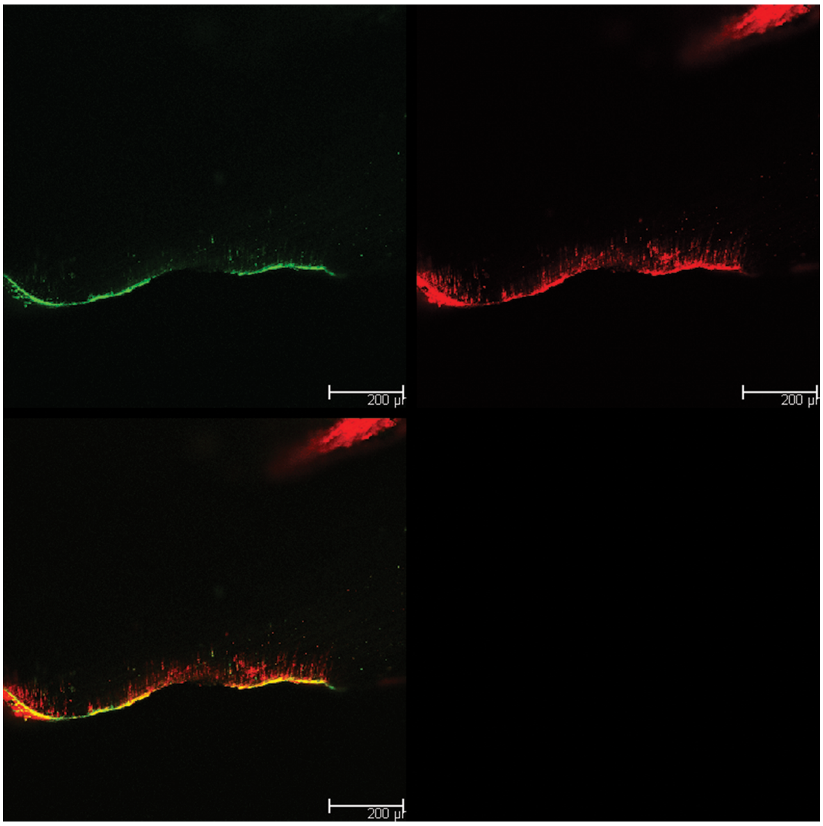

Figure 1. CLSM image of a sample (group 1). Images of channel 1 (primer in red), channel 2 (bond in green) and superimposed image between them (in yellow) (×10).

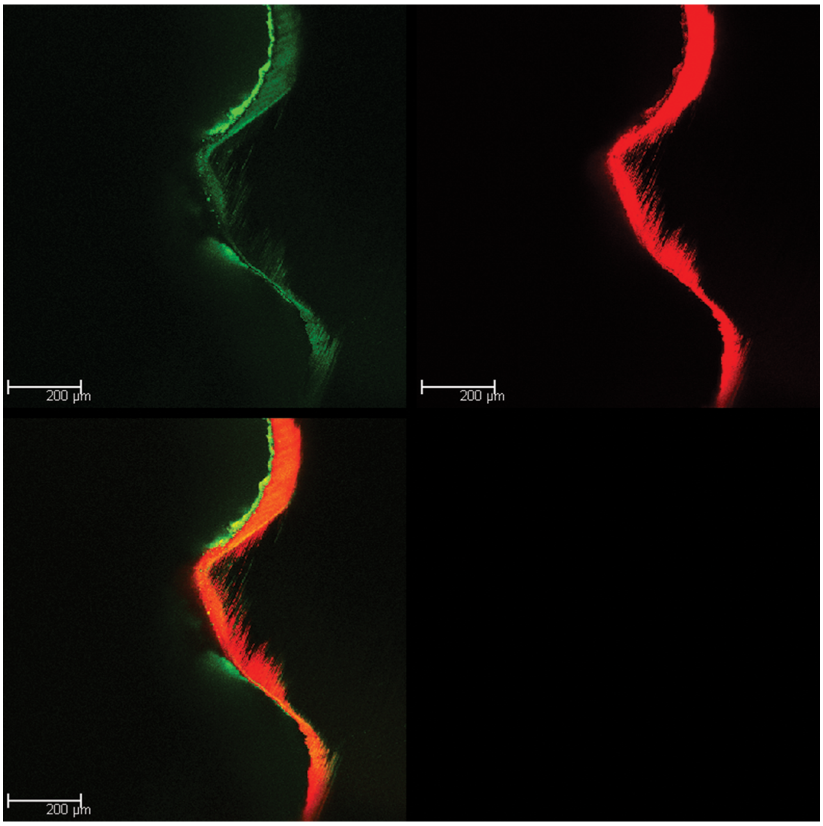

Figure 2. CLSM image of a sample of group 2. Images of channel 1 (primer in red), channel 2 (bond in green) and superimposed image between them (in yellow) (×10).

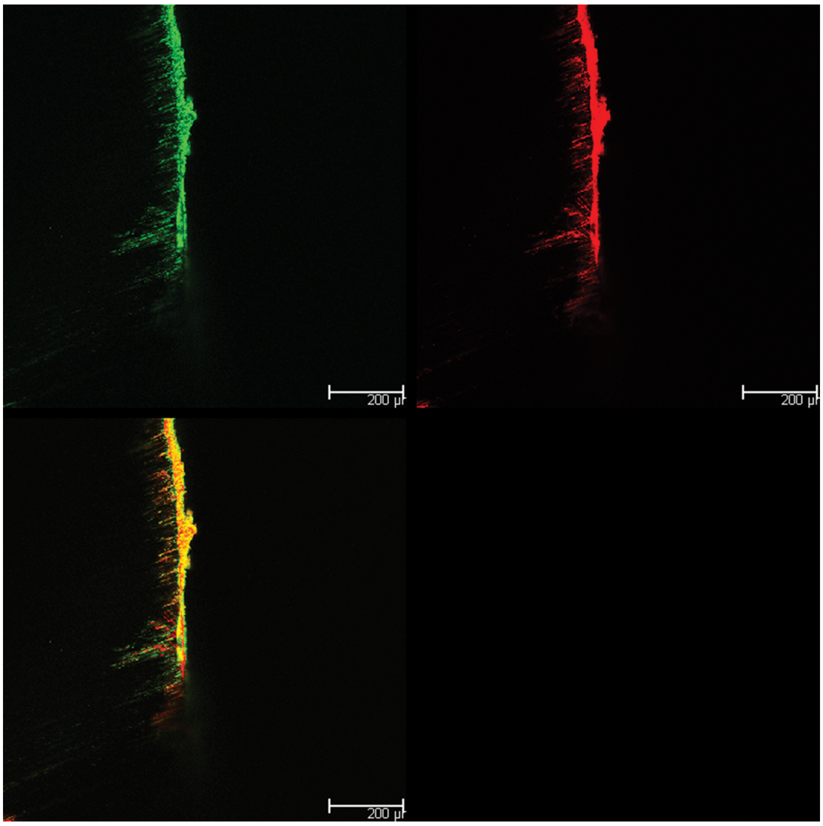

Figure 3. CLSM image of a sample from group 3 (controls). Images of channel 1 (primer in red), channel 2 (bond in green) and superimposed image between them (×10).

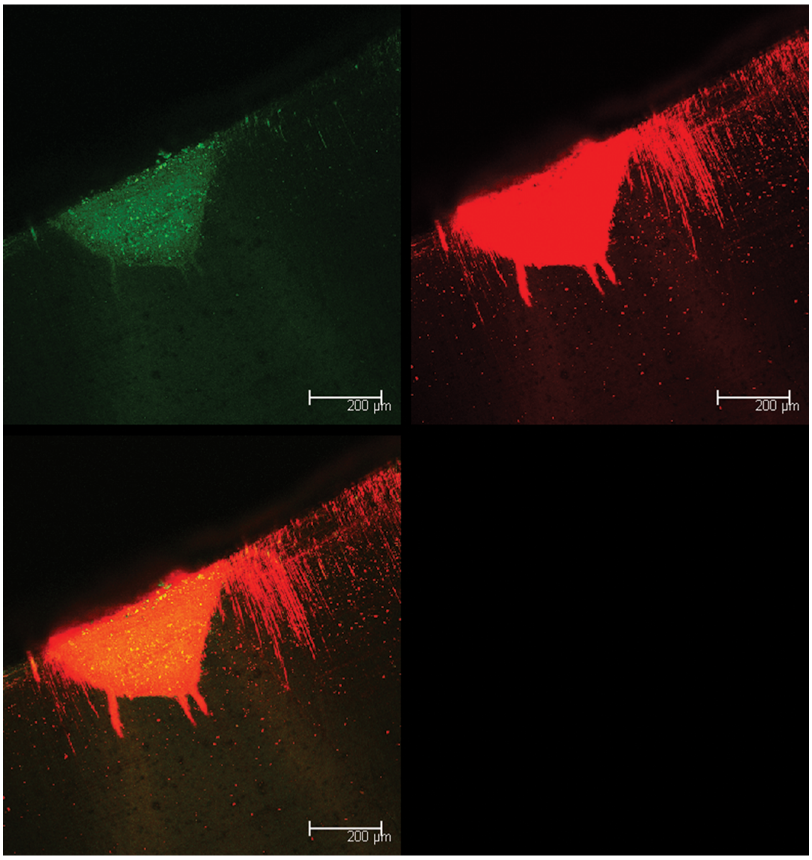

Figure 4. "Craters". CLSM image of a sample from group 1. Images of channel 1 (primer in red), channel 2 (bond in green) and superimposed image between them (×10).

the report of some studies of other authors. According to Ceballo et al., Er:YAG lasers create a laser-modified layer that adversely affects dentin adhesion, so it is not an alternative bonding strategy to conventional acid etching. Acid etching of dentin, according to them, gives values of shear bond strength, which are significantly higher than those achieved only by laser ablation or laser ablation in combination with acid etching.[26] In our study, we do not use devices to assess the strength of the adhesive bond and, accordingly, we can only indirectly evaluate it by visualizing the created sealing surface.

We noticed extremely thick resin-tags at the bottom of the "crater" **(Fig. 4)**, which are probably not in the dentinal tubules, due to the large diameter, and are cracks in the dentin. That may be the reason for the weak adhesive bond described by some authors.[26] According to the findings of Lohbauer et al., the length of the resin-tag does not affect the strength of the bonding.[27]

CONCLUSIONS

Based on our morphological study and the analysis of the dentinal surface performed with CLSM, we conclude that regarding dentin infiltration and hybrid layer formation on teeth prepared with Er:YAG laser, dentin has surfaces that are rougher than those prepared with metal burs. Shorter

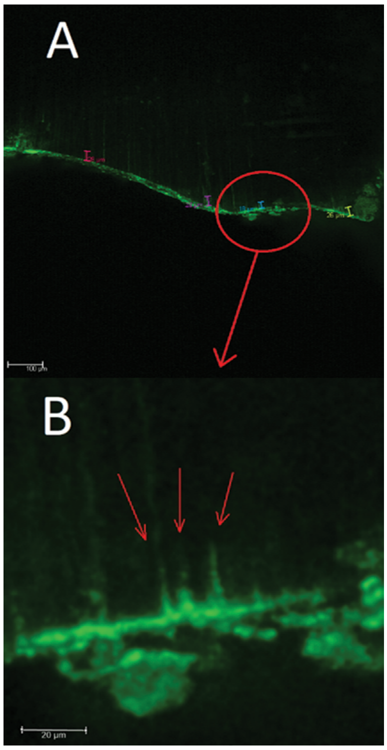

Figure 5. Image of a sample of group 3 (controls). **A**) Visualization of superimposed image from channel 2 (bond in green) (magnification ×10); **B**) area further enlarged digitally. Resin-tags are indicated by arrows.

resin-tags were observed in the control group. When using Er:YAG lasers for preparation, crater-like irregularities of the surface were observed. As the application time of the self-etching primer increased twice, we could not detect a noticeable morphological change in the formed hybrid layer compared to the samples in which we applied an adhesive system according to the manufacturer's instructions. The clinical role of the length and quality of tags as well as the ratio of infiltration must be further investigated.

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Микроморфология и анализ поверхности дентина после препарирования Er:YAG лазером и нанесения самопротравливающей адгезивной системы с 10-MDP. Исследование с применением конфокального лазерного сканирующего микроскопа

Николай Симеонов[1], Весела Стефанова[1]

[1] *Кафедра оперативного зуболечения и эндодонтии, Факультет дентальной медицины, Медицинский университет – Пловдив, Пловдив, Болгария*

Адрес для корреспонденции: Николай М. Симеонов, Кафедра оперативного зуболечения и эндодонтии, Факультет дентальной медицины, Медицинский университет – Пловдив, бул.“Христо Ботев“ № 3, 4002 Пловдив, Болгария; E-mail: n.m.simeonov@gmail.com; тел.: +359 882 705379

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Резюме

Цель: Целью данного исследования было проанализировать и сравнить микроморфологию гибридного слоя и поверхности дентина, когда дентин препарировали с помощью Er:YAG-лазера или боров, и выяснить, есть ли какая-либо разница, если время нанесения самопротравливающего праймера на дентин, подготовленном лазером Er:YAG, был удвоен.

Материалы и методы: Было отобрано 15 свежеудалённых человеческих зубов с сохранившимися коронками. На каждом удалённом зубе препарировали по три полости. Две полости были подготовлены стоматологическим лазером Er:YAG, а третья – борами. Наносили самопротравливающую адгезивную систему. Праймер клея был помечен Родамином Б, а связка была помечена флуоресцеином перед нанесением. Зубы разрезали мезиодистально через полости, и полость каждого образца исследовали с помощью конфокального лазерного сканирующего микроскопа. Инфильтрацию и микроморфологию определяли качественно на девяноста образцах.

Результаты: Поверхность дентина в образцах, подготовленных с помощью боров, была более гладкой и с более короткими смоляными метками, чем поверхность, подготовленная с помощью лазера Er:YAG. При использовании Er:YAG-лазера для препарирования наблюдались кратерообразные неровности поверхности. В пяти образцах, подготовленных борами, не было обнаружено смоляных меток. Мы не смогли обнаружить разницы в гибридном слое при увеличении времени нанесения адгезивной системы.

Заключение: Необходимо дальнейшее изучение клинической значимости длины и качества метки, а также степени инфильтрации.

Ключевые слова

конфокальный лазерный сканирующий микроскоп, Er:YAG, гибридный слой, MDP, смоляные метки