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MORPHOLOGICAL STUDY OF BORDER AREA OF PULP-CAPPING MATERIALS AND Er:YAG LASER PREPARED HARD DENTAL SURFACE

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МОРФОЛОГИЧЕСКОЕ ИССЛЕДОВАНИЕ ЗОНЫ СОПРИКОСНОВЕНИЯ ПУЛЬПОПОКРЫВАЮЩИХ МАТЕРИАЛОВ И ПРЕПАРИРОВАННОЙ ПРИ ПОМОЩИ Er:YAG ЛАЗЕРА ТВЁРДОЙ ТКАНИ ЗУБА

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ABSTRACT

Introduction: Vital pulp therapy involves biologically based therapeutic activities aimed at restoring health and preserving the vitality of cariously or traumatically damaged pulp. Adaptation of pulp-capping materials to the prepared tooth surface may be the key to the success of biological tooth treatment. **Aim:** To investigate the area of adaptation of synthetic tricalcium silicate cement, calcium hydroxide cement and mineral trioxide-aggregate to the dentin surface, prepared with the help of Er:YAG dental laser. **Material and methods:** Four extracted human tooth cavities were prepared with the help of Er:YAG dental laser (LiteTouch, Syneron, Israel), establishing microcommunication with the pulp chamber less than 1 mm in diameter. As pulp-capping materials in the cavities we used tricalcium silicate cement (Biodentine, Septodont, France), calcium hydroxide cement (Dycal) and mineral-trioxide aggregate (ProRoot MTA), stirred and administered according to manufacturers' instructions. The first material fills the whole cavity and the other two are spread in a thin layer and sealed with glass ionomer cement. Thus prepared, the samples were left for three days at 37°C in humidified environment. The samples were prepared for scanning electron microscopy (SEM) by standard methodology. The border area surfaces of the materials and the dentin were scanned using electron microscopy. **Results:** The morphological changes occurring to the Er:YAG laser prepared dentin and the structural characteristics of the studied pulp-capping materials are demonstrated using scanograms. The border areas where good contact of materials and dentinal tubules is established are thoroughly studied. Good adaptation is seen in three-calcium silicate cement, followed by mineral trioxide aggregate and calcium hydroxide cement. **Conclusion:** The dentin surface, prepared with Er:YAG laser demonstrates a very good adaptation of the three tested pulp-capping materials.

Key words: *scanning electron microscopy, Er:YAG laser, tricalcium silicate cement*

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РЕЗЮМЕ

Введение: Витальная терапия пульпы включает в себя биологически обоснованные терапевтические методы лечения, которые направлены на восстановление здоровья и сохранение жизнеспособности кариозно или травматически повреждённой пульпы. Адаптация пульпопокрывающего материала к препарированной поверхности зуба может оказаться решающей для успешного биологического лечения зуба. **Цель:** Исследование зоны адаптации синтетического трикальций силикатного цемента, кальций-гидроксидного цемента и минерального триоксидного агрегата к поверхности дентина, препарированной при помощи Er:YAG стоматологического лазера. **Материал и методы:** В четырёх экстрагированных человеческих зубах была осуществлена препарация полости при помощи Er:YAG стоматологического лазера (LiteTouch, Syneron, Izrael) с образованием точечного отверстия в пульпарной камере диаметром менее 1 мм. В качестве пульпопокрывающих материалов были использованы трикальций силикатный цемент (Biodentine, Septodont, France), кальций-гидроксидный цемент (Dycal) и минеральный

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триоксидный агрегат (ProRoot MTA), размешанные и нанесённые в соответствии с инструкциями производителей. Первым из материалов заполнена вся полость, а остальные два нанесены тонким слоем и покрыты стеклоиономерным цементом. Подготовленные таким образом пробы помещены на три дня во влажную среду при температуре 37 градусов по Цельзию. Образцы подготовлены для проведения СЭМ по стандартной методике. Проведена сканирующая электронная микроскопия поверхностей соприкосновения материалов и дентина. **Результаты**: Посредством скенограмм представлены морфологические изменения препарированного при помощи Er:YAG лазера дентина, структурные особенности исследуемых пульпопокрывающих материалов. Подробно исследованы и сопоставлены поверхности соприкосновения, на которых устанавливается хороший контакт материалов и дентинных трубочек. Хорошая адаптация установлена при трикальций силикатном цементе, за которым следуют минеральный триоксидный агрегат и кальций гидроксидный цемент. **Заключение:** Препарированная при помощи Er:YAG лазера дентиновая поверхность предоставляет возможность для очень хорошей адаптации трёх исследованных пульпопокрывающих материалов.

Ключевые слова: *сканирующая электронная микроскопия, Er:YAG лазер, трикальций силикатный цемент*

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INTRODUCTION

Vital pulp therapy is used in treating pulp tissue that has been compromised incipiently by caries or trauma. If treatment indications and clinical protocol are followed strictly, the dental pulp can preserve its vitality and practically recover.[1-3]

A large number of pulp-capping materials have been tested in preserving the vitality of dental pulp, specifically materials with anti-inflammatory, anti-allergic and dentinogenic effect. These include antiseptic agents, calcium hydroxide, antibiotics, sulfonamides, corticosteroids, enzymes, cyanoacrylates, dentin debris, adhesives, potassium nitrate in polycarboxylate cement, phytomedication etc.[1,4] Dental materials that are recognised in practice today are calcium hydroxide liners and mineral-trioxide aggregate (MTA).[2,3,5] In recent years entirely synthetic tricalcium silicate cement with good biological and manipulative qualities as well as a wide range of indications has been created.[6]

Dental lasers have been increasingly introduced into dental practice as an alternative to the conventional preparation of hard dental tissues with rotary instruments. The classic rotary instruments tend to leave a smear layer on the dental walls which contains microorganisms, saliva, debris, oil film and others. Diamond burs create rough cavity walls with deep furrows and smear layer which obturates most dentinal tubules. Metal burs create smooth and clean walls and a very fine smear layer.[1] Modern laser systems for removal of hard dental tissue (Er:YAG and Er, Cr: YSGG) limit to a great extent the damage caused by mechanical means of cleaning carious mass and increase patient comfort in biological treatment. On dentin walls after preparation with Er:YAG dental laser no smear layer is detected and dentinal tubules are open. The surface is retentive with no evidence of thermal damage, carbonification or melting.[7-9]

Adaptation of the pulp-capping materials to the prepared tooth surface may be the key to a successful vital pulp therapy of teeth. A crack left between the two structures would lead to micro leakage and penetration of microorganisms and toxins into the pulp which may result in disturbing or interrupting the healing process. The presence of smear layer and the lack of good mechanical or chemical adhesion of the pulp-capping materials to the dentin walls increase the chance of creating a leak.[5]

Lack of information about the adaptation of modern pulp-capping materials to the Er:YAG laser prepared tooth surface has necessitated this study.

AIM

To study the area of adaptation of synthetic tricalcium silicate cement, calcium hydroxide cement and mineral-trioxide aggregate to dentin surface, prepared with Er:YAG dental laser.

MATERIALS AND METHODS

In vitro experiments were performed to study the adaptation of synthetic tricalcium silicate cement, calcium hydroxide cement and mineral-trioxide aggregate to dentin surface, prepared with Er:YAG dental laser, by examining the border area surfaces using a scanning electron microscope (SEM).

The study was conducted in January 2013. The tooth preparation was done in FDM, MU-Plovdiv. Scanning electron microscopy was performed at the

Institute of Mineralogy and Crystallography of the Bulgarian Academy of Sciences. A study protocol was kept during the study and the electronic microscanograms were recorded electronically and analyzed qualitatively.

Tooth sample preparation

After 72-hour storage of four extracted human teeth in a physiological solution, four occlusal cavities were prepared by using a Er:YAG dental laser (LiteTouch, Syneron, Israel), together with establishing microcommunication with the pulp chamber less than 1 mm in diameter. A standard setup of the device for work in deep carious lesions was used for this purpose (Caries Removal - Deep Cavities HT, Non-Contact, 250 mJ, 30 Hz, and water spray 8). The cavities were prepared with handpieces provided with new sapphire tips 0.8 mm in diameter and 17 mm in length. The cavity with tricalcium silicate cement (Biodentine, Septodont, France) was filled completely, while those with calcium hydroxide cement (Dycal) and mineral-trioxide aggregate (ProRoot MTA) as pulp-capping materials were sealed with glass ionomer cement. The materials were prepared and administered in the cavities according to the manufacturers' instructions. After hardening of the materials, the samples prepared in this way were placed for three days at 37° C in a closed container with the root part thereof immersed in saline, and the crown part in humid environment so as to simulate the conditions in the oral cavity.

Preparation for SEM

To avoid rotary instrumentation, the teeth were mechanically split longitudinally in mesiodistal direction, with each tooth yielding two samples (n = 8). Scanning electron microscope (Philips, Holland) with incremental magnification (as high as x 9000) was used to scan the laser-prepared dentin, the pulp-capping agents, the border areas of the materials and hard tooth surfaces was performed with the help of. Thirty-two scanograms of the laser-prepared dentin, the materials used and the area of adaptation were made. The absence or presence of a gaps was evaluated at incremental magnification.

RESULTS

A. Dentin surface after preparation with Er:YAG laser

The scanning electron microscopy of Er:YAG laser prepared dentin surface shows longitudinally and transversely cut open dentinal tubules (Fig. 1). A surface free of smear layer and dentinal debris is exposed. Because of the lower degree of mineralization of intertubular dentin, its laser ablation is more intense compared to that of peritubular dentin, thereby dentinal tubules protrude above the surface

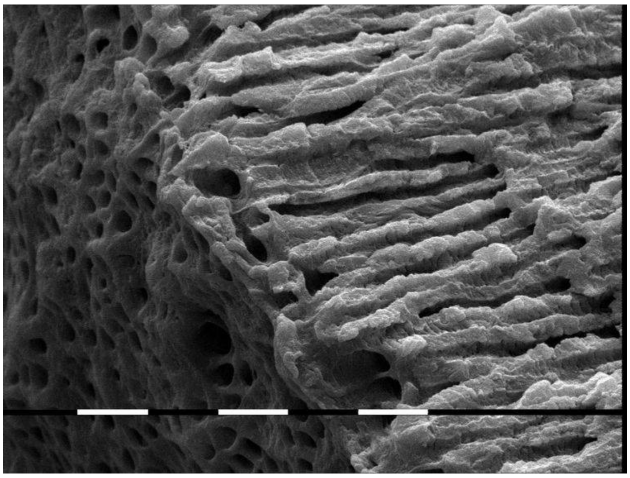

Figure 1. Er:YAG laser prepared dentin (x 1500).

in cross section. The changes that occur are the result of the impact of laser radiation on tissue.

B. Morphological characteristics and comparison of pulp-capping materials

Using SEM with incrementally increasing magnifications we studied the morphological characteristics of the three types of pulp-capping materials - the tricalcium silicate cement achieved good homogeneity of the particles (Figs 2a,b,c), which is most likely due to the fact that it is a fully synthetic product with controlled production characteristics. Even at 9000 magnification, a close contact was observed between individual particles without the presence of large air inclusions (Fig. 2c).

When the three scanograms of the pulp-capping material were analysed and compared at the same level of magnification (x5000), we found that the tricalcium silicate cement particles were the smallest, the calcium hydroxide particles bigger, and the largest particles were those of MTA. On the other hand, the presence of cavities was most pronounced in MTA, and the smallest number of cavities were observed in tricalcium silicate cement, with calcium hydroxide cement falling in the middle (Figs 2b,3,4).

C. Adaptation of pulp-capping material to Er:YAG laser prepared dentin

The border areas were studied by scanning electron microscopy with incremental magnification. The presence of a gap between the two structures

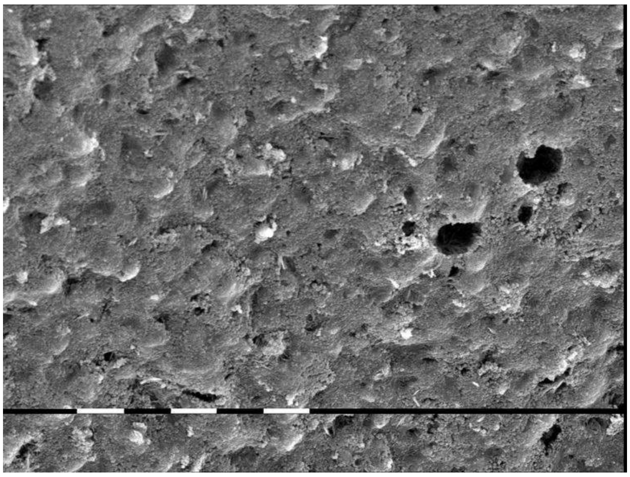

Figure 2a. Synthetic tricalcium silicate cement (x 1000).

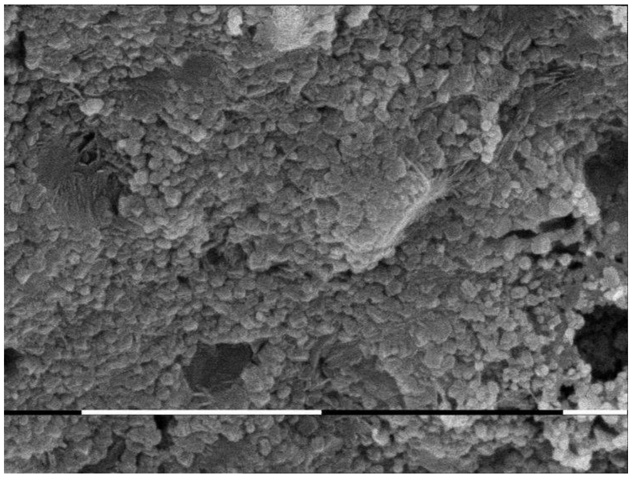

Figure 2b. Synthetic tricalcium silicate cement (x 5000).

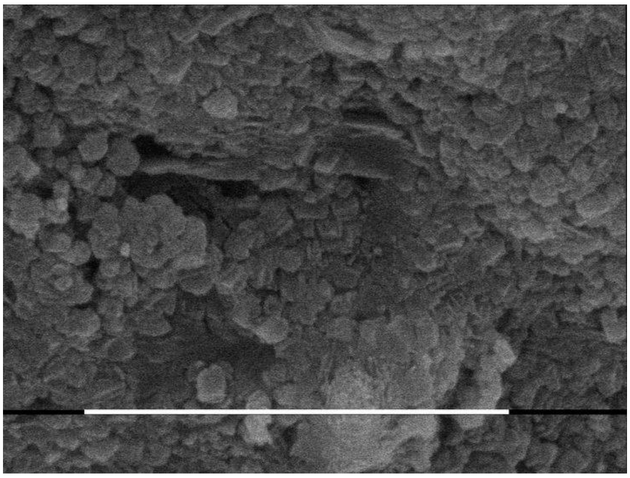

Figure 2c. Synthetic tricalcium silicate cement (x 9000).

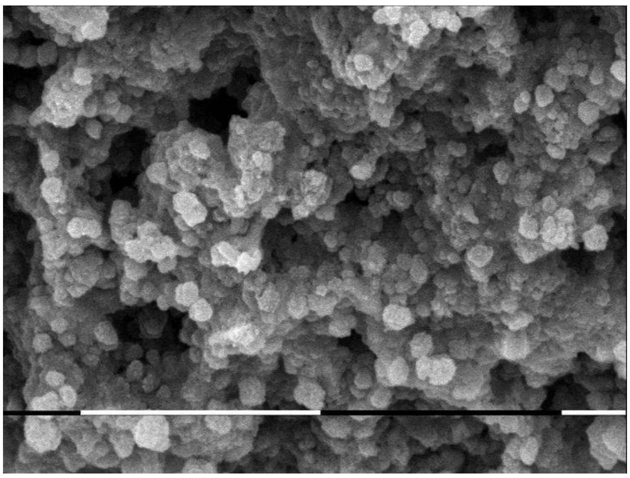

Figure 3. Calcium hydroxide cement (x 5000).

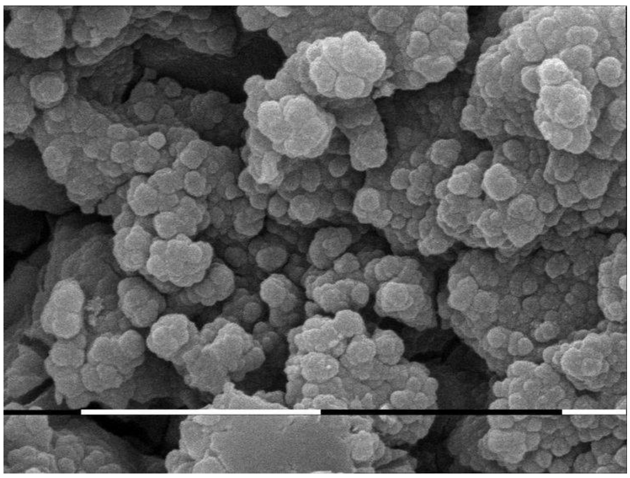

Figure 4. Mineral-trioxide aggregate (x 5000).

at the smallest magnification was documented and presented by a scanogram.

We performed for the first time in the present study an electronic microscopic examination of the border area between tricalcium silicate cement and laser prepared dentin (Figs 5a, b) and we found no evidence of a crack between them even at x1500 magnification.

In samples with calcium hydroxide cement as pulp-capping material (Fig. 6) there was evidence of a gap even at x 150 magnification.

When examining the samples with MTA at x 300 magnification we detected a gap which was not observed in all sections of contact with the dentin (Fig. 7).

DISCUSSION

Achieving hermetical sealing of dentin in the vital pulp therapy is crucial to the proper accomplishing of the healing process. Discontinuing the possibilities for reinfection and reintoxication of reversibly inflamed dental pulp creates optimal conditions for revealing regenerative and reparative pulp potential. Of crucial importance for the good marginal adaptation between the two surfaces are the physical and chemical interactions that occur in the area of contact between them. Ideally, ion exchange occurs between them establishing a true chemical bond on the border. The presence of such a bond is established in dentin and glass ionomer cements, tricalcium silicate cement and MTA to varying degrees and strength.[1,6,10] Undoubtedly, the adhesion of the two surfaces depends on their physical characteristics. The determining factors for the pulp-capping materials in this bond are the type, size and shape of the particles, their arrangement

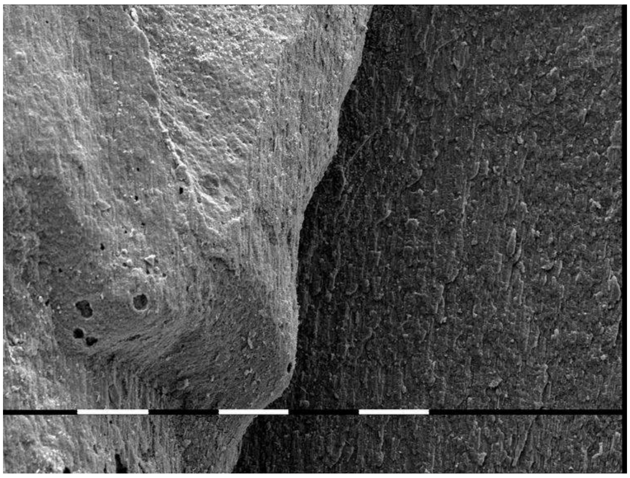

Figure 5a. Border area between tricalcium silicate cement and Er:YAG prepared dentin (x 150).

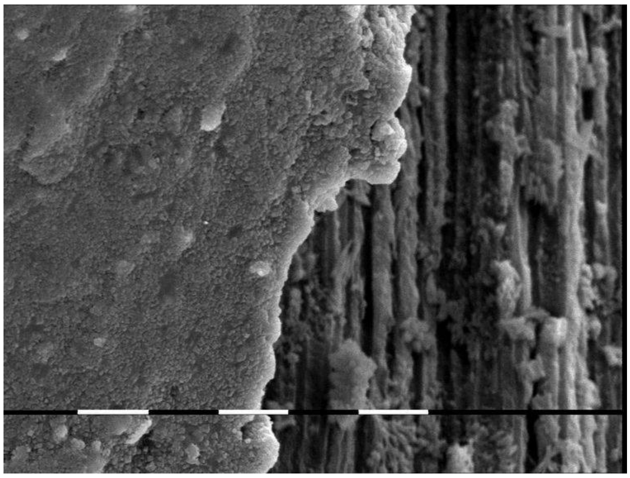

Figure 5b. Border area between tricalcium silicate cement and Er:YAG prepared dentin (x 1500).

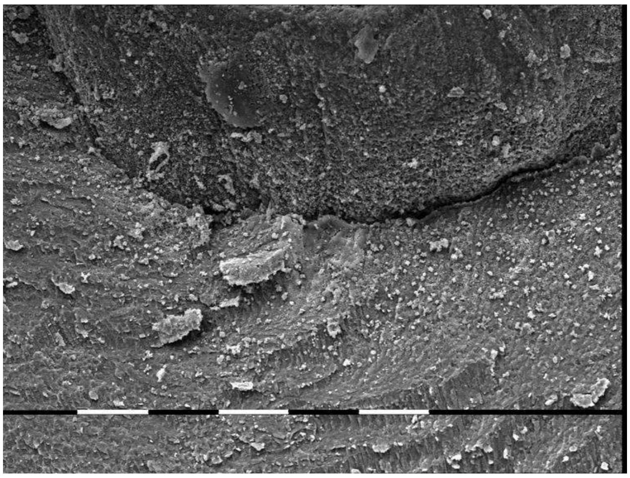

Figure 6. Border area between calcium hydroxide cement and Er:YAG prepared dentin (x 150).

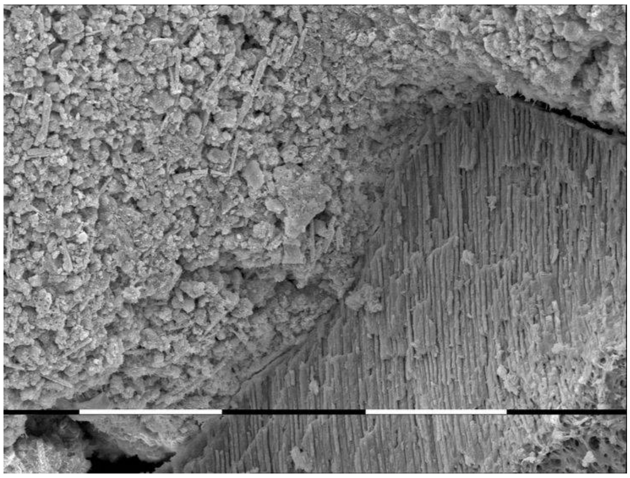

Figure 7. Border area between MTA and Er:YAG prepared dentin (x 300).

and the empty spaces between them. Dentin surfaces are also important for the good adaptation of these materials. The dentin near the pulp has a unique structure. The impact of the Er:YAG laser on the dentin with reduced mineral content and presence of a greater number and more dentinal tubules would lead to changes in the level of marginal adaptation, which necessitated its detailed study.

A. Dentin surface after preparation with Er:YAG laser

The used high energy pulsed Er:YAG laser has a wavelength (2940 nm), which is best absorbed by water and hydroxyapatite, which makes it most suitable for the removal of enamel and dentin.[7] Laser energy leads to microexplosions by which the structure of tissue is destroyed and craters emerge while the directed water stream fed by the apparatus to the site of interaction washes away the removed particles and reduces the risk of adverse thermal effects - melting and carbonation.[8]

Our findings are consistent with the research of other authors who also described the absence of smear layer after laser preparation of dentin in comparison by SEM with mechanically prepared dentin.[9] The open dentinal tubules allow direct contact of pulp-capping material and dentin lymph in them. Er:YAG laser prepared dentin surface showed more signs of unevenness that provides increased contact area and better mechanical retention for pulp-capping materials. The observed changes in the dentin close to the pulp after its preparation with Er:YAG dental laser give us reasons to believe that this method of removal of dentin creates optimal conditions for better adaptation of pulp-capping materials to the thus treated dentin surface.

B. Morphological characteristics and comparison of pulp-capping materials

The study of the area of adaptation would not be complete if a thorough monitoring of materials near their contact with dentin is not conducted. Magnification x 5000 allows us to compare the studied materials owing to the clear visualization of particles and their structural features. The fully synthetic nature of tricalcium silicate cement unlike the other two studied pulp-capping materials provides identical particle size and their close location to the already hardened material. Factory dosing of liquid and powder warrants the correct proportions of the ingredients in order to obtain a homogeneous material, while stirring in the capsule does not allow the inclusion of air bubbles and subsequent formation of cavities.[6] Because MTA is produced on the basis of Portland cement, which is comprised of various natural products, their particles are characterized by a large variety in shape and size. The mixing of these various materials gives rise to the emergence of a non-homogeneous mixture of heterogeneous particles, which in turn makes it difficult to spread and worsens the contact quality of the material to the dentin surface, which could affect negatively their marginal adaptation. Since calcium hydroxide cement is recognised as 'gold standard'[2,3,5] in vital pulp therapy, we also compared other materials to it in the present study.

C. Adaptation of pulp-capping materials to Er:YAG laser prepared dentin

The scientific literature discusses the significance of good dentin sealing and its importance for a successful vital pulp therapy[2], but until now there have been no studies that morphologically visualize, present and compare the border contact area between the laser prepared dentin and pulp-capping materials. The need for high tech equipment (high-energy dental laser, scanning electron microscope) on one hand and the heavy protocol for sample preparation, on the other, may be the cause for the lack of such studies. Proof of the close contact between the two surfaces or the presence of a gap between them can only be done at very large magnifications, which is possible using SEM. In SEM study of the adaptation of synthetic tricalcium silicate cement to conventionally prepared cavities with rotary tools, Dejou, Raskin, Pradelle reported very good marginal adhesion at x 1000 magnification between the two surfaces.[6] In our study, between Er:YAG laser-prepared dentin and tricalcium silicate cement, the observation with SEM shows no gap at even x 1500 magnification, which is a good sign of sealing and lack of micropermeability. In samples with pulp-capping materials of calcium hydroxide cement (Fig. 6) there is evidence of appearance of gaps even at magnification x 150, which may be caused by lack of microchemical adhesion of the calcium hydroxide preparations and dentin since their retention in the cavity is completely macroretentive.[1]

Although, according to the studies of several authors, MTA shows volume expansion to about 1.02% upon solidification[10] and better sealing properties than the tested calcium hydroxide cement, we found a gap in the latter at x 300 magnification (Fig. 7), which was not found in all areas of contact with the dentin.

The presented scanograms of the border area of the laser prepared dentin and the materials show that the smallest magnification to find a gap is in the calcium hydroxide cement (x 150) followed by the mineral trioxide aggregate (x 300), while in tricalcium silicate cement a gap was not even found at x 1500 magnification. The presence of a gap in the comparison of the border area in the studied samples is a sign of poor marginal adaptation. Larger gaps are evidenced at a smaller magnification. Therefore, the gap between calcium hydroxide cement and dentin is much greater than that of the MTA and accordingly the adaptation of the first one to the laser prepared dentin can be described as worse.

CONCLUSIONS

Er:YAG laser prepared dentin surface close to the pulp is with open dentinal tubules, no smear layer, which is a prerequisite for good adaptation of the studied pulp-capping materials to it. The small size of the particles, their close alignment and lack of cavities in the studied synthetic pulp-capping material create conditions for high adaptation and sealing to the prepared dentin surface.

In this study, a good level of adaptation to the Er:YAG laser prepared dentin surface was observed in samples with applied tricalcium silicate cement, followed by mineral-trioxide aggregate and calcium hydroxide cement.

This morphological study of Er:YAG prepared dentin close to the pulp and the structural features of modern pulp-capping materials as factors of quality marginal adaptation and comparison of observed border areas between them could form the basis of future investigations with a bigger sample size, which would allow testing the hypothesis with more precise quantitative statistical analyses.

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