ANÁLISE DOS RESULTADOS DE MICROSCOPIA ELETRÔNICA BASEADOS NA COMBINAÇÃO DO MÉTODO DE INFILTRAÇÃO COM DIFERENTES TECNOLOGIAS DE RESTAURAÇÃO E NA INVESTIGAÇÃO *IN VITRO* DO TRATAMENTO DA DESMINERALIZAÇÃO FOCAL DO ESMALTE EM ESTADO DEFEITUOSO**ANALYSIS OF ELECTRONIC MICROSCOPY RESULTS BASED ON COMBINING THE INFILTRATION METHOD WITH DIFFERENT RESTORATION TECHNOLOGIES AND *IN VITRO* INVESTIGATION OF ENAMEL FOCAL DEMINERALIZATION TREATMENT AT THE DEFECT STAGE****АНАЛИЗ РЕЗУЛЬТАТОВ ЭЛЕКТРОННОЙ МИКРОСКОПИИ ПРИ СОЧЕТАНИИ МЕТОДА ИНФИЛЬТРАЦИИ С РАЗЛИЧНЫМИ РЕСТАВРАЦИОННЫМИ ТЕХНОЛОГИЯМИ ПО РЕЗУЛЬТАТАМ ИССЛЕДОВАНИЯ *IN VITRO* ДЛЯ ЛЕЧЕНИЯ ОЧАГОВОЙ ДЕМИНЕРАЛИЗАЦИИ ЭМАЛИ В СТАДИИ ДЕФЕКТА**

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RESUMO

Atualmente, um grande número de métodos para prevenção e tratamento de cárie foi desenvolvido e implementado. No entanto, os problemas com a extensão e o desenvolvimento desse processo são redobrados a cada ano. Nesse contexto, é urgente o desenvolvimento de métodos que impeçam o desenvolvimento dessa patologia. Até o momento, foram obtidos resultados a longo prazo do tratamento de cárie no estágio de coloração por infiltração. Essa técnica permite interromper o desenvolvimento do processo carioso no estágio local impregnando tecidos patologicamente alterados com um medicamento à base de metacrilato, que estabiliza o desenvolvimento de cárie e cria condições para a restauração de áreas danificadas. Mas a questão do uso do Icon no tratamento da desmineralização focal do esmalte no estágio do defeito ainda é relevante. O objetivo desta pesquisa foi estudar a eficácia do tratamento de desmineralização focal do esmalte com base na aplicação do método de infiltração em combinação com várias tecnologias de restauração. A pesquisa foi realizada em 30 dentes selecionados, que foram removidos por várias razões e não haviam sido tratados previamente por via endodôntica. Após a limpeza mecânica da superfície dentária da placa na superfície vestibular de todas as amostras, foi criada a desmineralização artificial do esmalte no estágio do defeito. Um defeito pontual foi criado na superfície vestibular de todas as amostras dentro da zona de desmineralização usando boro esférico. A infiltração com Icon (DMG) foi realizada de acordo com as instruções do fabricante e os dentes foram divididos em 6 grupos de 5 amostras cada. O estudo revelou uma dependência entre a escolha do material de enchimento e o tamanho da cavidade preparada. Nos casos em que a cavidade é extensa, é preferível o uso de materiais compósitos com consistência e compômeros tradicionais. Se a cavidade for pequena, materiais fluidos (compósitos ou compômeros) podem ser usados para restaurar o defeito, porque esses materiais se espalham uniformemente pela cavidade dentária devido à sua consistência líquida. No modelo experimental, foi possível estabelecer a eficácia da combinação do método de infiltração com a preparação e restauração de Icon com materiais de preenchimento fotopolimerizáveis.

Palavras-chave: *desmineralização focal do esmalte, tratamento, método de infiltração, defeito, tecnologias de restauração.*

ABSTRACT

Currently, a large number of methods for caries prevention and treatment have been developed and implemented. However, the problems with the extension and development of this process are redoubling every year. Against this background, it is urgent to develop methods that prevent the development of this pathology.

To date, long-term results of treatment of caries in the staining stage by infiltration have been obtained. This technique makes it possible to stop the development of the carious process at the spot stage by impregnating pathologically altered tissues with a methacrylate-based drug, which stabilizes the development of caries and creates conditions for the restoration of damaged areas. But the question of using Icon for the treatment of focal demineralization of enamel in the defect stage is still relevant. The purpose of this research is to study the effectiveness of enamel focal demineralization treatment based on the application of the infiltration method in combination with various restoration technologies. The research was conducted on 30 selected teeth, which were removed for various reasons and had not been previously treated endodontically. After mechanical cleaning of the tooth surface from the plaque on the vestibular surface of all samples was created artificial demineralization of enamel in the defect stage. A point defect was created on the vestibular surface of all samples within the demineralization zone using spherical boron. Then the infiltration with Icon (DMG) was carried out according to the manufacturer's instructions, and the teeth were divided into 6 groups of 5 samples each. The study revealed a dependence between the choice of filling material and the size of the prepared cavity. In cases in which the cavity is extensive, using composite materials with traditional consistency and compomers is preferable. If the cavity is small, then fluid materials (composites or compomers) can be used to restore the defect because these materials spread evenly throughout the tooth cavity due to their liquid consistency. On the experimental model, it was possible to establish the effectiveness of the combination of the infiltration method with Icon preparation and restoration with light curing filling materials.

Keywords: enamel focal demineralization, treatment, infiltration method, defect, restoration technologies.

АННОТАЦИЯ

В настоящее время разработано и внедрено большое количество методик профилактики и лечения кариеса. Но проблема распространенности и развития данного процесса с каждым годом все сильнее усугубляется. На этом фоне имеет актуальность разработка методов препятствующих развитию данной патологии. На сегодняшний день получены долгосрочные результаты лечения кариеса в стадии пятна методом инфильтрации. Данная методика дает возможность остановить развитие кариозного процесса на стадии пятна за счет пропитывания патологически измененных тканей препаратом на основе метакрилата, что стабилизирует развитие кариеса и создает условия для восстановления поврежденных участков. Но вопрос применения препарата Icon для лечения очаговой деминерализации эмали в стадии дефекта по-прежнему остается актуальным. Целью настоящего исследования явилось изучение эффективности лечения очаговой деминерализации эмали путем применения метода инфильтрации в сочетании с различными реставрационными технологиями. Исследование проводилось на удаленных зубах, было отобрано 30 зубов, удаленных по различным показаниям, ранее эндодонтически не леченых. После механической очистки поверхности зубов от налета на вестибулярной поверхности всех образцов была создана искусственная деминерализация эмали в стадии дефекта. На вестибулярной поверхности всех образцов, в пределах зоны деминерализации, с помощью шаровидного бора, был создан точечный дефект. Затем провели инфильтрацию препаратом Icon (DMG) согласно инструкции производителя и разделили зубы на 6 групп по 5 образцов в каждой. Выявлена зависимость выбора пломбировочного материала от размера отпрепарированной полости. В том случае, когда полость обширная, более предпочтительнее использование композитных материалов традиционной консистенции и компомеров. Если полость небольшая, то возможно применение текучих материалов (композитов, компомеров) для восстановления дефекта, так как этот материал, благодаря своей жидкой консистенции равномерно распространяется по всей полости зуба. На экспериментальной модели удалось установить эффективность сочетания метода инфильтрации препаратом Icon с реставрацией пломбировочными материалами светового отверждения.

Ключевые слова: *очаговая деминерализация эмали, лечение, метод инфильтрации, дефект, реставрационные технологии.*

1. INTRODUCTION

Currently, a large number of methods for caries prevention and treatment have been developed and implemented [1-3]. However, the problems associated with the extension and development of this process are redoubling every

year. Therefore, methods are needed to prevent the development of this pathology. For many years, medicine has been focused on stopping the hard tooth tissue destruction process. Many invasive and non-invasive methods are used for this purpose [3-8]. Attempts have been made both to preserve tissues with the help of

remineralizing therapy and to excise the affected areas by means of preparation. These methods are widely used worldwide, but the development process is still underway [8-10].

To date, long-term results of caries treatment in the stain stage have been obtained using the infiltration method. This technique makes it possible to stop the development of the carious process at the stain stage due to impregnation of pathologically changed tissues with a drug based on methacrylate, which stabilizes the development of caries and creates conditions for the restoration of damaged areas. However, the question of whether the "Icon" drug can be used for the treatment of enamel focal demineralization in the defect stage remains relevant. Currently, laboratory [11-19] and clinical [20-25] investigations are being actively conducted worldwide to confirm the potential for the use of the infiltration method for the treatment of caries in the stain stage. In fact, the immediate and long-term results of enamel focal demineralization treatment in the defect stage can be improved by applying the infiltration method in combination with various restoration technologies [25-28]

The purpose of this research is to study the effectiveness of enamel focal demineralization treatment based on the application of the infiltration method in combination with various restoration technologies.

2. MATERIALS AND METHODS

The research was conducted on 30 selected teeth, which were removed for various reasons and had not been previously treated endodontically. After mechanical cleaning of dental plaque from the tooth surface (Figure. 1), artificial demineralization of the enamel in the defect stage was created on the vestibular surface of all patterns.

For this purpose, we isolated the entire tooth surface except for the light-cured composite material under investigation. This procedure was necessary to prevent direct penetration of the substance used. The teeth were immersed in hydrochloric acid (HCL) with a regular decrease in the percentage from large to small for 4 weeks (Figure. 2).

After a preset time, the patterns were removed, rinsed with water, and cleaned of filling material. A point defect was created on the vestibular surface of all patterns within the

demineralization zone using a spherical dental cutter (Figure. 3).

Then, the patterns were infiltrated with "Icon" (DMG) according to the manufacturer's instructions, and the teeth were divided into 6 groups of 5 patterns each. The first group was the control group because the effectiveness of the infiltration method needed to be evaluated within the limits of the solution of continuity of the enamel. The artificially created enamel focal demineralization in the defect stage was treated with Icon (DMG) without subsequent filling (Figure. 4).

In the second group, the defect was restored with EcuSphere Shine (DMG) using light-cured composite material in combination with the 5th generation adhesive system. In the third group, the defect was restored with the same EcuSphere Shine composite (DMG) with the 6th generation adhesive system (Figure. 5).

Thus, we not only evaluated the efficiency of applying the light-cured composite in combination with the infiltration method but also compared the adhesive force of the filling material to the tooth tissues through the use of two systems of different generations that are widely used globally. In the fourth group, the restoration was performed using Ketac Molar (3M ESPE) glass-ionomer cement. The cavities of the patterns of the fifth group were restored with the fluid composite EcuSphere Flow (DMG). In the last sixth group, the cavities were restored with the fluid compomer PrimaFlow (DMG) (Figure. 6).

Figure 1. Appearance of the extracted teeth included in the study (a) and sample preparation (b)

Figure 2. Sample preparation (a) and immersion in hydrochloric acid (b)

Figure 3. Purification of samples (a) and artificial creation of the defect with a bore (b)

Figure 4. Infiltration of all teeth with Icon according to the instructions and preparation of the control group

Figure 5. Restoration with the EcuSphere Shine composite in combination with the 5th generation adhesive system (a) and EcuSphere Shine composite in combination with the 6th generation adhesive system (b)

After the restoration of the defects, all teeth were prepared in the experimental cell pathology laboratory by immersing the patterns in nitric acid (7.5% HNO_3), followed by daily replacement of the solution with a smaller percentage ratio for 20 days. Then, the teeth were kept in alcohol ($\text{C}_2\text{H}_5\text{OH}$) of different percentages for 7 days with a daily change from 70% to 99%, followed by one hour in chloroform. Finally, the teeth were filled with paraffin for 24 hours for the subsequent manufacture of slices with a sledge microtome (HM 450).

After preparation of thin slices, microscopic examination of the patterns was carried out using scanning electron microscopy (SEM). The enamel surface and deep layers of the tooth hard tissues were evaluated using a

low-vacuum scanning electron microscope (model JEOL JSM-6380LV, Japan). The tooth microrelief was examined in the secondary electron emission mode with an accelerating voltage of 10–15 kV and a magnification ranging from 400 to 1300 times. A conductive coating — carbon or gold — was applied to chips of the extracted teeth to prevent electronic charging and thermal damage. The deposition of carbon or gold on a tooth with a thickness of 10–20 nm was carried out by thermal evaporation in a vacuum at 1.3×10^{-3} Pa. This protective coating has a low X-ray absorption coefficient and sufficient electrical conductivity, which is the basis for its use and significantly improves the image quality. The prepared object (tooth) was fastened to the holder and placed in the chamber of the scanning electron microscope.

3. RESULTS AND DISCUSSION:

The microscopic examination revealed significant differences among the patterns. Effective application of the infiltration method in combination with the light-cured composite EcuSphere Shine and the 5th and 6th generation adhesive system was achieved (Figure. 7).

The boundaries of the filling material transition in the tooth tissue are solid and have no macro and micro damages. During the examination of the tooth slices in the enamel focal demineralization zone, the presence of infiltration in the damaged area was clearly visible as a shinier and lighter area. In photomicrographs, these areas appear to be covered with frost in contrast to the more matte, healthy enamel. A microscopic examination of the control group patterns showed uniform impregnation of the tooth tissues with infiltration throughout the entire depth of the defect. The picture shows the presence of infiltration on the cavity paries of the tooth (Figure. 8).

Figure 6. Restoration with Ketac Molar glass ionomer cement, EcuSphere Flow fluid composite, and PrimaFlow fluid compomer

Figure 7. Microscopic examination of light-cured composite material-sealed EcuSphere Shine samples in combination with the 5th (a) and 6th (b) generation adhesive systems

Figure 8. Microscopic examination of the control group

Figure 9. Microscopic examination of samples recovered with the glass ionomer cement Ketac Molar

Visually, the presence of a filling material in the cavity of the tooth was distinctly noticeable on the slices and during microscopic examination of the patterns restored by the glass-ionomer cement Ketac Molar (Figure. 9). A partial detachment of the filling material from the cavity paries was observed, which indicated a lack of adhesion to the tooth tissues or its complete absence.

A detailed examination of the recovered defects using the fluid compomer PrimaFlow and the composite EcuSphere Flow determined the penetration of the infiltration into the tooth tissues along the entire length of the defect (Figure. 10).

Figure 10. Microscopic examination of the samples sealed with the fluid compomer PrimaFlow (a) and the fluid composite EcuSphere Flow (b)

In areas impregnated with infiltration, the penetration of the drug into the initial dentinal layers was confirmed. This finding is of very important clinical relevance since the probability of recurrence of the carious process becomes higher in cases of incomplete infiltration of the entire volume of the damaged tissues. Although in the in vitro study, we investigated drug infiltration on dry patterns, dentin fluid is present in the vital dentin and can become an insurmountable obstacle for the penetration of hydrophobic infiltration. During the course of the research, we obtained satisfactory results for the application of this method in combination with the light-cured composite EcuSphere Shine and the 5th or 6th generation adhesive system.

4. CONCLUSIONS:

The experimental model allowed to establish the effectiveness of the combination of the infiltration method with the “Icon” drug and the restoration of light-cured filling materials, such as the composite EcuSphere Flow, EcuSphere Shine, and compomer PrimaFlow. The obtained electron microscopic examination clearly show close contact of the filling material with the tooth tissues, which indicates high adhesion between them. The use of a combination of methods on the enamel surface did not result in damage, which confirmed the safety of using the presented treatment regimen in practice.

5. ACKNOWLEDGMENTS:

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6. REFERENCES:

1. Paris S., Meyer-Lueckel H., Kielbassa A.M. Resin infiltration of natural caries lesions. *J Dent Res.* **2007**; 86: 7: 662-666.
2. Paris S., Chatzidakis A.J., Meyer-Lueckel H. Influence of application time on caries infiltration in primary teeth. *Int J Paediat Dentist.* **2009**; 19(S1): 9.
3. Mueller J., Meyer-Lueckel H., Paris S. et al. Inhibition of lesion progression by the penetration of resins in vitro: influence of the application procedure. *Oper Dent.* **2006**; 313: 338-45.
4. Sevbitov A.V., Dorofeev A.E., Davidyants A.A., Ershov K.A., Timoshin A.V. Assessment of pain perception of elderly patients with different levels of dentophobia during surgical dental appointment. *Asian Journal of Pharmaceutics.* **2018**; 12 (S3): 1012-1016.
5. Sevbitov A.V., Brago A.S., Enina Yu.I., Dorofeev A.E., Mironov S.N. Experience in the application of hybrid ceramic restorations in the cervical region. *Asian Journal of Pharmaceutics.* **2018**; 12(S3): 1106-1109.
6. Yumashev A. V., Utyuzh A. S., Mikhailova M.V., Samusenkov V.O., Volchkova I.R. Selecting clinical and laboratory methods of manufacture of orthopaedic titanium alloy structures using a biopotentiometer. *Current Science (India).* **2018**; 114(4): 891-896.
7. Sevbitov A.V., Enina Yu.I., Derevyanchenko S.P., Dorofeev A.E. Comparative evaluation of effectiveness of restorations in cervical teeth region by direct and indirect method. *International Journal of Civil Engineering and Technology.* **2019**; 10(3): 3099-3105.
8. Enina Yu.I., Sevbitov A.V., Dorofeev A.E., Pustokhina I.G. Experimental substantiation of the choice of the restoration method in the cervical area of teeth with abfraction defects. *International Journal of Mechanical Engineering and Technology.* **2019**; 10(5): 41-47.
9. Álvaro Furtado, Gisela-Crippa Furtado, Ossam El Haje, Henrique-Damian Rosário, Ademir Franco, Irina Makeeva, Luiz-Renato Paranhos. Soft-tissue cone-beam computed tomography (ST-CBCT) technique for the analysis of skeletal, dental and periodontal effects of orthopedic rapid maxillary expansion. *J Clin Exp Dent.* **2018**; 10(9): e883-90.
10. Kuznetsova M.Yu., Nevдах A.S., Platonova V.V., Sevbitov A.V., Dorofeev A.E. Evaluation of effectiveness of a preparation on the basis of phytoecdysteroids for treatment of traumatic injuries of oral mucosa in orthodontic patients. *International Journal of Green Pharmacy.* **2018**; 12(S1): 297-300.
11. Doméjean S, Ducamp R, Léger S, Holmgren C. Resin infiltration of non-cavitated caries lesions: a systematic review. *Med Princ Pract.* **2015**; 24(3): 216-21.
12. Meyer-Lueckel H, Balbach A, Schikowsky C, Bitter K, Paris S. Pragmatic RCT on the Efficacy of Proximal Caries Infiltration. *J Dent Res.* **2016**; 95(5): 531-6.
13. Kielbassa AM, Ulrich I, Werth VD, Schüller C, Frank W, Schmidl R. External and internal resin infiltration of natural proximal subsurface caries lesions: A valuable enhancement of the internal tunnel restoration. *Quintessence Int.* **2017**; 48(5): 357-368.
14. Liang Y, Deng Z, Dai X, Tian J, Zhao W. Micro-invasive interventions for managing non-cavitated proximal caries of different depths: a systematic review and meta-analysis. *Clin Oral Investig.* **2018**; 22(8): 2675-2684.
15. Altarabulsi MB, Alkilzy M, Petrou MA, Splieth C. Clinical safety, quality and effect of resin infiltration for proximal caries. *Eur J Paediatr Dent.* **2014**; 15(1): 39-44.
16. Wright JT, Tampi MP, Graham L, Estrich C, Crall JJ, Fontana M, Gillette EJ, Nový BB, Dhar V, Donly K, et al. Sealants for preventing and arresting pit-and-fissure occlusal caries in primary and permanent molars: A systematic review of randomized controlled trials-a report of the American Dental Association and the American Academy of Pediatric Dentistry. *J Am Dent Assoc.* **2016**; 147(8): 631-645.
17. Ammari MM, Soviero VM, da Silva Fidalgo TK, Lenzi M, Ferreira DM, Mattos CT, de Souza IP, Maia LC. Is non-cavitated proximal lesion sealing an effective method for caries control in primary and permanent teeth? A systematic review and meta-analysis. *J Dent.* **2014**; 42(10): 1217-27.
18. Meyer-Lueckel H, Bitter K, Paris S. Randomized controlled clinical trial on proximal caries infiltration: three-year follow-up. *Caries Res.* **2012**; 46(6): 544-8.
19. Paris S, Hopfenmuller W, Meyer-Lueckel H. Resin infiltration of caries lesions: an efficacy randomized trial. *J Dent Res.* **2010**; 89(8): 823-6.

20. Ammari MM, Jorge RC, Souza IPR, Soviero VM. Efficacy of resin infiltration of proximal caries in primary molars: 1-year follow-up of a split-mouth randomized controlled clinical trial. *Clin Oral Investig*. **2018**. 22(3):1355-1362.
21. Bille J, Carstens K. Approximal caries progression in 13- to 15-year-old Danish children. *Acta Odontol Scand*. **1989**. 47(6): 347-54.
22. Phark JH, Duarte S Jr, Meyer-Lueckel H, Paris S. Caries infiltration with resins: a novel treatment option for interproximal caries. *Compend Contin Educ Dent*. **2009**; 30(3):13-7.
23. Lausch J, Askar H, Paris S, Meyer-Lueckel H. Micro-filled resin infiltration of fissure caries lesions in vitro. *J Dent*. **2017**; 57: 73-76.
24. Paris S, Lausch J, Selje T, Dörfer CE, Meyer-Lueckel H. Comparison of sealant and infiltrant penetration into pit and fissure caries lesions in vitro. *J Dent*. **2014**; 42(4): 432-8.
25. Paris S, Lausch J, Selje T, Dörfer CE, Meyer-Lueckel H. Comparison of sealant and infiltrant penetration into pit and fissure caries lesions in vitro. *J Dent*. **2014**; 42(4): 432-8.
26. Paris S, Lausch J, Selje T, Dörfer CE, Meyer-Lueckel H. Comparison of sealant and infiltrant penetration into pit and fissure caries lesions in vitro. *J Dent*. **2014**; 42(4):432-8. Epub 2014 Jan 18.
27. Manoharan V, Arun Kumar S, Arumugam SB, Anand V, Krishnamoorthy S, Methippara JJ. Is Resin Infiltration a Microinvasive Approach to White Lesions of Calcified Tooth Structures?: A Systemic Review. *Int J Clin Pediatr Dent*. **2019**; 12(1): 53-58.
28. Arslan S, Kaplan MH. The Effect of Resin Infiltration on the Progression of Proximal Caries Lesions: A Randomized Clinical Trial. *Med Princ Pract*. **2019**; 3.