DOI: https://doi.org/10.2298/SARH221228095M
UDC: 615.46:616.314-7; 616.31-002-085

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Effect of different demineralizing solutions and different exposing times on artificial initial caries lesion formation – an *in vitro* study

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Introduction/Objective Artificial enamel caries lesions are commonly created to simulate *in vivo* caries development and to examine the effect of non-invasive and microinvasive approaches in treatment of initial caries lesions.
The objective of the present study was to compare three different demineralizing solutions and exposing times in terms of the formation of artificial white spot lesions and to evaluate their demineralization effect through scanning electron microscopy observations.
Methods Twenty intact human premolars, extracted for orthodontic reasons, were thoroughly cleaned, stored in 0.1% thymol solution at room temperature and cut at the cementoenamel junction before demineralizing process. The specimens were randomly divided into three experimental groups, according to the used demineralization agent and the time of exposure: Group I (acetic acid; pH = 4.4; 96 hours); Group II (lactic acid; pH = 4.5; 120 hours); Group III (Lactic acid; pH = 4.3; 504 hours) and one control group (saline). After demineralisation, macroscopic appearance was checked and all specimens were observed under scanning electron microscope to evaluate the enamel characteristics and caries lesion depths.
Results In Group I and II enamel subsurface porosity with dissolution of enamel crystals is detected and the mean depths of white spot lesions were 48.55 μm (SD = 1.11) and 43.23 μm (SD = 6.74), respectively. In Group III structural integrity of enamel surface was not preserved.
Conclusion Demineralizing solutions used in experimental groups I and II resulted in artificial initial caries lesions with satisfactory characteristics and similar appearance on scanning electron microscopy. The outcome of demineralizing process which lasted 504 hours were cavitated enamel lesions.
Keywords: enamel; demineralization; white spot lesions; scanning electron microscopy

INTRODUCTION

Dental caries, one of the most common oral diseases worldwide, represents a major public health concern, as confirmed by the latest report on the Global Burden of Disease [1]. It is estimated that 2.5 billion people suffer from caries in permanent teeth, and more than 530 million children suffer from untreated caries in deciduous dentition [1, 2, 3]. Initial caries lesions, also known as white spot lesions (WSLs), are the earliest clinical signs of enamel caries. Subsurface enamel demineralization increases porosity and changes the optical characteristics of enamel. Consequently, the initial caries lesion appears as an opaque white spot, visibly different from the surrounding sound enamel due to differences in the refractive indexes (RI) [3, 4]. In the last decades, the prevalence of WSLs has increased, and some authors consider this increase a potential side effect of orthodontic therapy with fixed orthodontic appliances [5, 6]. If these lesions are not detected in the initial phase and are not properly managed in this reversible stage, they might progress into irreversible cavitated lesions. Additionally, WSLs might compromise the aesthetics of the anterior teeth [7, 8]. Contemporary dentistry established several non-invasive approaches in treatment of initial caries lesions to arrest caries lesions at an early stage and to improve its remineralization [9, 10, 11]. Fluoride-based and bioactive remineralizing agents such as casein phosphopeptide – amorphous calcium phosphate and calcium silicate-based materials are widely used [12–15]. As a modern, microinvasive approach, infiltration of the WSLs with a low viscosity composite resin, commercially available as ICON (DMG MORI, Hamburg, Germany), has been developed [16].

Accessible clinical experience points towards successful masking of whitish enamel discoloration in WSLs when treated with infiltrative resin [17]. However, there is still need for further examination of its impact on the other features and its effectiveness (such as surface roughness and enamel hardness, shear bond strength, penetration depth) [5]. *In vitro* studies

Received • Примљено: December 28, 2022
Revised • Ревизија: September 28, 2023
Accepted • Прихваћено: October 17, 2023
Online first: November 7, 2023

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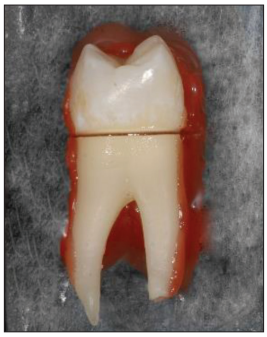

Figure 1. Before immersion into solutions, the roots of the teeth were separated at the cementoenamel junction with a low-speed diamond saw under water cooling; to perform the section, each tooth was fixed to glass plate using a sticky dental wax

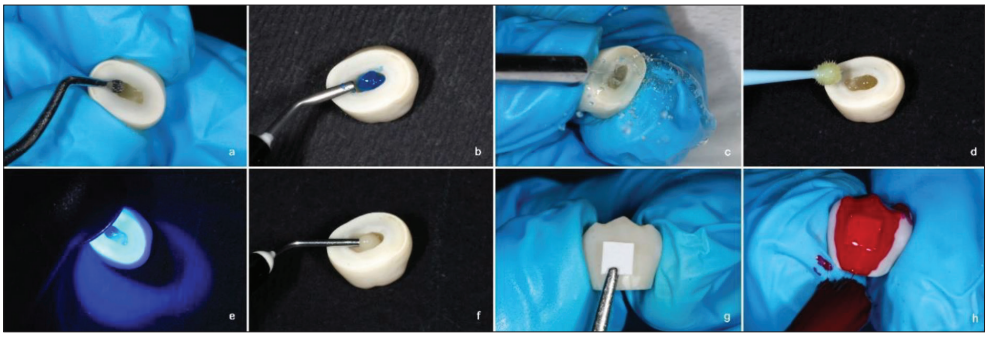

Figure 2. Preparation of the premolar crowns for the demineralizing process: a – pulp tissue was discharged; b – dentin was etched with 37% orthophosphoric acid; c – after 15 seconds, it was thoroughly rinsed and dried; d – an adhesive was applied on the dentin surface; e – polymerisation was performed; f – pulp chambers were obturated with flowable composite; g – the middle area of buccal and oral surface of all crowns was isolated using adhesive paper, sized 4 × 4 mm; h – the crowns were coated with an acid-resistant nail varnish

with resin infiltration of artificial initial caries lesions are a potential method to achieve this goal. There are numerous demineralization protocols for artificial WSLs which differ in the demineralization agents, the pH of the solutions/gels and the required time of the exposure to the demineralizing agent. They can contain lactic acid, acetic acid, methyl diphosphonate, acidified hydroxietilcellulose system, and in some of them fluoride might be added. The recent systematic review and meta-analysis reported that a duration of demineralization process, which simulated the formation of WSLs, varied from several to 1200 hours. In addition, pH of demineralizing solutions varied between four and five [5].

Developing adequate artificial initial caries lesion is needed to investigate the characteristics of different remineralizing protocols. Therefore, the objective of the present study was to compare the effect of three different demineralizing solutions and exposure times on the formation of artificial WSLs and evaluate their demineralizing effects through scanning electron microscopy (SEM).

METHODS

The present study was conducted in the Clinic for Pediatric and Preventive Dentistry and Department for Biochemistry, School of Dental Medicine, University of Belgrade and SEM evaluation was performed in the Department for Physics and Mathematics, Faculty of Agriculture, University of Belgrade.

The present work was approved by competent ethics committee (Ethics Committee of School of Dental Medicine, University of Belgrade, no 36/41) and conforms to the legal standards.

Twenty caries-free permanent maxillary or mandibular, first or second human premolars extracted upon orthodontic indication were used for this study. The teeth were visually examined using LED light to ensure that there are no enamel surface defects or microcracks.

Specimen preparation

The selected teeth were cleaned with periodontal curettes to remove any remaining soft tissue and with fluoride free prophylactic paste (Cleanic, Kerr, Orange, CA, USA), applied by a brush mounted in a low-speed hand piece, under water cooling. Subsequently, the teeth were washed in distilled water and stored in 0.1% thymol solution at room temperature until further use for no longer than 1 month.

Before demineralization process, roots were removed at the cementoenamel junction with a low-speed diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA) under water cooling (Figure 1). Pulp tissue was removed, and pulp chambers of the premolar crowns were obturated with flowable composite (Flow-It ALC, Pentron). The premolar crowns were randomly divided into three experimental groups and one control group (each group containing five teeth).

All crowns in experimental groups were coated with an acid-resistant nail varnish (Essence, Cosnova GmbH, Zulcbah, Germany), except for a middle area of buccal and oral surfaces which were isolated using adhesive paper, sized 4 × 4 mm (Figure 2). The adhesive papers were removed after the nail varnish had dried completely at room temperature. Each crown of the tooth gave two specimens (buccal and oral half), stored in saline solution at room temperature until SEM evaluation (Figure 3).

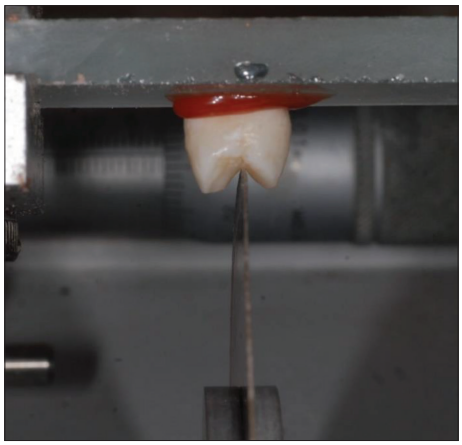

Figure 3. After demineralization, all premolar crowns were sectioned into two halves (buccal and oral) with a low-speed diamond saw under water cooling

Artificial initial caries lesions

The specimens were randomly divided into three experimental groups, according to the used demineralization agent, and one control group (n = 10):

Group I – demineralizing solution I – acetic acid based; pH = 4.4; for 96 hours [10, 18, 19];

Group II – demineralizing solution II – lactic acid based; pH = 4.5; for 120 hours [20];

Group III – demineralizing solution III – lactic acid based; pH = 4.3; 504 hours [21];

Group IV – control group (stored in saline solution at room temperature until SEM evaluation).

The composition of each demineralizing solution, its pH and exposure times are reported in Table 1.

Table 1. Characteristics of solutions used for demineralization

Features	Group I	Group II	Group III
Composition	2.2 mmol/L calcium chloride	12 mmol/L calcium chloride	18 mmol/L calcium chloride
	2.2 mmol/L monopotassium phosphate	10 mmol/L monopotassium phosphate	7.8 mmol/L monopotassium phosphate
	0.05 mmol/L acetic acid	50 mmol/L lactic acid	100 mmol/L lactic acid
	/	100 mmol/L sodium chloride	3 mmol/L sodium azide
pH	4.4	4.5	4.3
Exposure time	4 days / 96 hours	5 days / 120 hours	21 days / 504 hours

Each specimen was immersed in 20 ml of appropriate demineralizing solution in a sterile plastic container. The pH of demineralizing solutions was checked every day with a probe to maintain the initially defined values (Mi 150, pH/Temperature Bench Meter, Martini instruments; Figure 4). If needed, it was adjusted with potassium hydroxide. In Group I and Group III, specimens were stored at room temperature [10, 18, 19] and in Group II they were stored in 37°C incubator (Binder Gmbh Incubator, Tuttlingen, Germany) [21].

Subsequent to the artificial demineralization, nail varnish was carefully removed with oil-free acetone from the specimens, and they were thoroughly rinsed with distilled water. All specimens were visually inspected and macroscopic appearance of enamel lesion was evaluated.

Scanning electron microscopy evaluation of artificial white spot lesions

After completed demineralization procedure, all premolar crowns were sectioned into two halves (buccal and oral) with a low-speed diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA) under water cooling (Figure 3). In order to perform the section, each crown was fixed to glass plate using sticky dental wax (Galeo, Galenika, Belgrade, Serbia).

From obtained buccal and oral premolar halves, sections were made through the artificially created WSLs in experimental groups and from sound enamel in the control group. Each premolar half was sectioned in vestibulo-oral direction, into 1-mm-thick slices with a low-speed diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA) under water cooling. Sections were made through the middle area and two slices were obtained from each premolar half. All slices were polished with silicon carbide paper (600, 800, 1000, 1200 grit paper) under water cooling, rinsed with distilled water, dried and etched with phosphoric acid gel (Gel Etchant, Kerr) for 30 seconds. After abundant rinsing and drying, the specimens were dehydrated in an ascending ethanol series (25%, 50%, 70%, 90%, 95%). Drying was performed with oil free air spray. Specimens were mounted on metallic stubs, gold-sputtered (Bal-Tec SCD 005 Sputter Coater) and observed under a Scanning Electron Microscope (JEOL-JSM-6460LV, Tokyo, Japan) under different magnifications (40, 100, 700, 1000, 1500, 4000) to evaluate the characteristics of initial caries lesions – demineralization depth and pattern.

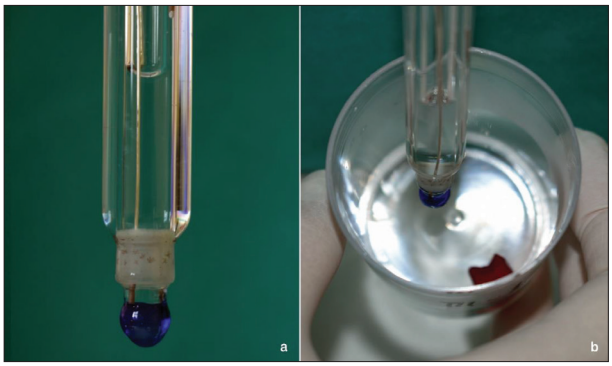

Figure 4. The pH of the demineralizing solutions was evaluated daily to maintain the initially defined values

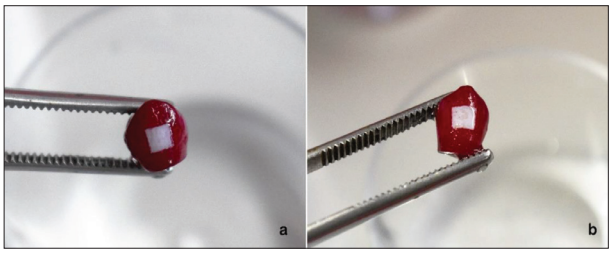

Figure 5. The areas exposed to the demineralizing solution appeared whitish-opaque on daily light at the end of the process

RESULTS

In the first and the second experimental group, areas exposed to the demineralizing solution appeared whitish-opaque on daily light at the end of demineralization process. In both groups, visual inspection of the specimens did not reveal any localized breakdown of the enamel (Figure 5). On the other hand, in the third experimental group the structural integrity of the enamel was not maintained.

Sound enamel in the control group (Group IV) on cross-sectional SEM micrograph showed physiological enamel rod appearance (Figure 6). The surface of sound enamel might appear with occasional depressions,

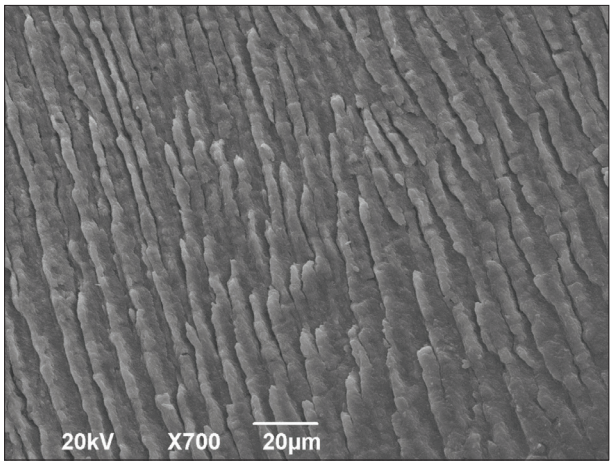

Figure 6. Scanning electron microscopy evaluation showed physiological enamel rod appearance in the control group

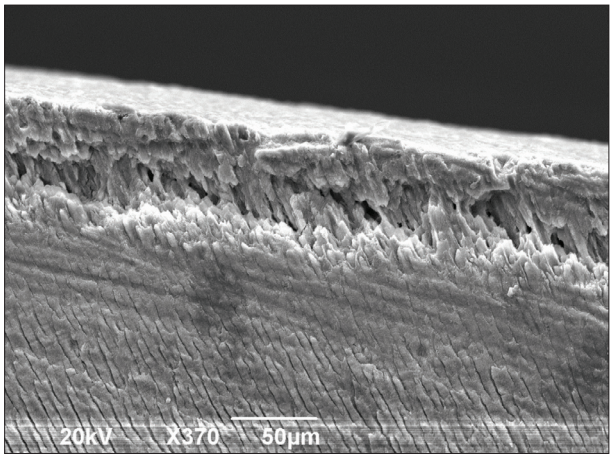

Figure 7. In the experimental group I enamel subsurface porosity with dissolution of enamel crystals was detected

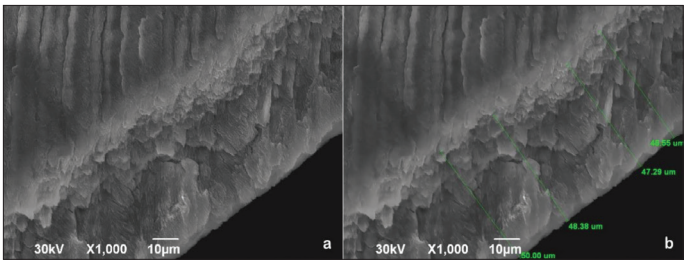

Figure 8. The depth of formed initial caries lesions was evaluated through scanning electron microscopy observations

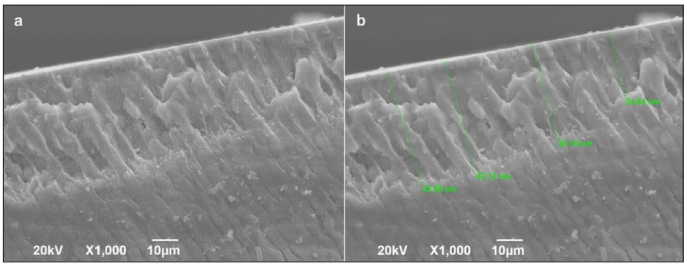

Figure 9. In the experimental group II caries-like subsurface lesions were obtained; they had similar enamel appearance as in the first experimental group

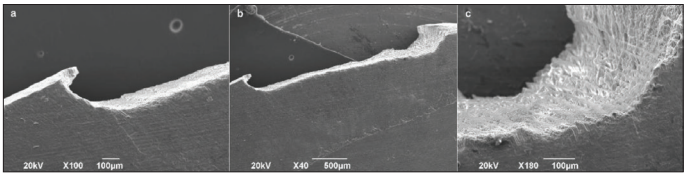

Figure 10. In the experimental group III structural integrity of enamel surface was not preserved; surface erosion with prismatic pattern of enamel surface dissolution can be seen

representing prism ends termination at the surface. These structures were usually located at the base of perikymata. The present SEM evaluation showed relatively smooth enamel without these morphological variations.

In Group I enamel subsurface porosity with dissolution of enamel crystals was detected (Figure 7). It indicated formation of caries-like subsurface lesion. The mean depth of lesion was 48.55 µm (SD = 1.11) (Figure 8). In Group II, the mean depth of the lesion was 43.23 µm (SD = 6.74). In Figure 9 similar enamel appearance as in the previous group was found. The appearance of these caries-like lesions might be similar to the surface of sound enamel except for surface depressions. Furthermore, in both groups (Group I and Group II), small number of specimens on microphotographs showed that some surface enamel areas of the WSLs were eroded while surrounded enamel surface was intact.

In the Group III structural integrity of enamel surface was not preserved. Figure 10 presents surface erosion with prismatic pattern of enamel surface dissolution. Also, this lactic acid based demineralizing solution resulted in loss of enamel prism cores with present porosities and preserved enamel prism peripheries.

DISCUSSION

Artificial enamel caries lesions are commonly created to simulate *in vivo* caries development. Enamel specimens are usually exposed to demineralizing solutions and gels composed of acetic or lactic acid, undersaturated regarding hydroxyapatite, to simulate the plaque fluid conditions and allow the formation of initial enamel lesions [22, 23].

Freitas et al. [24] used methylcellulose gel. Its high viscosity could compromise the diffusion rate of the acid, resulting in a reduced demineralization and fewer pores.

Issa et al. [25] concluded that presence of fluoride in the demineralizing process, especially when gel was used, significantly reduced the lesion depth and mineral loss in permanent teeth. In addition, typical subsurface lesions could be formed in the absence of fluoride. So, in the present study, demineralizing solutions without fluoride were used.

Considering previously mentioned solutions and availability of solutions' components, which is an important contributing factor in selection of demineralization agents [26], acetic and lactic acid-based protocols were selected for the present study. pH values of the solutions used in the study are also in accordance with a recently published systematic review and meta-analysis suggesting that the pH of demineralizing solutions should vary between four and five [5].

When it comes to duration of demineralizing process, less consistent findings are present in literature. As previously mentioned, this period varies from few to 1.200 hours. In the present study, solution used in the first experimental group lasted 96 hours as reported in several *in vitro* experiments [10, 18, 27]. Also, Behrouzi et al. [19] used solution for the same time, but it was acidified hydroxietilcellulose system. Group II in the present study had longer exposure time (120 hours).

On the other hand, in the third experimental group, it was significantly longer (21 day / 504 hours), as this protocol was suggested by Prajapati et al. [21]. These authors reported the mean depth of formed lesions of 341 μm. In the present pilot study, initial caries lesion with subsurface demineralization were not formed by this demineralizing solution. On the contrary, it resulted in localized enamel breakdowns with areas of prismatic pattern of dissolution.

The mean depth of artificial initial caries lesions obtained in the present study was 48.55 μm in the first group and 43.23 μm in the second group, which is not as deep as reported in some *in vitro* studies [10, 21], although there are similar findings in literature. Magalhães et al. [22] reported mean depth of subsurface enamel lesions between 35 and 52 μm, except for methyl diphosphonate lesions (86 μm).

In the present experiment, WSLs were visually detectable on the wet specimens and some authors stated that WSL was visible on a wet tooth surface when it had penetrated deeper into the enamel surface [28]. They could be described as whitish areas with rough enamel surface enamel.

Artificial WSLs induced in this experiment (Group I and Group II) demonstrated intact surface layer with subsurface prismatic demineralization and an enlargement of the prism sheaths. In some specimens, two types of enamel surface involvement were described: areas of erosion and other areas apparently intact, when compared to the sound enamel surrounding them. These two distinct sites of the WSL also presented diverse levels of enamel dissolution. Eroded areas showed irregular patterns of surface destruction, and, by some authors, they indicated an advanced stage of the dissolution of the enamel promoted by the carious process [29].

CONCLUSION

Based on the results of this study it could be concluded that different demineralizing solutions produce artificial initial caries lesions of variable features and depths. Demineralizing solution based on acetic acid of pH value 4.4- and 96-hour-long exposure time and solution based on lactic acid of pH value 4.5 and 120 hours exposure time provide artificial WSLs with satisfactory characteristics. The outcome of demineralizing process which lasted 21 days were cavitated enamel lesions.

Conflict of interest: None declared.

REFERENCES

1. Kassebaum NJ, Smith AGC, Bernabé E, Fleming TD, Reynolds AE, Vos T, et al. Global, Regional, and National Prevalence, Incidence, and Disability-Adjusted Life Years for Oral Conditions for 195 Countries, 1990-2015: A Systematic Analysis for the Global Burden of Diseases, Injuries, and Risk Factors. J Dent Res. 2017;96(4):380–7. [DOI: 10.1177/0022034517693566] [PMID: 28792274]
2. Nassar HM, Lippert F. Artificial Caries Lesion Characteristics after Secondary Demineralization with Theobromine-Containing Protocol. Molecules. 2021;26(2):300. [DOI: 10.3390/molecules26020300] [PMID: 33435546]
3. Theodory TG, Kolker JL, Vargas MA, Maia RR, Dawson DV. Masking and Penetration Ability of Various Sealants and ICON in Artificial Initial Caries Lesions In Vitro. J Adhes Dent. 2019;21(3):265–72. [DOI: 10.3290/j.jad.a42520] [PMID: 31093619]
4. Bandekar S, Patil S, Dudulwar D, Moogi PP, Ghosh S, Kshirsagar S. Remineralization potential of fluoride, amorphous calcium phosphate-casein phosphopeptide, and combination of hydroxylapatite and fluoride on enamel lesions: An *in vitro* comparative evaluation. J Conserv Dent. 2019;22(3):305–9. [DOI: 10.4103/JCD.JCD_13_19] [PMID: 31367119]
5. Soveral M, Machado V, Botelho J, Mendes JJ, Manso C. Effect of Resin Infiltration on Enamel: A Systematic Review and Meta-Analysis. J Funct Biomater. 2021;12(3):48. [DOI: 10.3390/jfb12030048] [PMID: 34449679]
6. Sundararaj D, Venkatachalapathy S, Tandon A, Pereira A. Critical evaluation of incidence and prevalence of white spot lesions during fixed orthodontic appliance treatment: A meta-analysis. J Int Soc Prev Community Dent. 2015;5(6):433–9 [DOI: 10.4103/2231-0762.167719] [PMID: 26759794]
7. Araújo GS, Naufel FS, Alonso RC, Lima DA, Puppin-Rontani RM. Influence of Staining Solution and Bleaching on Color Stability of Resin Used for Caries Infiltration. Oper Dent. 2015;40(6):E250–6. [DOI: 10.2341/14-290-L] [PMID: 26266656]
8. Perdigão J. Resin infiltration of enamel white spot lesions: An ultramorphological analysis. J Esthet Restor Dent. 2020;32(3):317–24. [DOI: 10.1111/jerd.12550] [PMID: 31742888]
9. Rana N, Singh N, Shaila, Thomas AM, Jairath R. A comparative evaluation of penetration depth and surface microhardness of Resin Infiltrant, CPP-ACPF and Novamin on enamel demineralization after banding: an *in vitro* study. Biomater Investig Dent. 2021;8(1):64–71. [DOI: 10.1080/26415275.2021.1919119] [PMID: 34179820]
10. Mandava J, Reddy YS, Kantheti S, Chalasani U, Ravi RC, Borugadda R, et al. Microhardness and Penetration of Artificial White Spot Lesions Treated with Resin or Colloidal Silica Infiltration. J Clin Diagn Res. 2017;11(4):ZC142–ZC6. [DOI: 10.7860/JCDR/2017/25512.9706] [PMID: 28571282]
11. De Almeida CM, da Rosa WLO, Meereis CTW, de Almeida SM, Ribeiro JS, da Silva AF, et al. Efficacy of antimicrobial agents incorporated in orthodontic bonding systems: a systematic review and meta-analysis. J Orthod. 2018;45(2):79–93. [DOI: 10.1080/14653125.2018.1443872] [PMID: 29504867]
12. Oliveira PRA, Barboza CM, Barreto LSDC, Tostes MA. Effect of CPP-ACP on remineralization of artificial caries-like lesion: an in situ study. Braz Oral Res. 2020;34:e061. [DOI: 10.1590/1807-3107bor-2020.vol34.0061] [PMID: 32609230]
13. Hamdi K, Hamama HH, Motawea A, Fawzy A, Mahmoud SH. Remineralization of early enamel lesions with a novel prepared tricalcium silicate paste. Sci Rep. 2022;12(1):9926. [DOI: 10.1038/s41598-022-13608-0] [PMID: 35705597]
14. Sebastian R, Paul ST, Azher U, Reddy D. Comparison of Remineralization Potential of Casein Phosphopeptide: Amorphous Calcium Phosphate, Nano-hydroxyapatite and Calcium Sucrose Phosphate on Artificial Enamel Lesions: An *In Vitro* Study. Int J Clin Pediatr Dent. 2022;15(1):69–73. [DOI: 10.5005/jp-journals-10005-2339] [PMID: 35528489]

15. Almansouri N, Bakry AS, Abbassy MA, Linjawi AI, Hassan AH. Evaluation of Resin Infiltration, Fluoride and the Biomimetic Mineralization of CPP-ACP in Protecting Enamel after Orthodontic Inter-Proximal Enamel Reduction. Biomimetics (Basel). 2023;8(1):82. [DOI: 10.3390/biomimetics8010082] [PMID: 36810413]
16. Ibrahim DFA, Venkiteswaran A, Hasmun NN. The Penetration Depth of Resin Infiltration Into Enamel: A Systematic Review. J Int Soc Prev Community Dent. 2023;13(3):194–207. [DOI: 10.4103/jispcd.JISPCD_36_23] [PMID: 37564169]
17. Wierichs RJ, Langer F, Kobbe C, Abou-Ayash B, Esteves-Oliveira M, Wolf M, et al. Aesthetic caries infiltration - Long-term masking efficacy after 6 years. J Dent. 2023;132:104474. [DOI: 10.1016/j.jdent.2023.104474] [PMID: 36878424]
18. Arora TC, Arora D, Tripathi AM, Yadav G, Saha S, Dhinsa K. An In-Vitro evaluation of resin infiltration system and conventional pit and fissure sealant on enamel properties in white spot lesions. J Indian Soc Pedod Prev Dent. 2019;37(2):133–9. [DOI: 10.4103/1319-2442.261345] [PMID: 31249175]
19. Behrouzi P, Heshmat H, Hoorizad Ganjkar M, Tabatabaei SF, Kharazifard MJ. Effect of Two Methods of Remineralization and Resin Infiltration on Surface Hardness of Artificially Induced Enamel Lesions. J Dent (Shiraz). 2020;21(1):12–7 [DOI: 10.30476/DENTJODS.2019.77864] [PMID: 32158780]
20. Sano H, Nakashima S, Songpaisan Y, Phantumvanit P. Effect of a xylitol and fluoride containing toothpaste on the remineralization of human enamel in vitro. J Oral Sci. 2007;49(1):67–73 [DOI: 10.2334/josnusd.49.67] [PMID: 17429185]
21. Prajapati D, Nayak R, Pai D, Upadhya N, K Bhaskar V, Kamath P. Effect of Resin Infiltration on Artificial Caries: An *in vitro* Evaluation of Resin Penetration and Microhardness. Int J Clin Pediatr Dent. 2017;10(3):250–6. [DOI: 10.5005/jp-journals-10005-1445] [PMID: 29104384]
22. Magalhães AC, Moron BM, Comar LP, Wiegand A, Buchalla W, Buzalaf MA. Comparison of cross-sectional hardness and transverse microradiography of artificial carious enamel lesions induced by different demineralising solutions and gels. Caries Res. 2009;43(6):474–83 [DOI: 10.1159/000264685] [PMID: 20016178]
23. Klaisiri A, Janchum S, Wongsomtakoon K, Sirimanathon P, Krajangta N. Microleakage of resin infiltration in artificial white-spot lesions. J Oral Sci. 2020;62(4):427–9 [DOI: 10.2334/josnusd.19-0321] [PMID: 32830164]
24. Freitas M, Nunes LV, Comar LP, Rios D, Magalhaes AC, Honorio HM, et al. In vitro effect of a resin infiltrant on different artificial caries-like enamel lesions. Arch Oral Biol. 2018;95:118–24 [DOI: 10.1016/j.archoralbio.2018.07.011] [PMID: 30099240]
25. Issa AI, Preston K, Preston A, Toumba K, Duggal M. A study investigating the formation of artificial sub-surface enamel caries-like lesions in deciduous and permanent teeth in the presence and absence of fluoride. Journal of Dental Research. 2002;81:A138-A. [DOI: 10.1016/s0003-9969(03)00095-5] [PMID: 12828985]
26. Zakizade M, Davoudi A, Akhavan A, Shirban F. Effect of Resin Infiltration Technique on Improving Surface Hardness of Enamel Lesions: A Systematic Review and Meta-analysis. J Evid Based Dent Pract. 2020;20(2):101405. [DOI: 10.1016/j.jebdp.2020.101405] [PMID: 32473796]
27. Ayad AH, AbdelHafez MI, AlGhandour RN, Mustafa DS, Nour KA. Effect of different surface treatments on the microhardness and colour change of artificial enamel lesions. Aust Dent J. 2022;67(3):230–8. [DOI: 10.1111/adj.12908] [PMID: 35224731]
28. Kidd EA, Fejerskov O. What constitutes dental caries? Histopathology of carious enamel and dentin related to the action of cariogenic biofilms. J Dent Res. 2004;83 Spec No C:C35–8. [DOI: 10.1177/154405910408301s07] [PMID: 15286119]
29. Haikel Y, Frank RM, Voegel JC. Scanning electron microscopy of the human enamel surface layer of incipient carious lesions. Caries Res. 1983;17(1):1–13. [DOI: 10.1159/000260643] [PMID: 6571803]

Утицај различитих раствора за деминерализацију и времена излагања на настанак и карактеристике почетних каријесних лезија глеђи – *in vitro* студија

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САЖЕТАК

Увод/Циљ Почетна каријесна лезија глеђи формирана у *in vitro* условима се често користи за испитивање ефикасности различитих средстава за реминерализацију.

Циљ овог истраживања био је испитивање утицаја три различита раствора за деминерализацију и различитог времена излагања на формирање белих мрља, као и процена карактеристика насталих почетних каријесних лезија глеђи помоћу скенирајућег електронског микроскопа.

Методе У овом *in vitro* истраживању коришћено је 20 премолара хуманог порекла који су извађени из ортодонтских разлога. Након детаљног уклањања остатака ткива, потопљени су у 0,1% раствор тимола, на собној температури. Пре процеса деминерализације извршено је одсецање корена у нивоу глеђноцементне границе. Методом случајног избора разврстани су у три експерименталне групе, у зависности од употребљеног раствора за деминерализацију и времена у току којег су били изложени дејству раствора: Група 1 (сирћетна киселина; *pH* = 4,4; током 96 сати); Група 2 (млечна киселина; *pH* = 4,5; 120 сати); Група 3 (млечна киселина; *pH* = 4,3; 504 сата) и контролна група (физиолошки раствор). Пресеци здраве глеђи у контролној групи и особине насталих каријесних лезија глеђи у експерименталним групама посматрани су на скенирајућем електронском микроскопу.

Резултати У првој и другој експерименталној групи уочен је настанак почетних каријесних лезија глеђи, са потповршинским зонама деминерализације и повећане порозности. У трећој експерименталној групи није очуван интегритет површине глеђи и дошло је до настанка кавитета.

Закључак Раствори за деминерализацију употребљени у првој и другој експерименталној групи дали су почетне каријесне лезије сличних особина, док је исход трећег раствора био настанак дефеката глеђи.

Кључне речи: глеђ; деминерализација; почетне каријесне лезије глеђи; скенирајући електронски микроскоп