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EFFECT OF pH CHALLENGE ON THE MICRO-HARDNESS OF ARTIFICIALLY INDUCED DE-MINERALIZED ENAMEL TREATED WITH RESIN INFILTRANT AND FLUORIDE VARNISH

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ABSTRACT

The purpose of this study was to evaluate the micro-hardness of artificially induced de-mineralized enamel after application of resin infiltrant and fluoride varnish and after exposure to pH challenges.

Material and methods: In the current study; sound permanent bovine incisors were used. A total of 40 sound enamel were then embedded in pre-cut metal cylinders. Two layers of acid-resistant nail varnish were applied to cover most of the enamel surfaces, leaving a window of 4×4 mm for demineralization. Each specimen was immersed in 32 ml of a de-mineralizing solution which contains 50 mM acetate buffer solution and 1.28 mM Ca(NO3)2_4H2O, 0.74 mM (NaH2PO4)_2H2O, and 0.03 ppm F at pH 5.0,10 for 24 hours at 37 C. The samples were then divided into two groups according to the material used to treat the de-mineralized enamel, each group consisted of 20 samples. Group 1: The samples of de-mineralized enamel were infiltrated with resin infiltration. Then the micro-hardness was recorded for all resin infiltrated samples before pH cycling challenge. The samples were then submitted to a pH cycling model at 37 C over 7 days. The pH cycling consisted of immersion of the samples in 35.5 ml of de-mineralizing solution: (2.0 mmol/ L Ca, 2.0 mmol/ L P, 0.075 mol/ L acetate buffer, 2.22 ml/mm2 of enamel surface) for 6 hours, alternated with immersion in 17.75 ml of re-mineralizing solution: (1.5 mmol/ L Ca, 0.9 mmol/ L P, 0.15 mol/ L KCl, 0.02 mol/ L cacodylate buffer, pH 7.0, 0.25 mL/mm2) for 18 hours for 5 days. The specimens were then kept for 2 more days in a fresh re-mineralizing solution, which completed 7 days of treatment. The samples were then washed in de-ionized water for 30 seconds among de-mineralizing and re-mineralizing cycles. Group 2: Fluoride varnish (NUPRO®WhiteVarnish, DENTSPLY) was applied as a thin layer by a brush and totally dried, then the micro-hardness was recorded [32] . The samples were then subjected to a pH cycling as in group 1. Then after challenge the micro-hardness measurements were performed as formerly described.

Results: The difference was highly significant between resin infiltrant and fluoride varnish treated enamel. The comparison among micro-hardness values of initial, de-mineralized enamel, resin-infiltrated enamel and resin infiltrated after pH cycling showed that there was a significant

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difference. The difference between micro-hardness values was highly significant when comparison was accomplished between initial and de-mineralized enamel ,initial and after pH cycling, de-mineralized enamel and resin infiltrated enamel ,de-mineralized enamel and after pH cycling and finally between resin infiltrated enamel and after pH cycling. The difference between micro-hardness values was not significant regarding initial and resin infiltrated enamel.

Conclusion: Under the limitations of the present study, it was concluded that the micro-hardness of resin infiltrated enamel was higher than that of de-mineralized enamel treated with fluoride varnish before and after pH cycling.

INTRODUCTION

The primary purpose in current dentistry is the quickly detection, safety and re-mineralization of incipient lesions[1, 2]. Conventional procedures have been changed to preventive, noninvasive, and minimal invasive methods. Recent methods of caries management which depend on early detection of carious lesions can facilitate to quit it early lesion which occurs within enamel. In recent times, caries control modalities have changed markedly [2].

The re-mineralization using fluoride varnish is considered one of the noninvasive treatments. It is well established that fluoride varnish is an efficient method to control caries in early stages [3]. By using fluoride varnish there is an increase in the duration of exposure of fluoride to the enamel to improve and enhance the outcome of topical fluoride therapies [4]. Fluoride varnish plays its anti–caries action via release of fluoride which leads to re-mineralization of enamel and decreases the process of demineralization [5]. Fluoride is known to be safe and effective, so its use has been increased recently [6]. The results of fluoride therapies depend upon patient's oral hygiene and it is not suitable for uncooperative patients [7, 8].

In recent years, an alternative management of early carious lesions was planned based on infiltration of caries with a hydrophobic resin material. These "infiltrant" materials occlude the micro-pore structures found in de-mineralized enamel and block the passages required by bacteria and acid to cause further dissolution of the enamel structure, thus inhibiting progression of the lesion [9, 10]. Infiltration of caries is well thought-out a noninvasive treatment choice for non-cavitated enamel lesions that extend to the outer third of dentin. It is not expected for these lesions to show re-mineralization or be arrested by other ways of non-invasive treatments[2].

The resin infiltration is composed mainly of hydrophilic triethyleneglycol-dimethacrylate (TEGDMA) [11, 12]. Resin infiltration differs from other restorative resin materials, as it is a liquid unfilled resin consisting of frequently TEGDMA. In addition, it doesn't need polishing steps after curing. TEGDMA is essential for preservation of the extremely low viscosity of the material that allows proper diffusion of the resinous material to the de-mineralized tooth structure [13, 14]. On the other hand, TEGDMA is recognized of having a significant water sorption rate[15].

The infiltrant resin plays an important role in masking white spot lesions by penetration of de-mineralized enamel by infiltrant resin material, as its refractive index is nearly similar to sound enamel.[16]. Also, the treatment of early carious lesions by resin infiltration leads to prevention of enamel demineralization and further progression of carious lesions, because the passage of cariogenic acids is blocked [17]. So, the infiltrant resin allows the management of early carious lesions in a minimally invasive approach [18].

The resin infiltration technique offers many advantages such as providing mechanical stabilization for the de-mineralized enamel structure, avoiding loss of structure of the affected or neighboring teeth, occluding the micro pore structures in the body of the lesion, arresting or reducing lesion progression, decreasing secondary caries, delaying the need for a restoration, avoiding postoperative sensitivity or pulp inflammation, reducing risk of gingivitis and periodontitis and producing good esthetic results in masking white spot [19, 20].

Bovine teeth are widely used in experimental studies as an alternative to human teeth due to the similarities between the two types of teeth regarding the chemical and physical characteristics, as its chemical structure and surface hardness [21]. In addition, the composition of bovine teeth shows less variations than human one, so the results obtained are more accurate and standardized [22]. The chemical structure of bovine enamel is nearly similar to human enamel, and the relatively large size of bovine teeth helps to provide sufficient enamel surfaces [3].

Hardness can defined as the resistance of a material or a surface against indentation or penetration. It is an important mechanical property of the material as the resistance to friction, abrasion and erosion increases with increasing hardness. This can be explained by the fact that high levels of mineral content in enamel are associated with low levels of abrasion compared with dentin [23]. There are constant chemical and mechanical challenges that may adversely affect tooth structure and restorative materials. Cycling models involving pH changes are used to evaluate treatment methods for early carious lesions and white spot lesions, as such models simulate pH changes in the oral environment[24, 25]. In general, resinous restorative materials show degradation in the oral environment to some extent, surface alteration due to different oral environment challenges affects characteristics of resinous materials [2, 26]. Surface micro hardness of many resin composites decreases following different challenges as pH changes [2].

Water sorption leads to plasticization of the resin matrix and hydrolytic breakdown of the resin–filler interface, so the surface hardness of resin based material markedly decreases[9]. Micro-hardness of enamel decreases obviously after demineralization. One of the primary functions of any material used in management of early carious lesions is to attain physical properties of sound enamel.

So, the purpose of this study was to evaluate the micro-hardness of artificially induced de-mineralized enamel after application of resin infiltrant and fluoride varnish and after pH challenges.

MATERIAL AND METHODS

In the current study; sound permanent bovine incisors were used. They were collected from steers aged 24 to 30 months old. They were stored in 0.1% thymol solution. Then the teeth were cleaned by slurry pumice and brush. All surfaces of the samples were examined using a stereomicroscope (SZ-CTY Olympus, Japan) to ensure absence of cracks or defects in the surface [2].

The tooth roots were removed under water irrigation. The teeth were sectioned using a water cooled diamond saw (Imicryl, Konya, Turkey) to remove their buccal surfaces giving one enamel specimen per tooth. A total of 40 sound enamel were then embedded in pre-cut metal cylinders using cold acrylic resin (Imicryl, Konya, Turkey) [27]. 400-, 600-, 800-, and 1200-grit sandpaper was used to flatten and polish the enamel surfaces [28]. The initial Vicker's hardness number (VHN) of each enamel surface in a sample was measured on four different points with a micro-indentation hardness tester (Zwick. roell, system 5153. UK) figure 1 that was fitted with a 50-g load for 15 seconds [10].

Fig. (1) Indentation hardness tester (Zwick. roell, system 5153. UK).

Two layers of acid-resistant nail varnish (nail polish, Amanda, Egypt) were used to cover the enamel surfaces, and a window of 4×4 mm for demineralization was left. The specimens were immersed in 32 ml of a de-mineralizing solution containing 50 mM acetate buffer solution and 1.28 mM Ca(NO3)2_4H2O, 0.74 mM (NaH2PO4)_2H2O, and 0.03 ppm F at pH 5.0,10 for 24 hours at 37 C. Then, the specimens were washed with de-ionized water [2]. Demineralization of the enamel surface were achieved. Micro-hardness measurements of de-mineralized enamel surface were recorded for each sample.

The specimens then were separated into two groups according to the material used to treat the de-mineralized enamel, each group consisted of 20 samples.

Group 1:

The samples of de-mineralized enamel were infiltrated with resin infiltration according to the manufacturer's instructions. Hydrochloric acid gel (15%) (Icon-Etch, DMG, Hamburg, Germany) was applied to the surface of de-mineralized enamel for 2 minutes, and afterwards it was rinsed with water and dried in air for 30 seconds previous to ethanol (Icon-Dry, DMG, Hamburg, Germany) was applied for. The sample was then subsequently air dried. A resin infiltrant (Icon-Infiltrant, DMG, Hamburg, Germany) was then applied to the surface for 3 minutes and the sample was light-cured for 40 seconds (P11060012A LED P5 Guilin, Guangxi, Medical instrument CO., China).

The resin infiltrant was reapplied for 1 minute, then the sample was light-cured [29]. Polishing of the samples was accomplished using medium, fine, and superfine aluminum oxide Sof-Lex discs (3M-ESPE Dental Products, USA) in a low-speed hand piece under air cooling for 20 seconds. The samples were kept for 7 days at 37 C .Then, the micro-hardness was recorded for all resin infiltrated samples before pH cycling challenge.

The samples were then submitted to a pH cycling model at 37 C over 7 days. The pH cycling consisted of immersion of the samples in 35.5 ml of de-mineralizing solution: (2.0 mmol/ L Ca, 2.0 mmol/ L P, 0.075 mol/ L acetate buffer, 2.22 mL/ mm2 of enamel surface) for 6 hours, alternated with immersion in 17.75 mL of re-mineralizing solution: (1.5 mmol/ L Ca, 0.9 mmol/ L P, 0.15 mol/ L KCl, 0.02 mol/ L cacodylate buffer, pH 7.0, 0.25 mL/ mm2) for 18 hours for 5 days. The specimens were then kept for 2 more days in a fresh re-mineralizing solution, completing 7 days of treatment. The samples were washed in de-ionized water for 30 seconds among de-mineralizing and re-mineralizing cycles[30, 31].

*Group 2:*Fluoride varnish ((NUPRO® White Varnish ,DENTSPLY,UK.) was applied as a thin layer using a brush and totally dried, then the micro-hardness was recorded [32]. The samples were then subjected to a pH cycling as in group 1. Then the micro-hardness measurements were performed as previously described. The data were collected and statistically analyzed using Statistical Package for the Social Sciences (SPSS Inc, Chicago, IL, USA) version 20.

RESULTS

The measured micro-hardness values were recorded as baseline (sound enamel), after demineralization, after treatment of de-mineralized enamel (infiltrated or fluoride varnish treated) and after pH cycling challenge for both groups. Comparison between group 1 and group 2 was carried out using t test.

Statistical analysis of mean micro-hardness values of sound (baseline) and demineralised enamel showed that there was no significant difference between group 1 and group 2, whereas the difference was highly significant between resin infiltrant and fluoride varnish treated enamel (table 1).

Table 2 shows the changes in micro-hardness values in the group 1 where the resin infiltration was used at different times of micro-hardness measurements.

The comparison among micro-hardness values of initial, de-mineralized enamel,resin infiltrated enamel and resin infiltrated after pH cycling using ANOVA test revealed that the difference was significant ($p<0.001$).

TUKEY'S Test showed that the difference between micro-hardness values was highly significant when comparison was made between initial and de-mineralized enamel ,initial and after pH cycling , de-mineralized enamel and resin infiltrated enamel, de-mineralized enamel and after pH cycling and finally between resin infiltrated enamel and after pH cycling where $p <0.001$. The difference between micro-hardness values was not significant regarding initial and resin infiltrated enamel using **TUKEY'S Test** where $p = 0.387$.

Table (1) Comparison of micro-hardness between group 1 and group 2 at different experimental steps.

Experimental steps		Groups						T-Test	
		Group 1			Group 2			t	P-value
Initial	**Range**	312.7	-	358	309	-	353	-0.465	0.645
	Mean ±SD	332.81	±	12.02	334.78	±	13.99		
De-mineralization	**Range**	200	-	255	200	-	253	0.223	0.825
	Mean ±SD	226.21	±	15.86	224.97	±	18.48		
Enamel treatment	**Range**	300	-	350.5	209	-	300.5	14.952	<0.001*
	Mean ±SD	324.87	±	13.93	239.16	±	20.75		
pH cycle	**Range**	101	-	181	89	-	100.3	6.611	<0.001*
	Mean ±SD	124.14	±	18.65	95.24	±	3.93		

Table (2) Comparison of micro-hardness among different experimental steps of group 1.

Experimental steps	Group 1						ANOVA	
	Range			Mean	±	SD	F	P-value
Initial (a)	312.7	-	358	332.81	±	12.02	779.104	<0.001*
De-mineralization (b)	200	-	255	226.21	±	15.86		
Resin infiltration(c)	300	-	350.5	324.87	±	13.93		
Resin infiltration followed by pH cycle(d)	101	-	181	124.14	±	18.65		

TUKEY'S Test

a&b	a&c	a&d	b&c	b&d	c&d
<0.001*	0.387	<0.001*	<0.001*	<0.001*	<0.001*

Mean and standard deviation of micro-hardness values in group 2 where the fluoride varnish was used to treat the demineralised enamel at different times of micro-hardness measurements is shown in Table 3.

ANOVA test revealed that there was a significant difference among micro-hardness values of initial, de-mineralized enamel, resin infiltrated enamel and resin infiltrated after pH cycling where ($p<0.001$).

Despite that the values of micro-hardness of enamel after fluoride varnish application were higher than that of de-mineralized enamel, **TUKEY'S Test** revealed that the difference was not significant ($p=0.033$). The same test revealed that the difference between micro-hardness values was highly significant when comparison was made between initial and de-mineralized enamel ,initial and after pH cycling, and de-mineralized enamel and after pH cycling and finally between fluoride varnish treated enamel and after pH cycling where $p <0.001$.

TABLE (3) Comparison of micro-hardness among different experimental steps of group 2.

Experimental steps	Group 2						ANOVA	
	Range			Mean	±	SD	F	P-value
Initial (a)	309	-	353	334.78	±	13.98	749.307	<0.001*
De-mineralized enamel (b)	200	-	253	224.96	±	18.48		
Fluoride varnish (c)	209	-	300.5	239.15	±	20.74		
Fluoride varnish followed by pH cycle (d)	89	-	100.3	95.23	±	3.93		

TUKEY'S Test

a&b	a&c	a&d	b&c	b&d	c&d
<0.001*	<0.001*	<0.001*	0.033*	<0.001*	<0.001*

DISCUSSION

Fluoride varnish has been used as a replacement to traditional topical fluorides [33]. It is an efficiently adherent useful material which contains high fluoride concentration in the form of salt or silane preparation in a rapid drying, alcohol, and resin-based solution [34].

Fluoride varnish has been used as a caries defensive method for many years. It is considered as a physical barrier, as it prevents the direct contact between acids and enamel. In addition, it discharges fluoride in the oral cavity [35]. The varnish is distinguished from other fluoride products as easy to use, safe and effective method. Fluoride varnish can be applied every three or six months to increase its efficiency. This is one of its main drawbacks [36]. There are three mechanisms of fluoride varnish which explain its anti-caries effect. First mechanism, the fluoride ions pass into dental tissues and lead to formation of fluoro-apatite by the available calcium and phosphate ions in saliva[37]. The dissolving salts containing manganese and carbonate are replaced by insoluble sediments, which were formed and lost during de-mineralization by action of bacteria. This leads to increased resistance of enamel to acidic challenge. Second mechanism, a similar process leads to re-mineraliztion of non-cavitated lesions. Third, fluoride is characterized by its antimicrobial activity [38].

Resin infiltration is a treatment option that was developed to prevent the progression of initial carious lesions. The resin infiltration concept depends upon penetration of low viscosity resinous material inside enamel and further occlusion of pathways of acids and dissolved minerals [39]. Additionally , strengthening the enamel structure mechanically can be achieved by resin matrix which penetrates the enamel structure, and therefore prevents collapse of the enamel surface [9].

Hardness is considered an essential mechanical property of a biomedical material and is defined as the resistance of a material or surface against indentation or penetration [40]. Measurement of micro-hardness is a suitable method to determine the strength of a hard tissue as enamel [41]. This method of assessment is fairly simple with accurate measurement and is non-invasive but needs special preparation of the samples for accurate measurements, prismless enamel layer should be removed and we get this through preparing a flat polished enamel surface [42] .

High levels of mineral content in enamel are related to abrasion resistance and this is related to resistance of enamel to caries. The objectives of the current study were to determine the micro-hardness of an intact enamel surface at the baseline, then after creating the early enamel carious lesions and finally after treatment of lesions and subjecting to pH cycling. Therefore, measuring VHN (Vickers Hardness Number) was calculated in four steps [43]. In both tested groups, there was a significant reduction in micro-hardness of de-mineralized enamel when compared with the sound one due to the loss of mineral content. After treatment, the micro-hardness values increased in both tested groups due to occlusion of micro-pores created by demineralization. In the current study, the surface micro-hardness of resin-infiltrated enamel was higher than that treated with fluoride varnish. This result coincided with Aziznezhad et al [44]. Increased surface hardness of enamel after application of resin infiltration is noticeably not a role of resin matrix because TEGDMA hardness measured (26 VHN) [45]. The action of resin infiltration depends upon the penetration of the resinous material into the porous enamel lesion which is formed after application of acid etching and then the resin material seals the voids and spaces of the de-mineralized area. So, additional demineralization and lesion progression is prevented [46]. A somewhat uniform resin-hydroxyapatite complex was formed and characterized by high surface hardness. Resin-hydroxyapatite complex was formed as a result of encapsulation of the hydroxyl-apatite crystals by resin infiltration material [45, 47]. The obtained results confirmed the capability of low-viscosity resin in resin infiltration to occupy and block the voids and spaces which were created between the residual crystals of de-mineralized enamel and produced a barrier, not only confined to the surface, but also inside the enamel lesion body [48]. So, a layer of enamel infiltrated by resin showed increase in the strength of the de-mineralized enamel and avoided advanced wear and cavitations [49]. Previous clinical studies have concluded that resin infiltration of initial carious lesions was considered an efficient and safe management to arrest the carious lesions and conserve the de-mineralized enamel [9]. The results of the current study agreed with those reported by Paris et al [29] and Torres et al [30] in that the micro-hardness of de-mineralized enamel was significantly enhanced after application of resin infiltration.

In addition, Taher et al [42] indicated that enamel surface micro-hardness significantly increased after application of resin infiltration when compared with micro-hardness of de-mineralizd enamel treated with fluoride varnish.

It is argued that the micro-hardness of porous lesion bodies is increased when they are filled with a resin than when they are simply untreated or re-mineralized carious lesions. In addition, the difference between micro-hardness of sound enamel showed no significant difference when compared with that of treated enamel in group I.

Although the micro-hardness of enamel treated with fluoride varnish increased, the difference in micro-hardness between it and sound enamel was significant. This result conveys that, the re-mineralization was incomplete and less than that achieved by resin infiltration. This may be due to the decreased time of contact of fluoride varnish with the enamel surface in present study, as the fluoride varnish can have chemical reaction with enamel over 24 hours. On the other hand, more frequent application of fluoride varnish can improve the action of anti-caries properties. But, in the current study, use of fluoride varnish one-time was used to simulate professional clinical situation. Despite resin infiltration markedly increased the micro-hardness of de-mineralized enamel, it was unable to protect enamel from pH cycling. This result is compatible with previous studies [2, 9, 30]. On the contrary, it does not agree with other studies. This may be due to the difference in pH cycle model where it used long-term (50 days) pH cycling models under weaker challenge conditions [9, 13, 24]. This was different from the short-term protocol used in the current study. Another reason can be the polishing procedure after application of resin infiltrant, as it was established that resin-based materials were able to defend enamel against erosion when they were found over enamel as a physical barrier [50].

The same was found in group 2 where fluoride varnish did not provide a physical or mechanical barrier against pH cycle. The result is compatible with Bayrak et al., [51] despite the difference in acidic challenge. Tricalcium phosphate which is added to flouride varnish is a hybrid material formed by a milling technique that fuses beta tricalcium phosphate and sodium lauryl sulfate or fumaric acid [4]. This blending produces a functionalized calcium and a free phosphate, which is considered to increase fluoride retention in enamel and facilitate re-mineralization [6]. When tricalcium phosphate becomes in contact with surface of the tooth and is moistened by saliva, the protective barrier breaks down, making calcium, phosphate, and fluoride ions available to the teeth [5] . The tricalcium phosphate with fluoride can promote the protective effect of enamel against any pH changes. The fluoride varnish used in the current study does not contain tricalcium phosphate. This may explain the results of current study. Magalhaes et al., 2007 stated that the commercial fluoride varnishes chemically reacted with enamel decreasing the softening of enamel, but this was not sufficient to decrease the enamel loss aggravated by the acidic challenge. Despite the fact that the NaF varnishes caused a significant reduction of softening of enamel, it might not be effective in the clinical situation. This result coincides with our study [52]. On the other hand, many other studies showed a resistance of commercial varnishes to acidic challenge in vitro and in situ protocols [1,3,53]. This may due to the use of different challenges and different types of fluoride varnish.

The reduction in micro-hardness after pH cycle of resin infiltrated enamel was significantly less than that recorded in group 2. The current results are compatible with the results of a study carried out by Aziznezhad et al. [44]. This may be attributed to the difference in chemical composition of the two tested materials.

Conclusion: Within the limitations of the present study, it is concluded that the resin infiltrated enamel micro-hardness was higher than that of de-mineralized enamel treated with fluoride varnish before and after pH cycling.

REFERENCES

1. Vieira, A., et al., Inhibition of erosive wear by fluoride varnish. Caries Research, 2007. 41(1): p. 61-67.
2. Neres, É., et al., Microhardness and Roughness of Infiltrated White Spot Lesions Submitted to Different Challenges. Operative Dentistry, 2017.
3. Comar, L.P., et al., TiF4 and NaF varnishes as anti-erosive agents on enamel and dentin erosion progression in vitro. Journal of Applied Oral Science, 2015. 23(1): p. 14-18.
4. Rirattanapong, P., et al., Effect of various forms of calcium in dental products on human enamel microhardness in vitro. Southeast Asian Journal of Tropical Medicine and Public Health, 2012. 43(4): p. 1053.
5. Karlinsey, R.L., et al., In vitro evaluation of eroded enamel treated with fluoride and a prospective tricalcium phosphate agent. Journal of Dentistry and Oral Hygiene, 2009. 1(4): p. 052-058.
6. Shellis, R., et al., Methodology and models in erosion research: discussion and conclusions. Caries research, 2011. 45(Suppl. 1): p. 69-77.
7. Esfahani, K.S., R. Mazaheri, and L. Pishevar, Effects of treatment with various remineralizing agents on the microhardness of demineralized enamel surface. Journal of dental research, dental clinics, dental prospects, 2015. 9(4): p. 239.
8. Mohd Said, S.N., M. Ekambaram, and C.K. Yiu, Effect of different fluoride varnishes on remineralization of artificial enamel carious lesions. International Journal of Paediatric Dentistry, 2017. 27(3): p. 163-173.
9. Paris, S., et al., Micro-hardness and mineral loss of enamel lesions after infiltration with various resins: influence of infiltrant composition and application frequency in vitro. Journal of dentistry, 2013. 41(6): p. 543-548.
10. Horuztepe, S.A. and M. Baseren, Effect of resin infiltration on the color and microhardness of bleached white-spot lesions in bovine enamel (an in vitro study). Journal of Esthetic and Restorative Dentistry, 2017.
11. Ionta, F.Q., et al., Penetration of resin-based materials into initial erosion lesion: A confocal microscopic study. Microscopy research and technique, 2016. 79(2): p. 72-80.
12. Gajewski, V.E., et al., Monomers used in resin composites: degree of conversion, mechanical properties and water sorption/solubility. Brazilian dental journal, 2012. 23(5): p. 508-514.
13. Gelani, R., et al., In vitro progression of artificial white spot lesions sealed with an infiltrant resin. Operative dentistry, 2014. 39(5): p. 481-488.
14. Paris, S., et al., Comparison of sealant and infiltrant penetration into pit and fissure caries lesions in vitro. Journal of dentistry, 2014. 42(4): p. 432-438.
15. Belli, R., et al., Wear and morphology of infiltrated white spot lesions. Journal of dentistry, 2011. 39(5): p. 376-385.
16. Zhao, X. and Y. Ren, Surface properties and color stability of resin-infiltrated enamel lesions. Operative dentistry, 2016. 41(6): p. 617-626.
17. Altarabulsi, M., et al., Clinical safety, quality and effect of resin infiltration for proximal caries. Eur J Paediatr Dent, 2014. 15(1): p. 39-44.
18. Senestraro, S.V., et al., Minimally invasive resin infiltration of arrested white-spot lesions: a randomized clinical trial. The Journal of the American Dental Association, 2013. 144(9): p. 997-1005.
19. Borges, A., et al., Is resin infiltration an effective esthetic treatment for enamel development defects and white spot lesions? A systematic review. Journal of dentistry, 2017. 56: p. 11-18.
20. Arslan, S., et al., Effect of resin infiltration on enamel surface properties and Streptococcus mutans adhesion to artificial enamel lesions. Dental materials journal, 2015. 34(1): p. 25-30.
21. Urabe, I., et al., Physical properties of the dentin-enamel junction region. American Journal of Dentistry, 2000. 13(3): p. 129-135.
22. LEVY, F., et al., The erosion and abrasion inhibiting effect of TiF4 and NaF varnishes and solutions on enamel in vitro. International journal of paediatric dentistry, 2012. 22(1): p. 11-16.
23. Catelan, A., et al., Effect of artificial aging on the roughness and microhardness of sealed composites. Journal of Esthetic and Restorative Dentistry, 2010. 22(5): p. 324-330.
24. Buzalaf, M.A.R., et al., pH-cycling models for in vitro evaluation of the efficacy of fluoridated dentifrices for caries control: strengths and limitations. Journal of Applied Oral Science, 2010. 18(4): p. 316-334.
25. Queiroz, C.S., et al., pH-cycling models to evaluate the effect of low fluoride dentifrice on enamel de-and remineralization. Brazilian Dental Journal, 2008. 19(1): p. 21-27.
26. Zhao, X., et al., Effectiveness of resin-based materials against erosive and abrasive enamel wear. Clinical oral investigations, 2017. 21(1): p. 463-468.
27. EL-WASSEFY, N.A., Remineralizing effect of cold plasma and/or bioglass on demineralized enamel. Dental materials journal, 2017. 36(2): p. 157-167.

28. Gurdogan, E.B., D. Ozdemir Ozenen, and N. Sandalli, Evaluation of Surface Roughness Characteristics Using Atomic Force Microscopy and Inspection of Microhardness Following Resin Infiltration with Icon®. Journal of Esthetic and Restorative Dentistry, 2017. 29(3): p. 201-208.

29. Paris, S., W. Hopfenmuller, and H. Meyer-Lueckel, Resin infiltration of caries lesions: an efficacy randomized trial. Journal of dental research, 2010. 89(8): p. 823-826.

30. Torres, C., et al., Effect of caries infiltration technique and fluoride therapy on microhardness of enamel carious lesions. Operative dentistry, 2012. 37(4): p. 363-369.

31. Oliveira, G., et al., In vitro effects of resin infiltration on enamel erosion inhibition. Operative dentistry, 2015. 40(5): p. 492-502.

32. Vicente, A., et al., Efficacy of fluoride varnishes for preventing enamel demineralization after interproximal enamel reduction. Qualitative and quantitative evaluation. PloS one, 2017. 12(4): p. e0176389.

33. Delbem, A.C.B., et al., Effect of fluoridated varnish and silver diamine fluoride solution on enamel demineralization: pH-cycling study. Journal of Applied Oral Science, 2006. 14(2): p. 88-92.

34. Shafi, I., Fluoride varnish reduces white spot lesions during orthodontic treatment. Evidence-based dentistry, 2008. 9(3): p. 81-81.

35. Muñoz Millán, P., et al., Effectiveness of fluoride varnish in preventing early childhood caries in rural areas without access to fluoridated drinking water: A randomized control trial. Community Dentistry and Oral Epidemiology, 2017.

36. Chu, C. and E. Lo, Uses of sodium fluoride varnish in dental practice. Annals of the Royal Australasian College of Dental Surgeons, 2008. 19: p. 58.

37. Buzalaf, M.A.R., et al., Mechanisms of action of fluoride for caries control, in Fluoride and the oral environment. 2011, Karger Publishers. p. 97-114.

38. Ten Cate, J., Contemporary perspective on the use of fluoride products in caries prevention. British dental journal, 2013. 214(4): p. 161-167.

39. Crombie, F., et al., Resin infiltration of developmentally hypomineralised enamel. International journal of paediatric dentistry, 2014. 24(1): p. 51-55.

40. Mahoney, E., et al., The hardness and modulus of elasticity of primary molar teeth: an ultra-micro-indentation study. Journal of dentistry, 2000. 28(8): p. 589-594.

41. Mahoney, E.K., et al., Mechanical properties and microstructure of hypomineralised enamel of permanent teeth. Biomaterials, 2004. 25(20): p. 5091-5100.

42. Taher, N.M., H.A. Alkhamis, and S.M.l. Dowaidi, The influence of resin infiltration system on enamel microhardness and surface roughness: An in vitro study. The Saudi Dental Journal, 2012. 24(2): p. 79-84.

43. Mueller, J., et al., Surface tridimensional topography analysis of materials and finishing procedures after resinous infiltration of subsurface bovine enamel lesions. Quintessence international, 2011. 42(2).

44. Aziznezhad, M., et al., Comparison of the effect of resin infiltrant, fluoride varnish, and nano-hydroxy apatite paste on surface hardness and streptococcus mutans adhesion to artificial enamel lesions. Electronic physician, 2017. 9(3): p. 3934.

45. Venter, S.A.d.S., et al., Hardness and degree of conversion of dental restorative composites based on an organic-inorganic hybrid. Materials Research, 2013. 16(4): p. 898-902.

46. Paris, S. and H. Meyer-Lueckel, Masking of labial enamel white spot lesions by resin infiltration--A clinical report. Quintessence international, 2009. 40(9).

47. Paris, S., et al., Penetration coefficients of commercially available and experimental composites intended to infiltrate enamel carious lesions. Dental Materials, 2007. 23(6): p. 742-748.

48. Baek, C.-J., et al., The effects of light intensity and light-curing time on the degree of polymerization of dental composite resins. Dental materials journal, 2008. 27(4): p. 523-533.

49. Tuncer, S., et al., The effect of a modeling resin and thermocycling on the surface hardness, roughness, and color of different resin composites. Journal of Esthetic and Restorative Dentistry, 2013. 25(6): p. 404-419.

50. Tereza, G.P.G., et al., Influence of removing excess of resin-based materials applied to eroded enamel on the resistance to erosive challenge. Journal of dentistry, 2016. 47: p. 49-54.

51. Bayrak, S., et al., Effect of Fluoride Varnish Containing CPP-ACP on Preventing Enamel Erosion. Scanning, 2017. 2017.

52. Magalhaes, A., et al., Effect of an experimental 4% titanium tetrafluoride varnish on dental erosion by a soft drink. Journal of Dentistry, 2007. 35(11): p. 858-861.

53. Murakami, C., et al., Effect of fluoride varnish and gel on dental erosion in primary and permanent teeth. Archives of oral biology, 2009. 54(11): p. 997-1001.