EFFECT OF RESIN INFILTRATE AND ENAMEL PROVARNISH ON PRIMARY TEETH ENAMEL MICROHARDNESS AND *STREPTOCOCCUS MUTANS* ADHESION: INVITRO STUDY

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ABSTRACT

Aim: this study aimed to assess surface microhardness of Enamel ProVarnish (Amorphous calcium phosphate (ACP)* (Premier Dental Products, PA, USA) containing fluoride varnish) and Icon resin infiltration (Icon-Infiltrate) ** (Resin Infiltration *Icon* DMG, Hamburg, Germany) after remineralization of artificial enamel lesion of extracted primary anterior teeth

Materials and Methods: eighty primary anterior teeth, which were randomly assignment into two equal groups: Group A and Group B (*n*=40 per group). Each group was further divided into two subgroups (*n*=20 per subgroup) according to sealant type: Group I: Enamel ProVarnish (control) and Group II: Icon Resin Infiltrate (intervention). In each group Microhardness test and *Streptococcus Mutans* adhesion were evaluated.

Results: Group II Icon resin infiltrate showed significantly higher microhardness and bacterial adhesion than Group I Enamel ProVarnish (p < 0.05).

Conclusion: Icon resin infiltrate showed significantly superior performance than Enamel ProVarnish in surface Microhardness while Enamel ProVarnish presented significant difference than Icon Resin infiltrate in *Streptococcus Mutans* adhesion.

KEYWORDS: Icon, Enamel ProVarnish, Microhardness, *Streptococcus Mutans*.

INTRODUCTION

The most mineralized and hardest tissue in the human body is tooth enamel **(Rodrigo *et al*, 2017)**. However, acidic attack of enamel, typically, due to the presence of acidogenic bacteria on enamel's surface, can cause a dramatic reduction in enamel's degree of mineralization and its mechanical strength. Decrease buy acidogenic bacteria on enamel's surface that leads to development of an incipient carious lesion also known as "white spot lesion" which can eventually penetrate deep into the enamel and dentin of a tooth **(Khoroushi and Marzie, 2017)**.

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White-spot lesions in the upper primary incisors at the gingiva margin are the first signs of early childhood caries (ECC). If the condition progresses, caries can cause the crown to be completely destroyed. **(Sukumaran *et al,* 2017***)*. The goal of modern dentistry strategy is to manage non-cavitated carious lesions through remineralization and reduction of bacterial adhesion to prevent disease progression that will eventually will improve aesthetics, strength and function **(Ulien *et al*, 2013)**.

This is especially important in the context of prophylaxis and treatment using minimally invasive methods. If the rate of remineralization exceeds the rate of demineralization, a carious lesion may stop developing or heal, which results in a smoother surface**.** On the other hand, demineralization leads to an increase in the porosity and roughness of the enamel **(Ekambaram *et al*, 2010)**.

Fluoride is the most important agent used in caries prevention measures, and teeth become more resistant to dental caries as a result of fluoride treatment **(Horst *et al*, 2018)**. For this reason, fluoride varnish was developed in the late 1960s to extend the adhesion of fluoride to teeth surfaces in order to increase its effectiveness. On the basis that fluoride varnishes are easy to apply and well tolerated, they have been described as the most practical method of having children take professionally applied topical fluoride **(Zabokova *et al*, 2014)**.

In the process of developing mineralized tissue, amorphous calcium phosphate (ACP) is produced as an intermediate product. The material characteristics are similar to crystalized hydroxyapatite, but it is smaller and has the ability to grow in aqueous conditions, where it can change into apatite and octa-calcium phosphate (OCP) **(Oliveira *et al*, 2017)**. In addition to enhancing remineralization and suppressing demineralization and fluoride salts, ACP also buffers free calcium and phosphate ion activities, resulting in increased bioavailability **(Fahimeh *et al*, 2019)**. These new polymers must

therefore release bioavailable calcium, phosphate and fluoride and protect enamel against acid demineralization, and hopefully considerably better, than conventional fluoride-alone dental varnishes. These new calcium phosphate and fluoride varnishes were tested in this study to investigate if it might release calcium, phosphate, and fluoride ions and prevent enamel demineralization. **(Benzian *et al*, 2012)**.

To treat non-cavitated lesions by application of remineralizing agent or low-viscosity light curing resins (so called Icon Infiltrate). The porosities of enamel in smooth surfaces lesions and proximal non-cavitated caries have been altered and commercialised in Germany using this principle. Since remineralization cannot occur unless the patient is willing and able to adhere to their treatment plan. This treatment seeks to seal the lesion without covering the resin layer by obstructing pores inside the lesion body, which acts as diffusion paths for acids and dissolved minerals. Due to this, the placement of a crown or bridge may be delayed because of resin intrusion **(Domejean *et al*, 2015)**.

The null hypothesis (H0) of the current study suggested that there was no difference in the microhardness and bacterial adhesion of Enamel ProVarnish and icon after remineralization of artificial enamel lesion of anterior primary teeth.

MATERIALS AND METHODS

1-Teeth selection

Total of eighty sound human primary anterior teeth extracted at the time of their exfoliation were collected. From outpatient's clinic, Pedodontic department, Faculty of Dentistry, Minia University. The study protocol conformed to the principles outlined in the Ethics Committee's statement for the use of human body material in medical research. Using a stereomicroscope (Leica, Switzerland), all teeth were carefully assessed to ensure that they

were free of decay, hypo-calcification, cracks, abrasion, or any restorations on the buccal and lingual surfaces.

Based on the findings of a pilot study included 10 primary anterior teeth the sample size was calculated using the following formula:

$$N= \frac{(Z_{\alpha/2}+Z_{\beta})^2 * 2 * \sigma^2}{D^2}$$

Where $Z_{\alpha/2=1.96}$ $Z_{\beta=0.84}$ $\sigma=1.25$ $_{d=0.56}$

$_{So}$ N=80 (**Pratap, 2012**).

2-Preparation of Enamel surface

Enamel surfaces were coated with acid resistant nail varnish except for a 3x3 mm window on enamel surface; that was left at the middle surface of teeth. For standardization small squares of pink wax were cut into 3x3 mm and applied over the enamel surfaces before the varnish application (**Mohanty et al, 2013**) and digital caliber was also used after application nail varnish to ensure the correct size.

3-Teeth mounting

The acrylic molds were constructed in a polyvinyl chloride (PVC) ring. The ring's dimensions were 15 mm in diameter, and 10 mm in depth. Separating medium was painted to the ring. Following manufacturer's directions, the cold cure acrylic resin material was mixed in a glass container and poured into a PVC ring using a spatula. To achieve a flat base, the PVC ring was set on a glass slab.

Statistical analysis: Data were analyzed using Statistical Package for Social Science (IBM SPSS Statistic for window version 22.0. Armonk, NY: IBM Crop.). After testing data for normality, paired means of surface Microhardness and number of adhered colonies of bacteria for each group (Enamel ProVarnish and ICON groups) were analyzed using Paired Samples t-test. Independent Student t-test was used to compare means of surface Microhardness

and number of adhered colonies of bacteria of both groups. Statistical significance level was set to 5% at 95% CI.

Block randomization and allocation into two study groups: Group 1 and Group 2 (n=40 per group).

Group I: (40 Teeth)

Teeth received Enamel ProVarnish and divided into two subgroups:

Subgroup A: 20 teeth were used to determine microhardness test

Subgroup B: 20 teeth were used to determine bacterial adhesion test.

Group II: (40 Teeth)

Teeth received Icon Resin Infiltration and divided into two subgroups

Subgroup A: 20 teeth were used to determine microhardness test

Subgroup B: 20 teeth were used to determine bacterial adhesion test.

Part I: Microhardness test

Forty teeth were placed in the acrylic molds, facing upwards, while the acrylic resin set. Acid resistant nail varnish was applied around the 3x3 mm square area on enamel surface.

The mounted teeth were measured for micro-hardness at 3 successive occasions (before demin-eralization or baseline T0), (after demineraliza-tion T1) and (after application of remineralizing agent T2).

Teeth were indented using Wilson Microhardness tester (Tukon 1102 Wilson Microhardness tester Buehler). Three indentations were performed per tooth at 100 grams' load with a dwell time of 10 seconds. The average score of the three readings were recorded for each tooth (**Amaechi et al, 2013**).

All teeth were immersed in a quarter liter of demineralizing solution in a glass container for six hours, followed by immersion in a remineralizing solution. This technique was repeated every day for 14 days to create an artificial enamel lesion (**Sano *et al*, 2007**). Teeth were rinsed with tap water for 30 seconds, then tested for the microhardness as mentioned before.

Demineralizing Solution: Acetic acid solution with a concentration of 50 mM ($C_2H_4O_2$), Calcium Chloride (CaCl2) 2.2 mM, Sodium Dihydrogen Phosphate (NaH_2PO_4) and Potassium Hydroxide (KOH) were used to bring the pH of the demineralizing solution to 4.4. (**Itthagarun *et al*, 2011**).

Remineralizing Solution: 1.5 mM Calcium Chloride ($CaCl_2$) 0.9 mM Sodium Dihydrogen Phosphate (NaH_2PO_4) and 0.15 mM Potassium Chloride (KCl) were used to bring the pH of the remineralizing solution to 7.0 (**Itthagarun et al, 2011**).

For the control group (Enamel ProVarnish) was applied according to the manufacturer's guidelines on each tooth surface using a microbrush, then it was left undisturbed for 4-6 hours, and then the excess was removed using cotton buds, then tested for the microhardness as mentioned before

For the intervention group (Icon Resin Infiltrate) was applied according to the manufacturer's guidelines on each tooth surface, Icon-Etch was applied for 2 minutes, teeth were water rinsed, air dried using airway syringe for 30 seconds. Icon-Dry was applied for 30 seconds and air-dried using airway syringe. Afterwards Icon-Infiltrate was applied twice, the first time for 3 minutes and the second time for 1 minute. Light cured for 40 seconds, LED light cure was used for both applications, then tested for microhardness as mentioned before

Part II: Adhesion of Streptococcus Mutans Bacterial preparation

Streptococcus Mutans ATCC 25175 was refreshed in Brain Heart Infusion Broth (BHI), then incubated at 37°C overnight.

Teeth preparation and sterilization

Enamel surfaces were coated with acid resistant nail varnish except for a 3x3 mm window; that was left at the middle surface of each enamel surface. For standardization pink wax was cut into small squares of 3x3 mm in dimensions and placed over the enamel surfaces before the varnish application and removed using tweezers after hardening of the varnish. Teeth were sterilized using ultraviolet light with sterilized air for 20-30 min in Laminar Air Flow (EuroClone Aura Mini Laminar Flow, Australia) after nail varnish application.

The prepared teeth were measured for bacterial adhesion at 3 successive occasions (before demineralization T0), (after demineralization T1) and (after application of remineralizing agent T2).

Streptococcus Mutans was cultured on the surface on each tooth in a sterile Eppendorf tube, then incubated at 37°C for 48 hours. After incubation, each tooth was vortexed for 1 minute.

Serial dilutions (1/10,1/100, and1/1000) were performed on the samples after colonies removed with sterile cotton Swabs (**AvishaiBen and Davidson, 2014**) using a sterile 1 ml pipette to transfer precisely one ml of BHI media into a sterile 9 ml saline tube via micropipette.

Each of the tubes was mixed (by means of a new 1 ml pipette) to insure even distribution of the organism, with a sterile 1 ml pipette, aseptically 1 ml of each dilution was transferred into sterile Petri dishes.

Each plate was marked with the dilution present BHI medium was poured into Petri dishes, and the bacterial suspension was mixed with the medium. The plates were incubated at 37°C for 48 hours. Following incubation, the number of colonies were counted and express the average for the three plates in terms of the number of colonies per ml of specimen.

All teeth were immersed in a quarter litre of demineralizing solution in a glass container for six hours, followed by immersion in a remineralizing solution. This technique was repeated every day for 14 days to create an artificial enamel lesion (**Sano et al, 2007**). Teeth were rinsed with tap water for 30 seconds then tested for the bacterial adhesion as mentioned before.

For the control group (Enamel ProVarnish) was applied according to the manufacturer's guidelines on each tooth surface using a micro brush, then it was left undisturbed for 4-6 hours, and then the excess was removed using cotton buds, then tested for the bacterial adhesion as mentioned before.

For the intervention group (Icon Resin Infiltrate) was applied according to the manufacturer's guidelines on each tooth surface, Icon-Etch was applied for 2 minutes, teeth were water rinsed, air dried using airway syringe for 30 seconds. Icon-Dry was applied for 30 seconds and air-dried using airway syringe. Afterwards Icon-Infiltrate was applied twice, the first time for 3 minutes and the second time for 1 minute. Light cured for 40 seconds, LED light cure, was used for both applications, then tested for the bacterial adhesion as mentioned before.

RESULTS

Surface Microhardness:

Before starting the laboratory steps, average scores of surface microhardness of extracted teeth (i.e. at baselines) was as follows; (1) for ProVarnish [M= 306.33 ± 8.24; 95% CI 301.77; 310.90], and (2) for ICON [312.20 ± 7.47; 95% CI308.07; 316.33]. After demineralization, remarkable decrease in the average values of surface microhardness was reported. The mean of microhardness of ProVarnish was 212.27 ± 9.41 with 95% CI of 207.06; 217.48. While, the mean of microhardness of ICON was 209.80 ± 9.01 with 95% CI of 204.81; 214.79.

Fig. (1) Wilson microhardness tester.

Fig. (2): Enamel surface 3x3 mm

Finally, after remineralization with Enamel ProVarnish and ICON, the average scores were 241.93 ± 6.07 (95% CI 238.5; 245.29) and 283.80 ± 6.22 (95% CI 280.35; 287.25) respectively (Table 1).

Comparing the paired means of ICON group and Enamel Provarnish showed a statistically significant difference between them for Icon group (p < 0.05).

Bacterial adhesion

The number of average colonies adhered to the tooth surface at the baselines of the Enamel ProVarnish and ICON were 57.33 ± 7.75; 95% CI 52.77; 61.36, and 55 ± 2.77; 49.07; 60.93 respectively. After demineralization, the average number of colonized bacteria on the demineralized tooth surfaces increased. The mean of colonies adhered on demineralized surfaces for Enamel ProVarnish was 82.93 ± 7.38 with 95% CI of 78.85; 87.02. While the mean of colonies adhered on demineralized surfaces for ICON was 83.53 ± 12.4 with 95% CI of 76.87; 90.20. Finally, after remineralization with Enamel ProVarnish and ICON, the average scores were increased; for Enamel ProVarnish group, the mean of adhered colonies was 19.07 ± 1.03 (95% CI 16.86; 21.28) and 43.87 ± 6.80 (95% CI 40.10; 47.63). respectively (Table 2). Comparing the paired means of ICON group and Enamel Provarnish showed a statistically significant difference between them for Enamel ProVarnish group (p < 0.05).

TABLE (1) Pretreatment, after demineralization and post treatment surface microhardness mean scores of Enamel ProVarnish and Icon resin infiltrate.

Treatment	Mean±SD	SE	95% CI	Minimum	Maximum
Before treatment					
Enamel Pro Varnish	30633 ± 8.24	2.13	301.77; 310.90	288	322
ICON	312.20 ± 7.47	1.93	308.07; 316.33	302	325
Demineralization					
Enamel Pro Varnish	212.27 ± 9.41	2.43	207.06; 217.48	197	230
ICON	209.80 ± 9.01	233	204.8; 214.79	197	224
After treatment					
Enamel Pro Varnish	241.93 ± 6.07	1.57	238.57; 245.29	233	253
ICON	283.80 ± 6.22	1.61	28035; 287.25	275	295

TABLE (2) Mean scores of pretreatments, after demineralization and post treatment adhered colonies of bacteria of Enamel ProVarnish and Icon resin infiltrate.

Treatment	Mean±SD	SE	95%Cl	Minimum	Maximum
Before treatment					
Enamel Pro Varnish	57.33 ±7.75	2.01	52.77; 61.36	42	74
ICON	55 ± 2.77	2.77	49.07; 60.93	40	78
Demineralization					
Enamel Pro Varnish	82.93 ± 7.38	1.41	78.85; 87.02	64	98
ICON	83.53 ± 12.4	3.11	76.87; 90.20	55	100
After treatment					
Enamel Pro Varnish	19.07 ± 1.03	1.03	16.86; 21.28	10	25
ICON	43.87 ± 6.80	1.76	40.10; 47.63	32	60

DISCUSSION

Esthetics is an important aspect in dentistry and dental education. With the continuing advances in dentistry and dental technology, there are now an abundance of options that make it easy to perform esthetic dental procedures. The most conservative of all esthetic procedures is one that involves no tooth removal at all. This can be achieved by "minimal intervention dentistry." (**Krishna** *et al*, **2018**).

The microhardness can be evaluated using two different methods: Knoop and Vickers indenter. In the current study, The Vickers indenter with 100 gm load for 10 seconds was selected to measure microhardness because it provided an appropriate size of the indentations for an accurate measurement with the available equipment and the present experimental design. Specimens demonstrated flat surfaces, since the slightest tilt would have had affected the accuracy of the Microhardness testing (**Ramy and Mohamed, 2017**).

Two commonly used remineralizing agents were evaluated in this study (Enamel ProVarnish) and resin infiltration(Icon) on surface microhardness of incipient enamel lesions, Microhardness was significantly increased when using icon resin infiltrate than an enamel varnish, the average scores were 241.93 ± 6.07 kg/mm^2 for enamel provarnish and for icon 283.80 ± 6.22 kg/mm^2.

After the application of Icon, microhardness significantly increased, the findings of the current study are in agreement with (**Wiegand** *et al*, **2011**). Due to TEGDMA, has low-molecular-weight polymer included in the ICON's chemical composition, may be to blame. A reduced viscosity and easier penetration of the ICON resin matrix result in a better bonding of the resin to the enamel. In addition, the two-minute application of HCL etching removes much of the hypermineralized surface layer, which improves Icon's penetration

This study's findings are in agreement with my own findings (**Zankalouny** *et al*, **2016**). Resin infiltration had a greater impact on mechanical strength and microhardness in samples treated with resin infiltration. Resin infiltration considerably increased the microhardness of carious lesions as compared to untreated artificial lesions after demineralization, results found in resin infiltrate (Icon) ranged from (174.0-208.0kg/mm), followed by ACP (165.5-182.6kg/mm).

This study's findings are not in agreement with my own findings (**Parastou** *et al*, **2020**) using ICON had no effect on microhardness, which remained stable over the course of the 20-week research. This is because Icon can only form a barrier layer on the itched enamel surface and didn't penetrate it.

Regarding bacterial adhesion there are 3 methods turbidimetric, direct microscopic count and a viable cell count. According to the current study, to determine the quantity of bacteria present, a viable cell count was performed. that was actively developing in a sample. The colony could be seen with the naked eye, and the number of colonies on the plate could be counted, making it a simple and quick process (**Kuangwen** *et al*, **2018**).

This study's findings are in agreement with the current findings (**Mahdiye** *et al*, **2017**). Significant reductions in bacterial count were recorded. Fluoride ions reduce the production of Glycosyltransferase enzymes, which inhibits the adhesion of *S. mutans* on enamel surface.

This study's findings are in agreement with the current findings (**Denzy and Puja, 2020**). That is due to amorphous calcium phosphate with +5% fluoride varnish pastes had antimicrobial property and high bioavailability against *S. mutans* have shown significant reductions in the counts at one-hour post application.

This study's findings are in agreement with the current findings (**Rocha** *et al*, **2011**). Enamel pro varnish show low microhardness value than Icon, this may be due to the fact that hyper mineralized surface layer, which is characteristic for the incipient lesions, acts as a barrier for the diffusion of ACP+5%sodium fluoride. Ideally, a remineralization

system would favor subsurface mineral gain rather than deposition only in the surface layer not infiltrate in to the body of the lesion.

This study's findings are not in agreement with the current findings **(Basak and Banu, 2018)**. Due to icon show lower surface microhardness was found for the resin infiltrated group demonstrated reduced lesion progression compared with untreated artificial lesions after further demineralization Possibly, two applications of infiltrates might compensate for Second-degree polymerization shrinks the lesion and fills up the porosities, which may increase hardness and prevent mineral loss. This could also explain the hardness values obtained after further demineralization.

CONCLUSIONS

1. Icon Resin Infiltrate has shown the ability to improve microhardness of the artificial enamel lesions compared to Enamel ProVarnish.

2. Although Icon Resin Infiltrate can infiltrate deeply into artificial enamel lesions with no apparent demineralization zones, the Enamel ProVarnish aggregate was only confined to the surface of the artificial enamel lesion with no evidence of subsurface infiltration.

3. Enamel ProVarnish has shown the ability to decrees Streptococcus Mutans adhesion of the artificial enamel lesions compared to Icon Resin Infiltrate.

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