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RESEARCH ARTICLE

MICROHARDNESS OF THREE DIFFERENT TECHNIQUES FOR TREATMENT OF ARTIFICIAL WHITE SPOT LESIONS AN IN VITRO COMPARATIVE STUDY

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ABSTRACT

Background: Clinically appearing early caries are referred to as white spot lesions (WSLs). These lesions compromise esthetics and precede cavitation; therefore they must be halted by effective materials. **Aim:** To evaluate and compare microhardness of artificial white spot lesions treated with icon infiltrant, single bond universal adhesive and green tea. **Materials and methods:** Forty extracted human premolars were selected for this study. The teeth were debrided and stored in deionized water at room temperature from the day of extraction until used. All premolars were coated with a nail varnish, leaving a 4 mm × 4 mm window on the middle of buccal surface covered by label stickers. Then all premolars were demineralized with phosphoric etching acid material with 1 minute phosphoric acid H3PO4 37% to create artificial white spot lesions on the buccal surface. 10 of the demineralized teeth were kept as control group, 10 teeth we retreated with low viscosity resin (Icon Infiltrant, DMG, Hamburg, Germany), 10 single bond universal adhesive (3M Espe^{TM}) and 10 with green tea (Ahmed Tea London). Enamel surfaces were treated with these three materials approaching three different techniques then microhardness of specimens were measured and compared. **Statistical analysis:** One-way ANOVA followed by Tukey post hoc test were used to compare between more than two groups in non-related samples. **Results:** It showed that; there was a statistically significant difference between all the groups where ($p<0.001$). A statistically significant difference was found between (Icon) and each of (Control), (Adhesive) and (Green tea) groups where ($p<0.001$), ($p=0.001$) and ($p=0.002$) respectively. No statistically significant difference was found between (Control) and each of (Adhesive) and (Green tea) groups where ($p=0.118$) and ($p=0.059$) respectively. **Conclusions:** Within the limitations of this study; resin infiltration technique is a promising micro invasive approach for management of noncavitated lesions and is expected to increase the span of micro invasive dentistry.

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INTRODUCTION

Caries is a dynamic, complex, multifactorial process involving the gradual loss of mineral compounds from hard dental tissue, it is considered a world-wide chronic disease, easily detectable, and reversible at an early stage. White-spot lesions are the earliest macroscopic evidence of enamel caries (Silverstone, 1977). Typically, the enamel surface layer stays intact during subsurface demineralization, but, without treatment, will eventually collapse into a full cavity (Mann and Dickinson, 2006). White spot lesions occur when the pathogenic bacteria have breached the enamel layer and organic acids produced by the bacteria have leached out a certain amount of calcium and phosphate ions that may or may not be replaced naturally by the remineralization process.

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This loss of mineralized layer creates porosities that change the refractive index (RI) of enamel that is usually translucent (Kidd and Fejerskov, 2004). The decrease in microhardness after demineralization was reported, as the process softens the enamel by chemical dissolution of enamel rods and creates voids (Torres *et al*., 2012). Several techniques have been proposed for treatment of the white spot lesions. The common treatment strategy for white spot lesions comprises adhesive resins, improvement of remineralization using remineralizing agents, fluoride containing products, micro abrasion, argon-laser irradiation, herbal products and by resin infiltration technique through stopping the advance of the lesion by low viscosity resin materials (Cha *et al*., 2001). Recently, 'resin infiltration technique' was introduced with the development of flowable resin material. Caries infiltration involves the low-viscosity light curing resins (Icon) composed of triethylene glycol dimethacrylate (TEGDMA) which completely fills pores within the tooth, replacing lost tooth structure and

stopping caries progression (Paris and Meyer-Lueckel, 2010). It penetrates into the lesion by capillary forces and creates a diffusion barrier inside the lesion and not only on the lesion surface (Meyer-Lueckel and Paris, 2008). Therefore, success of caries infiltration technique, depends on the efficacy of this low viscosity resin or caries infiltrant to penetrate up to the depth of the white spot lesion and not just mask the lesion (Paris *et al*., 2010). Also, single bond universal adhesive is used in the placement of direct restorations (composites, compomers) as well as in the adhesive luting of indirect restorations (all-ceramics, composites) involving light-curing and it could be used in the arresting of demineralized enamel.

A lot of chemical agents and herbal extracts are being tested nowadays for their remineralizing effect; one of these examples is green tea. Green tea is an aqueous extract of various dried and processed leaves (Yam *et al*., 1997). It has numerous medicinal properties, mainly attributed to the antibacterial and antioxidant properties, and the bioactive chemicals, including; polyphenols, alkaloids, mineral, volatile oils (Chopade *et al*., 2008). From dental point of view, tea has preventive effect against tooth decay (Mbata, 2007). It reduces both dental plaque and caries as a result to its high fluoride and organic constituents that inhibit bacterial activity and re-mineralize enamel (Niu *et al*., 2008). It is noteworthy that there is a large amount of fluoride in the tea plant around 100–200 mg/kg**,** 98% of which has been accumulated in its leaves and can be easily released into the tea infusion. Thus, tea liquor can be a major source of fluoride (Maleki *et al*., 2016).

Aim of the study: Since there is no ideal method until now to treat white spot lesions; therefore this study aimed to evaluate and compare microhardness of artificial white spot lesions treated with icon infiltrant, single bond universal adhesive and green tea.

MATERIALS AND METHODS

Materials Compositions and manufacturers of the materials used in the study are shown in Table 1. The authors of the present study followed the ethical rules and regulations of the ethical committee of Future University in Egypt. Forty extracted human premolars, indicated for orthodontic extraction and periodontal reasons, were selected for this study. Teeth with cracks, caries or restorations were excluded. Calculus and stains were removed with hand scaler, rubber cup and polishing paste. The teeth were debrided and stored in deionized water at 37˚C from the day of extraction until used. Compositions and manufacturers of the materials used in the current study are shown in table 1.

Artificial white spot lesions: All premolars were coated with two layers of colored nail varnish, leaving a 4 mm × 4 mm window on the middle of buccal surface covered by label stickers. The sticker were removed, then all premolars were demineralized with phosphoric acid etching material (H3PO4 37%) for 1 minute to create artificial white spot lesions on the buccal surface (Marny *et al*, 2018, Al Khateeb *et al*., 2000). For microhardness investigation, the forty premolars with artificial white spot lesions were divided randomly into 4 groups with 10 specimens each according to the material used in each group as follows:-

Group I: white spot lesions left untreated (control group).

Group II: white spot lesions treated with Icon.

Group III: white spot lesions treated with single bond universal adhesive.

Group IV: white spot lesions treated with green tea.

Materials application methods

Icon treatment: The surface of the lesion was etched using (Icon etch^{TM}) 15% hydrochloric acid gel for 2 minutes. The etching gel thoroughly washed for 30 seconds using a water spray. Following etching the lesion desiccated by applying (Icon-Dry ^{TM}) ethanol for 30 seconds and air dried according to the manufacturer's instructions. Low viscosity resin (Icon) was applied on the lesion surface using a micro brush and allowed to penetrate for 3 min. The excess material were removed using a cotton roll and the surface was light cured for 40 seconds using (Elipar^{TM}, 3m ESPE, USA) LED light curing unit according to manufacturer's instructions.

Single bond universal adhesive treatment: The surface of the lesion was etched using 34% phosphoric acid (Scotch bond Universal) for 15 seconds. The etching gel thoroughly washed for 10 seconds with water and excess water was blotted using a piece of gauze until the surface appeared glistening without pooling of water. The white spot lesion was treated with single bond universal adhesive. It was rubbed for 20 seconds using a disposable applicator, gently air dried for 5 seconds, then light cured for 10 seconds using (Elipar TM, 3m ESPE, USA)LED light curing unit according to the manufacturer's instructions.

Green tea treatment: Teeth were subjected for testing solution named green tea (ready prepared tea bags, Ahmed trademark). Each tea solution was prepared daily by brewing 2g crushed dried leaves in 100 ml de-ionized water for 30 seconds. Teeth were immersed in 20 ml for 4 minutes in the testing tea, then rinsed with and kept in de-ionized water at 37°C for next day. This procedure was repeated daily for 7 days (Al-Ubaidi, 2007).

Microhardness investigation: All the specimens were embedded in auto polymerizing acrylic resin with outer buccal surface exposed. The surface microhardness of the specimens were determined by using Vickers microhardness tester (Wilson Tuken, 6000series, Norwood, MA., USA) with a diamond indenter at 1000g (HV 0.1) load for (10) seconds. The physical quality of the indenter and the accuracy of the applied load were controlled in order to get the correct results. Three indentations at spacing of 100 microns were made on the middle of the exposed window on the buccal surface. Digital readings were recorded and the average value was considered the mean microhardness for each specimen.

RESULTS

The mean and standard deviation values were calculated for each group in each test. Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests, data showed parametric (normal) distribution. One-way ANOVA followed by Turkey post hoc test were used to compare between more than two groups in non-related samples. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows. There was a statistically significant difference between (Control), (Icon), (Adhesive) and (Green tea) groups where ($p<0.001$).

Table 1. Materials used in the current study

Materials	Compositions	Manufacturers
Icon (caries infiltrant)	1. Icon-Etch (HCl 15%) 2. Icon-Dry (99% ethanol) 3. Icon-Infiltrant (methacrylate-based resin matrix, initiators, additives)	DMG – Hamburg, Germany
Single bond universal adhesive	• Scotchbond Universal etchant 34%phosphoric acid.	3MEspe™
	• MDP Phosphate Monomer Dimethacrylate resins HEMA, Vitrebond Copolymer Filler Ethanol Water Initiators Silane	
Green tea	50 gr (25x2g)	Ahmed tea trademark, London

Table 2. The mean, standard deviation (SD) values of microhardness of different groups

Variables	Microhardness	
	Mean	SD
Control	112.59 [b]	8.03
Icon	191.00 [a]	26.35
Adhesive	139.30 [b]	27.35
Green tea	143.24 [b]	18.02
p-value	<0.001*	

Means with different letters indicate significant difference * significant (p<0.05), ns; non-significant (p>0.05)

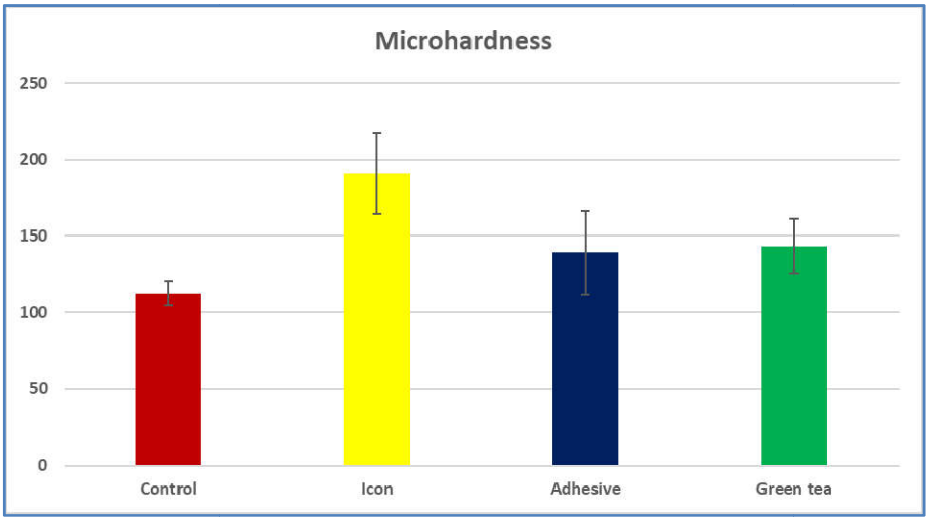

Figure 1. Bar chart representing microhardness for different groups

A statistically significant difference was found between (Icon) and each of (Control), (Adhesive) and (Green tea) groups where (p<0.001), (p=0.001) and (p=0.002) respectively. No statistically significant difference was found between (Control) and each of (Adhesive) and (Green tea) groups where (p=0.118) and (p=0.059) respectively. No statistically significant difference was found between (Adhesive) and (Green tea) groups where (p=0.986) as shown in table 2 and figure 1.

DISCUSSION

Dental caries is a multi-factorial disease process caused by acids, from bacterial metabolism, dissolving the mineral content of enamel and dentine. It is defined as a "complex disease caused by an imbalance in physiologic equilibrium between tooth mineral content and biofilm fluid i.e., saliva" causing demineralization of teeth (Bowes and Murray, 1935). Demineralization is a process in which the inorganic content of the enamel structure is lost leading to occurrence of white spot lesions.

Therefore initial carious lesions are so-called "white spot" lesion (WSL) which implies that there is a subsurface area with most of the mineral loss beneath a relatively intact enamel surface (Roopa *et al*., 2015). Identification of these early caries lesions and treatment with nonsurgical methods is a contemporary approach that has been well received and adopted. The concept of minimally invasive dentistry of diseased tooth is of great significance for retaining remaining tooth structure through different methods as resin infiltration, adhesives and remineralization (Chen *et al*., 2015). One of these approaches is resin infiltration through the tiny pores within the enamel lesion body which act as diffusion pathways for acids and provide path for acid to dissolve minerals which ultimately leads to cavitation which is considered as alternative and novel approach to arrest such carious lesions infiltrating these pores with light-curing resins. This approach not only seals the microporosities and blocks the access of acids to any remaining pores, but also significantly increases surface hardness and provides significant mechanical support to tooth tissue (Panigrahi*et al*., 2015). An infiltrant resin used for this purpose must possess very low viscosity, a high

surface tension, and a low contact angle with the enamel, all of which are important properties for penetration of the resin into the body of an incipient enamel lesion (Paris *et al*., 2007a). Many demineralizing agents have been used to produce in vitro enamel demineralization models for example, phosphoric etching acid, acetic acid, lactic acid, or acidified hydroxy-ethylcellulose system. Phosphoric acid H3PO4 37% was selected for our study due to ease and simplicity of this demineralization technique (Al Khateeb *et al*., 2000). Regarding specimens treated with icon; specimens were prepared according to manufacturer's instructions in all surface conditioning and dehydration procedures, then the infiltrant resin was applied twice over the lesion in order to occlude the space generated by the shrinkage of material after the first application. The application time was kept at 3 minutes (Robinson *et al*., 2001). Microhardness tests are widely used to measure the hardness of teeth. It is a reliable method for obtaining indirect information about mineral content changes of dental hard tissues. This method is easy, quick, and requires only a tiny area of specimen surface for testing. Vickers hardness tests are preferred for tooth hardness testing as against the Knoop hardness tests. Accordingly, Vickers hardness test was preferred for this study (Chuenarrom *et al*., 2009). Regarding enamel microhardness, the results of the present study showed that the Icon group revealed the highest value followed by green tea group followed by Adhesive group then control group. The high microhardness result of Icon infiltrant may be attributed to the ability of low-viscosity resin to fill the spaces between the remaining crystals of porous lesions and create a diffusion barrier not only at the surface, but also within the enamel lesion body. Therefore, a resin-infiltrated layer should be able to strengthen the demineralized enamel structure, and prevent further wear and cavitation. Some clinical studies have reported that micro-invasive caries treatment with resin infiltration was an effective and safe approach to arrest initial caries lesions and preserve demineralized enamel (Meyer *et al*., 2012) (Altarabulsi *et al*., 2014).

The findings of the present study agreed with those reported by (Torres *et al*., 2012 and Paris *et al*., 2013) in that the microhardness of carious lesions was significantly increased with resin infiltration (Taher *et al*., 2012) also indicated that enamel surfaces treated with an infiltrant showed significantly higher surface hardness than treatment with fissure sealant. Regarding Single bond universal adhesive, the recorded VHN in adhesive group was significantly lower than those of resin infiltrant group. These lower results may be attributed to the fact that; in order to get adhesion on moisture conditions and increase fluidity, adhesive systems have solvents in their composition but these could affect their polymerization degree and their mechanical properties. The kind of solvent in tested adhesives systems could also influence on depth penetration (Van Landuyt *et al*., 2007). This results could be also attributed to the adhesive resin layer remaining on the top of the enamel surface and had insufficient material penetration, while the use of longer acid conditioning with Icon (2 min with hydrochloric acid) erodes the surface layer more effectively and could have led to deeper resin penetration than etching with phosphoric acid gel (Paris *et al*., 2007b). According to the microhardness results of this study, the use of green tea increased microhardness of enamel but it was not statistically significant than the control group. Although tea plants accumulated fluoride in their leaves in level comparable to that recommended in preventive dentistry (Cao *et al*., 2004, Mahvi *et al*., 2006), there was only remineralization of surface area, i:e incomplete re-mineralization. This can be explained that only low level of fluoride is required to trigger the mechanism of remineralization, raising the fluoride level dose not result in greater degrees of mineralization (Abdulraheam and Ghareeb, 2011) i;e only free exchangeable one can react with calcium ion. This attracts the attention to find a way to make much of fluoride in any tea in its reactable state to get the best benefit or to prevent the competition of different ions in the single solution to get reaction with the appatite crystals. This finding agrees with the result of (Jaâfoura *et al*., 2014) which stated that sugar-free green tea protects the enamel from further demineralization and surface loss by its ability to increase surface microhardness and decreases surface roughness of enamel. The percentage of improvement of surface microhardness after immersion of eroded tooth in green tea was increased to 55%. While the percentage of reduction in the surface roughness of eroded tooth was 30%.

Conclusions

Resin infiltration technique was found to be a promising, noninvasive approach and might be considered as an additional option to nonoperative and operative treatment approaches and is expected to increase the span of microinvasive dentistry. It can prevent the progression of lesion body and increase the dental tissue hardness and resistance to acid attacks.

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