The Effect of Non-Invasive Treatment Techniques on the Colour Masking and Surface Roughness of Induced Enamel Lesions

(In Vitro Study)

By

Nada Ali Almah Obead

Supervisor

Prof. Nagat Hassan Bubteina

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Faculty of Dentistry

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University of Benghazi **Faculty of Dentistry**

Department of Conservative dentistry and Endodontics

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This Thesis was Successfully Defended and Approved on: 00.00.2021

Supervisor

Dr. **Nagat Hassan Bubteina**

Signature: .....

Dr. **Internal examiner**.....

Dr. **External examiner**.....

.....

Dean of Faculty

.....

Director of Graduate studies and training

بسم الله الرحمن الرحيم

{وَقُلِ ٱعْمَلُواْ فَسَيَرَى ٱللَّهُ عَمَلَكُمْ وَرَسُولُهُ وَٱلْمُؤْمِنُونَ}

صدق الله العظيم

سورة التوبة (الآية رقم 105)

Dedication

It is with my genuine gratefulness and warmest regard that I dedicate this work to my beloved parents, my loving husband and lovely kids, **Aws, Aseel, and Ayham**.

I express my very profound gratitude to my parents, **Mrs. Zakia Shoiab** and **Dr. Ali Obead**, and to my loving husband **Mohamed Alshowaihdy** my siblings for providing me with unfailing support to pursue my aspirations not only through the process of researching and writing this thesis but throughout all my years of study. This accomplishment would not have been possible without them. Thank you!

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First, I thank **ALLHA** Almighty for giving me the ability to complete this research.

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Nada Ali Obead

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List of Abbreviations

Abbreviation	Meaning
WSL	White spot lesion
ICDAS	International Caries Detection and Assessment System
F	Fluoride
HCL	Hydrochloric acid
3D	Three-dimensional
MIT	Minimal invasive treatment
DC	Dental caries

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Abstract

Aim of the study: The aim of this in vitro study was to evaluate the effect of the resin infiltration technique and remineralization of induced enamel caries with fluoride solution on the color masking of white spot lesions and the surface roughness. **Materials and Methods:** A total of 45 sound teeth were used in this study. All the teeth were sectioned along the long axes into two halves lingual and buccal halves to get 90 specimens. All specimens were immersed in demineralized solution for seven days. The specimens were devided randomly into three equal groups (n=30) according to the type of treatment; **Group 1** (n=30) was treated with fluoride varnish(Clinpro). **Group 2** (n=30) was treated by resin infiltration (Icon); **Group 3** (n=30): was used as a control group with no treatment. The color and surface roughness were measured three times at baseline (T1), directly after artificial WSLs (T2), directly after application of the treatment options (T3). The colors were measured using a portable reflective spectrophotometer and the 3D surface roughness was measured using a Light Sectioning Vision System. Data were collected and statistically analyzed using T test and Mann Whitney u test. **The results:** Surface roughness was almost equal in the study groups with no statistically significant differences reported. Icon

showed slightly higher color scores than that of ClinPro. **Conclusions:** The Icon produced favorable esthetic results compared to the fluoride therapy while no significant differences were reported regarding the surface roughness. **Key words:** WSL, resin infiltration, fluoride, color masking, surface roughness.

Chapter 1

Introduction

Dental caries (DC) is one of the most prevalent chronic diseases globally. Early recognition of the disease process, before cavitation, is important to implement intervention in an attempt to stop and even reverse the disease process (1). The first clinically visible stage of the carious enamel lesions is characterized by enamel demineralization without cavitation. Such diseased enamel usually presents a near intact surface layer of 10–100 μm thickness with a subsurface porous area called the body of the lesion. The pores are formed as a consequence of partial dissolution of hydroxyapatite crystals due to organic acids produced by biofilm cariogenic bacteria metabolizing carbohydrate (2). Due to the significant difference of the refractive indices of the medium inside the acid-created pores of the demineralized area, a whitish opaque appearance of these lesions can be observed. This phenomenon is called a white spot lesion (WSL) (3). The demineralized enamel surface characterized by roughness and porosity which has an important role in plaque retention and bacterial adherence, over time colonization of acidogenic bacteria resulted in an active caries lesion of the enamel, such roughness and porosity causing enamel discoloration that can lead to unsatisfactory aesthetic (4).

Within the modern concept of dental caries management, prevention and hard tissue preservation are the primary goals. The treatment of choice for non-cavitated lesions should be based on the diagnosis of the lesions, both its extension and the status of active or inactive, the caries risk at the patient level, and the best evidence available to support the treatment decision (5). Currently, the most internationally recognized and used visual caries detection system, is the International Caries Detection and Assessment System (ICDAS). The main aim of the ICDAS methodology is to detect and classify the small variations in visual signs that occur at the

tooth level throughout the progression of the caries disease (6). Overall management of WSL involves methods of preventing demineralisation as well as methods of encouraging remineralisation of existing lesions (7).

Starting with the least invasive, most preventative treatments, which is fluoride therapy (8). Fluoride slows down caries progression by interfering with the dynamics of the caries process, reducing enamel demineralization and enhancing its remineralization (9).

Resin infiltration is a novel treatment option for incipient carious enamel lesions that aim to fill, reinforce and stabilize demineralized enamel, without drilling and sacrificing healthy tooth structure, it has also been shown to inhibit caries progression in lesions on smooth surfaces that are too advanced for remineralization therapy (10). Furthermore, the advantages of these minimally invasive treatments are virtually painless as it requires no anesthesia and meets the compliance of young patients (3).

Therefore, this study was conducted for evaluation of the effect of resin infiltration and fluoride therapy on color masking and surface roughness of incipient enamel lesions.

Chapter 2

Literature Review

2.1. Overview of Dental Caries

Despite the fact that the prevalence of dental caries has decreased over the past decades, it still remains one of the most prevalent disease worldwide. It is defined as a multifactorial, infectious oral disease characterized by localized demineralization of tooth structure caused by acid that is generated as byproduct of bacterial metabolism in dental plaque biofilm (11).

Featherstone 2001, declared that in the demineralization process acids can dissolve the calcium phosphate mineral of tooth structure, and if this process is not halted or reversed via remineralization by redeposit ion of mineral via saliva it may progress from demineralization to non-cavitated lesions, and finally to cavitated lesions (4). Demineralization and remineralization happen simultaneously in the oral cavity and are considered as a dynamic and continuous process that occurs throughout the whole life of the tooth (12). Also, **Featherstone 2001** described the categories of dental caries that are mostly considered by clinicians and researchers are:

- Smooth-surface caries.
- Pit and fissure caries.
- Enamel caries.
- Dentinal caries.
- Secondary caries.
- Early childhood caries.
- Root caries.

Other subdivisions may also be considered and described clinically or histologically. The basic mechanism of dental caries is the same for all of

these so-called "types of caries". The mineral is lost through attack by acid generated by bacteria (4).

Featherstone 2004, postulated that the natural body repair mechanism for dental caries is remineralization related primarily to minerals from saliva or other topical sources diffusing back into the porous subsurface region of the caries lesion (13).

Chen and Wang 2010, reported that in the demineralization process, the bacteria produces organic acids that diffuse into the tooth through the water surrounding the hydroxyapatite crystals, which are the major constituents of tooth enamel and dentin, calcium and phosphate are dissolved and transferred into the surrounding aqueous phase between the crystals so, if the diffusion of calcium, phosphate and carbon ate out of the tooth is allowed to continue without proper remineralization, cavitation will eventually take place(14).

2.2. Incipient Caries Lesion in Enamel

Moreno and Zahradnik 1979, described the histological feature of the incipient enamel lesion as it has relatively sound surface layer of enamel overlying the demineralized zone and the bulk of the mineral loss in the early stages of the demineralization occurs at some distance from the enamel surface for this reason the term subsurface lesion is used. Another term is used to describe the appearance of the lesion which is white spot (15).

Mariotti, Angelo 2007 described thin ground sections of teeth with incipient enamel lesion visualized by polarized light microscopy. Illustrated in Figure 1, the process of demineralization and remineralization often associated with white spot lesions. The outermost 30 microns of the white

spot lesion often is called the surface zone. This area of ground section appears relatively intact but may be more porous than sound enamel. The surface is relatively intact as a result of the remineralization of calcium, phosphate in saliva. Subjacent to the surface zone is the "body of the lesion" which is the most demineralized part of the lesion. If the lesion continues to progress the surface zone will develop small defects allowing acids to more rapidly diffuse below the surface. If a demineralization environment persists, surface enamel will be undermined, and cavitation will occur. Once cavitation occurs, bacteria can readily invade the underlying dentin and are less likely to be affected by preventive treatments (16).

Azizi 2015, explained that the loss of enamel translucency in these areas was attributed to the extensive subsurface porosity caused by demineralization (12).

M0hamed et al., 2018 declared that demineralization of enamel resulted in a visible change in the appearance of tooth enamel. It starts to lose its gloss and shine (translucency) and takes an opaque and chalky-white appearance called white spot lesion (WSL) which is denoted the earliest evidence of caries on smooth enamel surfaces (17).

M0hamed et al., 2018,also mentioned that the demineralized enamel surface also characterized by roughness and porosity which plays an important role in plaque retention and bacterial adherence, overtime colonization of acidogenic bacteria resulted in an active caries lesion and also causing enamel discoloration that can lead to unsatisfactory esthetic (17).

Roig-vanaclocha et al., 2020 reported in their study that dental enamel is a highly mineralized tissue; it is composed of a high percentage

of minerals (98%), with some organic components and water (2%) so, when the composition of dental enamel undergoes a reduction in minerals, and an increase in organic components, dental discoloration appeared as white spots and characterized by an intact exterior surface and a deeper demineralized area. These spots are perceived by the human eye as opaque white-colored lesions due to light refraction that falls on the demineralized areas, the effect of scattering is the function of the difference in refractive indices of two involved component which are enamel prism surrounded by a fluid medium, the refractive index of enamel is 1.65 while the refractive index of water is 1.33 and of air is 1.00. The larger the differences in the refractive indices the greater scattering are produced at the enamel/air interface (18).

Chen et al., 2010, reviewed that whether dental caries development is progressive, static or reversal is dependent on a balance between demineralization and remineralization. Therefore, any factor that can push this balance towards remineralization happening can be utilized as a weapon in the battle against dental caries (14).

According to **Pitts 2004** the international trend in caries management is to move away from the surgical model which involve to excise and replace diseased tooth tissue, towards a preventive approach aiming to control the initiation and progression of the disease process over a person's lifetime (medical model) (19). The medical model for caries management is a philosophy where optimizing dental health by eliminating caries is the primary aim. Under this model, the onset of caries disease should be prevented, ongoing current disease processes should be arrested and eliminated, and damage should be reversed as far as possible. Prevention is

better than cure the medical model of caries management addresses the preventive aspect of the caries disease (20).

Ayad et al., 2020 evaluated the effect of different remineralizing agents and resin infiltration on resistance to demineralization of artificial enamel lesions using a pH cycling model. They found that if the demineralizing process is not interrupted and reversed, WSLs might progress from demineralization to non-cavitated lesions and eventually become cavitated. Consequently, remineralization of white spot lesions instead of restoring such lesions is considered one of the important aspects of minimal intervention in modern dentistry. Fluoride is the basis of the non- invasive management as well as casein phosphopeptide-amorphous calcium phosphate-containing products. Additionally, resin infiltration is also one of the treatment modalities. It is considered as a micro-invasive treatment, that eliminates the need for conventional restoration (21).

Tyas et al., 2000 suggested the following important strategies for keeping teeth free from carious lesions: (i) early caries detection and assessment of caries activity and caries risk with validated instruments, (ii) remineralisation of demineralised enamel and dentine, (iii) optimal caries-preventive measures, (iv) minimally invasive operative interventions, and (v) repair rather than replacement of defective restorations (22).

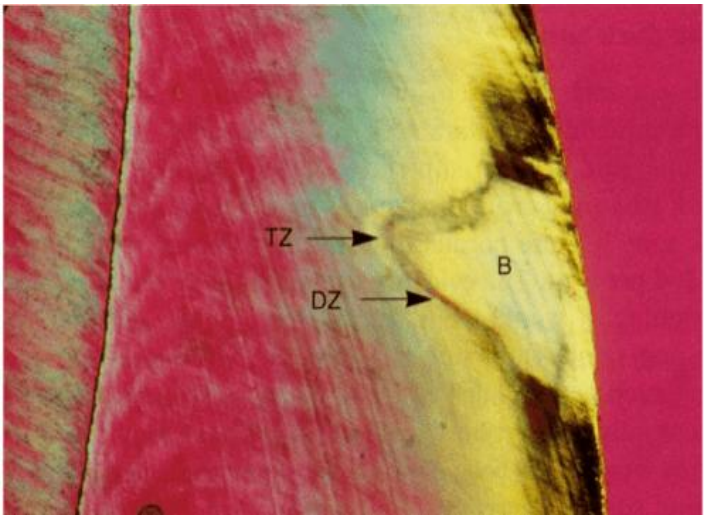


Figure 1: A thin ground section of a white spot lesion visualized with polarized light microscopy demonstrated the zones of enamel caries – body (B), dark zone (DZ) and translucent zone (TZ).(23)

2.3. Role of Fluoride in Dentistry

2.3.1. What is the fluoride?

Fluoride is the ionic form of the trace element fluorine, a member of the halogen group of elements, fluorine is common in the environment, reaching the hydrosphere by leaching from soils and rocks into the groundwater, most ionic forms of fluoride are readily soluble in water. Generally, the majority of fluoride is absorbed in the stomach and the remainder in the upper small intestine (24).

2.3.2. The discovery of fluoride

Fluoride has been used in dentistry for over 100 years for the purpose of preventing dental caries(24). Fluoride was discovered as the side effect of fluorosis in teeth in areas with elevated levels of fluoride in the drinking water (25).

The pioneers in this field were Black and McKay. They started observing and describing the effects of fluoride in the late 19th and the beginning of the 20th century (26). During the period prior to the discovery of fluoride as the etiological agent of mottled enamel, **McKay 1929** studied brown stains on enamel in population from different parts of the USA and he described them as "Colorado stains" (27).

Dean 1938, discovered that patients with this "Colorado stain" had a lower prevalence of dental caries. Also, he found out that the percentage of carious teeth was less than in non-endemic areas (28).

Churchill 1931, discovered the causative factor of mottled enamel on his study and he approved that the defect occurred in certain geographical areas, and the causal factors seem to be associated with the water supply of those areas, which contained fluorides (29).

Furthermore, a study was performed by **Short 1944** regarding the inverse relationship between the fluoride content of the public water supply and the dental caries experience. He found that drinking water with 1ppm of fluoride can prevent dental caries, increase tooth strength, and does not have a negative impact on enamel (30). **In the mid 1940s**, the first experimental trials of water fluoridation, to bring the fluoride concentration up to an optimal level to prevent dental caries, were conducted in the USA and Canada (31).

Bull 1950, compared enamel changes to fluoride concentration in drinking water and the relation between fluoride content and the quantity of enamel spots**;** he documented that dental fluorosis was prevalence in the USA and on comparing it to caries prevalence in children, he discovered that fluoride was a potent cariostatic agent (31).

Whitford 1987 reviewed 95 water fluoridation studies in 20 countries between 1945 and 1978. They reported that higher caries reductions of 40-50% of primary and 50-60% of permanent teeth were observed in this earlier period, these observations and discoveries triggered massive drinking water fluoridation, the use of fluoridated salt and milk and an increase in dietary supplement production pills, drops, chewing gum, lozenges (32).

2.3.3. How fluoride works? (Topical vs Systemic)

Bibby et al., 1950 conducted a study comparing the efficacy of fluoride-coated pills intended to be swallowed, with fluoride lozenges intended to be dissolved slowly in the mouth. Their findings were interpreted to indicate that the use of fluoride lozenges might contributed to the control of the dental caries and that the caries reduction produced by such lozenges could be the result of fluorine acting on the external surfaces of the teeth (33).

Dirks 1966 were the first to report clinical remineralization in the famous Tiel-Culemborg study of water fluoridation. This study was also the first in which active and arrested lesions or cavities were diagnosed and recorded separately, as well as the conversions among these various states during the six years of the study. Remineralization was observed in study cities, but it was more obvious in the fluoridated city Tiel (34).

Koulourides et al., 1974 developed the intraoral cariogenicity test. The initial study revealed that enamel preformed lesions could be hardened or remineralized in the mouth, and this process also enhanced by fluoride. Due to the incorporation of a large amount of fluoride in the newly formed hydroxyapatite from the environmental fluid (35).

Ten Cate and Featherstone 1991 postulated that the caries inhibiting effect of fluoride were thought to be primarily due to the incorporation of fluoride by the minerals during development of the teeth (36). Although Fluoride incorporated during tooth development is insufficient to play a role in the mechanisms involved in caries protection, the primary effect of fluoride is post-eruptive. Fluoride is needed regularly throughout life to protect teeth against caries (4).

Authers have stressed the importance of fluoride interactions at the interface between the tooth and the oral fluids (36).

2.3.4. Mechanisms of fluoride on caries control

It was reported that the fluoride ion fits well in the structure of a hydroxyapatite crystal, better than the hydroxyl group, which resulted in lower solubility of fluoridated apatite when compared with fluoride-free apatite (37). The important effect of fluoride was borne from the systemic ingestion of fluoride and its incorporation into developing enamel. Since the 1980s, research has started to concentrate on the topical effects of fluoride on the caries process which was considered to be the most significant (24).

The fluoride ion (F) has been widely used topically in the treatment of dental caries for its anticariogenic and antimicrobial properties. The antibacterial action of fluoride is due to the acidification of the bacterial cytoplasm through the formation of H+ and F- ions from hydrogen fluoride and the disruption the bacterial metabolism by inhibition of vital bacterial enzymes such as proton releasing adenosine triphosphatase and enolase (38). There are three principal methods that have been suggested by which fluoride can have a topical effect on dental caries: 1) in the presence of fluoride remineralization is encouraged. 2) The apatite formed in the

presence of fluoride is more resistant to acid attack. 3) Fluoride may inhibit bacterial metabolism when it diffuses into an acidified plaque as hydrogen fluoride. Hence, fluoride promotes remineralization, discourages demineralization and may reduce the action of plaque bacteria by inhibiting their growth (4,26).

2.3.5. Fluoride application

The different ways of using fluoride are classified, as shown in Figure 2, according to the strategy used to deliver fluoride to the oral cavity: community based, individual, professional, and the combinations of these (19).

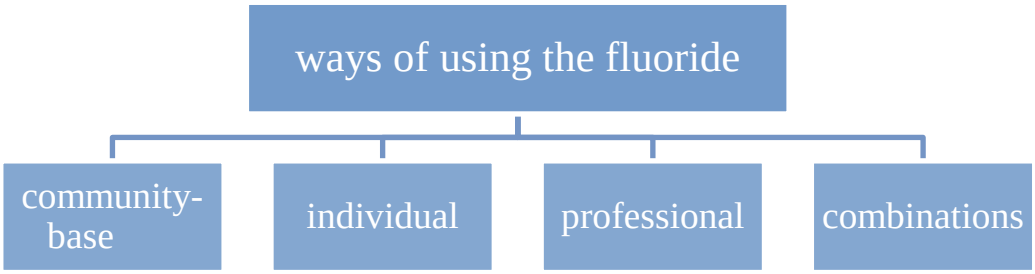


Figure 2: Different ways of using Fluoride

The WHO oral health report noted that dental caries can be controlled by the joint action of communities, professionals and individuals aimed at reducing the impact of sugar consumption and emphasizing the beneficial impact of fluoride (39). Fluoride has been delivered to the oral cavity by a number of methods and mediums including fluoridated water, salt, tea and milk, toothpaste, mouth washes, gels and varnishes, dental restorative materials, fluoride particle coated prostheses, and various fluoride delivery devices (38).

2.3.6. Overview of fluoride varnish

Fluoride varnishes have been used for the prevention and control of dental caries since the 1960s (38). Fluoride solutions in higher concentrations are applied by a dental professional from two to four times a year (40).

2.3.6.1. The advantages of varnishes (38)(41)

- Their simple application.
- Prolonged contact with the demineralized surface of the enamel
- Relatively low-cost and easily operated treatment and has been used to arrest active dental caries.
- Requiring no special equipment.
- Well tolerated by patients.

2.3.6.2. Indication of fluoride varnish

Varnishes are indicated for therapeutic applications to control active caries, root surface caries, xerostomia patients, hypersensitive areas of enamel and dentine, for orthodontic patients and physically or mentally handicapped patients (38).

2.3.6.3. Application of fluoride varnish

Fluoride varnishes are not intended to adhere permanently to a tooth, but to remain in close contact with enamel for several hours. During application, the clinician uses a brush, a cotton-tip applicator or a syringe-type applicator (included with the product) to apply varnish directly onto the teeth. Application time is one to four minutes, depending on the number of teeth present. Because the varnish sets in contact with intraoral moisture, thorough drying is not required before application. To maximize contact

between the varnish and the teeth, patients are instructed to avoid eating for two to four hours after the application and to avoid brushing their teeth the night of the application (42). Topical fluoride varnish contains 5% sodium fluoride and was developed to enhance the retention of fluoride on tooth surfaces. It is applied directly onto the tooth surface with the property of adhering to the tooth surface. It allows continuous release of fluoride ions into enamel, dentine, plaque, and saliva. It offers an effective means of not only preventing caries, but also arresting early enamel lesions (43).

A commercial formula which is used in this study, ClinPro™ (3MESPE), contains f TCP and 5% sodium fluoride. Manufacturer of ClinPro™ has claimed that the protective fumaric acid barrier present in the product helps in the coexistence of calcium and fluoride ions but preventing unwanted reaction between them during its storage. When the agent comes into contact with saliva, the protective barrier breaks, and the ions are released for effective tooth remineralization (44).

The caries preventive effect of topical fluoride therapy depends on an adequate supply of calcium and phosphate ions. Although calcium and phosphate ions are supplied naturally by saliva, the concentration of such ions is low. Low concentration of salivary calcium and phosphate ions leads to mineral deposition only at the surface of the enamel as a result of the low ion concentration gradient. Deposition of minerals at the surface of enamel alone may not improve the structural properties of the deeper part of the white spot lesion (45).

Gao et al., 2016 showed that the use of a 5% sodium fluoride varnish can remineralize incipient caries lesions, thus making this option an important method to inhibit enamel demineralization (46). Sodium fluoride

was chosen as the fluorine vehicle because its activity in prevention of dental decay has been so well demonstrated (19). Additional ingredients or chemical agents such as chlorhexidine or calcium fluoride were added into the NaF varnish, but the addition had no significant effect on remineralising early enamel caries. Thickening agents such as colophony resin, gums and waxes have been added to varnishes to promote and prolong their contact time with the tooth surface (41)(46).

Elkassas et al., 2014 conducted a study to assess enamel remineralization of ClinPro^{TM} white varnish, Tooth Mousse Plus and VanishTMXT and evaluated their remineralizing efficiency at baseline, after demineralization, after 2 and 4 weeks remineralization and after final acid challenge. They concluded that ClinPro^{TM} varnish presented the highest remineralization tendency with the greatest resistance for the final acid challenge (47).

Gontijo et al., 2007 concluded that fluoride varnish could be considered an efficient preventive method to enhance enamel resistance against cariogenic challenges during orthodontic therapy. Their findings demonstrated that significant calcium fluoride-like material (CaF2) depositions were found and acted as a reaction product of fluoride varnish when applied adjacent to orthodontic brackets. Furthermore, the study reported that the application of fluoride varnish was even more beneficial for less compliant patients compared with those resorting to fluoride mouth rinse on a daily basis (48).

Yetkiner et al., 2014 performed a study to compare the color-masking effect of infiltration, fluoride remineralization, and micro-abrasion treatments of WSLs plus, the resistance of these treated surfaces against discoloration. Colour measurements were assessed at standardized ambient

conditions using a spectrophotometer and they found that low-concentration fluoride treatment improved the WSL appearance more than the clinical detectable limit, but the stability was not different than the effect of saliva remineralization (49).

Fluoride is not able to replenish the porous area inside the enamel with minerals, but will help impair the process, resulting in the arrestment of the caries lesion progression. The white spot will eventually have a shiny surface, as a result of surface polishing and remineralization in the presence of fluoride, but the white aspect, from the porous areas underneath, will partially remain (9).

2.4. Resin Infiltration as Microinvasive Approach

2.4.1. Resin Infiltration Concept:

Caries resin infiltration represents a new concept in dentistry, offering beneficial clinical applicability for clinicians and high acceptance by patients; from the available in vitro, in vivo, and in situ studies, it seems convincing that the resin infiltration of enamel lesions is effective in arresting and stabilizing the progress of WSLs (50). This novel technique might bridge the gap between noninvasive and minimally invasive treatment of initial dental caries, postponing as long as possible the need for a restoration. Early studies began in the 1970s with experiments involving commercially available adhesives and sealants demonstrated that these adhesives and sealants are effective infilterant of artificially created lesions (51).

Dayila 1975 performed a study of the new materials and techniques developed to bond adhesives to pit and fissure areas might be useful in arresting the progressive development of white spots. Adhesives have been

shown to infiltrate spaces created in normal enamel by acid conditioning. These materials may seal the lesion entrances and spaces arresting demineralization and obviating cavity formation. They assessed in vitro the ability of an ultraviolet light- polymerized adhesive to impregnate artificial and natural white spots. The result of the study indicated that liquid adhesives of low molecular weight polymerizable monomers could flow through the lesion to fill partially or completely the microspaces (52).

Robinson et al., 2001 have examined the artificial lesions of enamel were generated in extracted human teeth using acidified gels; a range of currently available adhesive materials was then used to infiltrate the porosities. The extent of occlusion of the lesion porosities was determined both qualitatively using light microscopy and quantitatively using a chloronaphthalene imbibition technique. Results showed that up to 60% of the lesion pore volume had been occluded following infiltration with some of the materials and that this treatment was capable of reducing further acid demineralization (53).

Meyer-Lueckel et al., 2006 illustrated that sealing of enamel lesions by infiltration with low viscous resins seem to be a promising approach in non-operative dentistry and should bear advantages compared to remineralization or invasive treatment. The in vitro study was to evaluate the penetration ability of five dental adhesives and a fissure sealant into initial enamel lesions for an application time of either 15’s or 30’s, It could be shown that sealants penetrate the body of the lesion up to 95% and reduce the accessible pore volumes within the lesions significantly. Moreover, it was observed that sealants were capable to inhibit further lesions progress under demineralizing conditions (54).

In 2009 the concept of caries infiltration was first developed in the Charité Berlin and the University of Kiel in close cooperation with the two leading developers Dr. H. Meyer-Lückel and Dr. Sebastian Paris, as a microinvasive approach for the management of smooth surface and proximal non-cavitated caries lesions. It is marketed under the name Icon® (DMG America Company, Englewood, NJ) (12), (55).

The material has become a promising minimally invasive treatment for non-cavitated initial caries lesions (International Caries Detection and Assessment System (ICDAS code 1&2). ICDAS code 1&2 natural enamel caries lesions are characterized by a pseudo-intact surface layer and a demineralized subsurface body of the lesion (56). Since the year 2000, caries infiltration was first examined in vitro and the first in vivo study developed by Meyer-Lückel H, 2010 to assess the efficacy of resin infiltration of proximal carious lesions with a low-viscous resin (55).

2.4.2. The mechanism of action of resin infiltration:

The caries infiltration technique was introduced with the aim of filling the intercrystalline spaces of the early enamel lesion by capillary action with a low-viscosity resin without drilling, to arrest lesions. Since porosities of enamel caries lesions act as diffusion pathways for acids and dissolved minerals, infiltration of these pores with low-viscosity resins occlude the pathways and thus hamper or arrest caries progression. In contrast to the application of sealants, where the diffusion barrier remains on the enamel surface as a covering resin coat, the resin infiltration creates a diffusion barrier within the enamel lesion and enables the strengthening of the demineralized enamel structure with the resin matrix, preventing cavity formation (17)**,**(57)**,**(58).

Taher et al., 2012 investigated the effect of a resin infiltrant on the surface roughness of healthy enamel and compared it with a fissure sealant, Sound enamel surfaces were treated with a resin infiltrant (Icon) or fissure sealant (Seal-Rite). The average roughness of the specimens were measured with a profilometer. In contrast to fissure sealing, in which a diffusion barrier is placed on top of the lesion surface, the infiltration technique aimed to create a diffusion barrier inside the lesion, by replacing lost minerals with resin. The fissure sealant used in the above mentioned study showed good roughness results, with a lower roughness than those of the controls and infiltrate groups. The obtained results might be related to the remaining resin layer on top of the enamel surface. The infiltrant group demonstrated no significant difference in roughness compared to the control group (59). The roughness and porosity characteristics of the demineralized enamel surface, usually contribute to pigment precipitation, rough surfaces are vulnerable to dye uptake from coffee, tea and become darker over time leading to unsatisfactory aesthetic (17).

Arnold et al., 2016 evaluated surface roughness of initial enamel caries lesions in human teeth after resin infiltration to determine changes in roughness for resin infiltrated enamel lesions after short-term thermocycling, the surface roughness was determined with an Infinite Focus G3 optical profilometer. The differences between the untreated surface and the surfaces after resin infiltration as well as the first thermocycle were not significant. No significant differences were found in the negative control surfaces (56).

2.4.3. Color masking properties of Icon

In addition to its action in the control of caries progression, the resin infiltration of white spot lesions produces a positive effect in masking the color of teeth, because the lesions tend to lose their whitish appearance, looking similar to sound enamel (60).

Paris et al., 2013 performed an in vitro study to assess the influence of various refractive indices of experimental infiltrants on the appearance of artificial enamel caries lesions. The aim of this in vitro study was to evaluate if refractive index RI of infiltrants correlated with their ability to mask enamel caries lesions using Digital photographs were obtained under standardized conditions, and he found that the masking effect of enamel caries was caused by infiltrating the lesions using resins with a similar refractive index (RI of infiltrant: 1.52) to sound enamel. Thus, light scattering is reduced and visual color differences to enamel decreased (61).

Yetkiner et al., 2014 checked the Color improvement and stability of white spot lesions following infiltration, micro-abrasion, or fluoride treatments in vitro, in which the effects of different treatments were evaluated by the change in color components (L*, a*, b*). The standard quantification of color change was performed using a spectrophotometer. Based on the obtained results, infiltration and micro-abrasion treatments performed better in diminishing the opaque WSL appearance compared with the fluoride treatment and control. This effect was stable only for the infiltration treatment under discoloring effects (49).

2.4.4. The composition of Icon, DMG

The infiltration treatment consists of three easy steps Etching, Drying, Infiltrating. All the materials required are contained in every Icon package.

1. Icon-Etch: Prepares the tooth for infiltration. The HCl-Gel is applied to the affected area with the aid of our special applicator tip, removing the pseudo-intact surface layer. Only when this layer has been removed can the infiltrant penetrate into the pore system of the tooth.
2. Icon-Dry: For the next step of the infiltration process, a dry environment is necessary. To this end, the lesion is dried with Icon-Dry (ethanol) and air.
3. Icon-Infiltrant: The low viscosity infiltrant is applied and penetrates deep into the enamel through capillary action and it is then light cured. The infiltrated lesion has similar mechanical and visual properties to healthy enamel, which is composed of triethylene-glycol-dimethacrylate–based resin, bisphenol A glycerolate dimethacrylate, camphorquinone, and ethyl 4-(dimethyl- amino) benzoate and ethanol which has an extremely high penetration coefficient that facilitates deeper penetration (51).

2.4.5. Resin infiltration steps and procedure

The procedure for resin infiltration is fairly simple and acceptable by operators and patients. ICON® is marketed in two different forms: Proximal surface and vestibular surface kits. The principle of usage in both is similar except for the need for separation in case of proximal lesion treatment. As the mineralized surface layer of the non-cavitated lesions hampers resin from penetrating into the lesion. This layer should be

removed. Hydrochloric acid gel (15%) (Icon Etch) applied for 120 seconds. The surface is then dehydrated with 99% ethanol (Icon Dry) to facilitate the drying process. Then the application of infiltrant resin with brushing motions. The last step involves light curing of the resin following a three-minute application time. The excess resin is then removed, and the surface is polished (12) (55).

2.5. Color Assessment

Color measurements in dentistry often involve nonhomogeneous layers of both natural and prosthetic materials with varying color and translucency (62). In the clinic, these materials often present with an external curved surface. By contrast, color and reflectance standards are flat and opaque, mainly due to the complicating effects of both translucency and non-planar surfaces on color measurement (63).

Color perception by human observers involves the spectrum of visible light entering the eye and stimulating the three types of color receptors in the eye's retina, which in turn communicate to the brain via the optic nerve (64). Given the three types of color receptors in the eye, it is most common to specify the color an observer would detect with three color parameters. Although other color order systems described color as being three-dimensional (65).

Kuehni 2002 was the first to separate into perceptually uniform and independent dimensions of hue, value and chroma, and was the first to systematically illustrate the colors in three-dimensional space (66). For materials, it is the interaction of the illuminant and the material which allows for light to be either reflected or transmitted to a human observer,

depending on the relative placements of the illuminant, the material, and the observer (64).

2.5.1. Spectrophotometric and colorimetric measurements:

The conversion of spectrophotometric measurements to three color parameters (68) is described by the International Commission on Illumination. (Known as the CIE for its name in French) and has been well exemplified (65). This conversion requires knowledge of: 1) the spectrum of light from the illuminant, 2) the spectrum reflected or transmitted by the material, and 3) the three spectral observation characteristics of the human observer (67). Some colorimeters approximate with filters the light from a specific illuminant and the specific observation characteristics of the human observer. This color data is then valid only for that illuminant and observer.

Significant advantages to spectrophotometric measurements include the ability to analyze the principal components of a series of spectra and the ability to convert spectrophotometric measures to various color measures (69). The ability to convert a reflectance or transmittance spectra to multiple colorimetric data is facilitated by the available publication of much illumination and the two major observer characteristics (68).

Computer programs are often supplied with spectrophotometric measuring devices to facilitate the conversion of reflectance to the various color parameters (70). The conversion of spectrophotometric measures to color parameters involves a specification of both the illuminant and the observer that was used in the conversion and should be included with the resultant color parameters. There are multiple illuminants possible, including the CIE Illuminant A; represents a glass-enclosed tungsten-

filament bulb, the CIE Illuminants B and C are no longer recommended for use in describing daylight, various CIE D illuminants used to describe various daylight conditions (e.g., D50, D55, D65, and D75), and various CIE F illuminants for fluorescent lamps (68).

Further, CIE describes a transformation of tristimulus values into the CIE L* a* b* color space, which was intended to be uniform in each of its three directions of color, L*, a* and b*. A distinct advantage of the CIE L* a* b* color space was the simplicity of calculating a color difference between two colors using:

$$\Delta E_{CIELAB} = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{½}$$

Color difference has been used extensively in dental research and applications, including descriptions of coverage error of dental shade guides (71), the magnitude of color instability of dental polymers, the change caused by processing dental materials, the mismatch caused by inadequate color specification for prosthetic materials, color accuracy and repeatability (72), color perceptibility and acceptability, and as a translucency parameter. It can be expected, therefore that the revised color difference formula will be used also for these applications (73).

2.6. The Importance of Surface Roughness

The enamel surface presents a natural roughness due to the presence of Retzius grooves, pits and small defects, as well as mineral deposits occur in the oral environment (74). Surface roughness plays an important role in plaque retention and bacterial adherence (75). The demineralized surface of the enamel usually characterized by roughness and porosity causing enamel discoloration and pigment precipitation, that can lead to unsatisfactory

esthetic (76). The significance of the surface roughness of enamel in caries progression and remineralization is critical. Moreover, roughness is essential property of teeth, which influence the attachment of foreign materials to their surfaces. Surface roughness is one of the commonly used tests to assess the effect of different materials on dental hard tissues and is well recognized for quantifying surface texture (77). It has been suggested that remineralizing agents have anti-erosive and anticariogenic properties, when placed on the human enamel surface, it can interact with hydrogen ions, form calcium hydrogen phosphate, which releases calcium and phosphate ions, which prevents the acid dissolution and protect the enamel (78). Remineralization concept is based on compensation of lost minerals from enamel tooth structure by improving the natural ability of saliva to remineralize enamel surfaces (79).

To occlude the tiny porous openings and widened intercrystalline spaces of incipient non- cavitated carious lesions a new technique was developed to infilterate these pores with a low viscous resin. This resinous dental biomaterial encapsulates the remnants of enamel prisms and interacts with the oral environment by re-forming the previously demineralized and roughened enamel into new surface areas (80). Any remaining roughness resulting from the demineralized tooth surface or from overlying material remnants could facilitate biofilm accumulation. Consequently, at these areas, the risk for initiation and/or continuation of the carious process and of inflammation of the gingival tissues could increase. Driven by capillary action, resin infiltrants are capable to penetrate deeply into the porous bed of an initial lesion, and this leads to a hybrid mixture of demineralized enamel prisms and a circumfluent polymerized network of the resin infiltrant. It seems clear that the resin infiltrant will not form a smooth coat

on the lesion surfaces but renders exposed enamel prism remnants uncoated (81).

Ulrich et al., 2015 concluded that the resin infiltrate is not capable to fill up micro porosities, nor to sufficiently flatten the roughness of subsurface lesions to a clinically acceptable grade (75).

No study reported human enamel three-dimensional roughness measured at a similar magnification has been published for comparisons. Furthermore, it is not possible to compare roughness values obtained with contact profilometer along one line of the specimen with those values obtained with the noncontact optical interferometers as surface area. As measurement of surface roughness determined by measurement method, the research protocol for roughness is vital (82). A contact profilometer with a stylus that moves in line is used for the quantitative investigation of roughness, may induce misconception due to holes on the surface, and may injury enamel because of its contact with the surface (74). Other instruments are available to measure roughness at a much higher resolution and over a larger area such as non-contact optical interferometers and atomic force microscopes (AFM). The optical interferometry noncontact profilometer was used to measure surface roughness. Compared with a stylus profilometer, the optical interferometry noncontact profilometer is faster, nondestructive, and allow repeatability. In addition, it provides a larger field and does not need sample preparation in comparison with AFM (83).

Chapter 3

Aim and Objectives of the Study

3.1. The Aim

The aim of this an in vitro study was to evaluate the effect of the resin infiltration technique and remineralization of induced enamel caries lesions with fluoride solution on the color masking of white spot lesions and the surface roughness.

3.2. The Objectives

1- To assess the effect of resin infiltration on the color masking and surface roughness on induced white spot lesion on enamel surface.

2- To assess the effect of fluoride varnish on the color masking and surface roughness on induced white spot lesion on enamel surface.

3- To evaluate and compare the effect of these non invasive techniques.

Chapter 4

Materials and Methods

4.1. Study Design

Artificial WSLs was induced on enamel surface of 45 extracted premolars (N=90, n=30 per test group) were treated forming the following groups: resin infilteration (Icon), fluoride (ClinPro), and control (remain untreated). All the specimens were subjected to demineralized solution for seven days. Color component and surface roughness were measured at baseline (prior to demineralization), after WSL formation, and after the treatment. The experiment design illustrated in Figure 3.

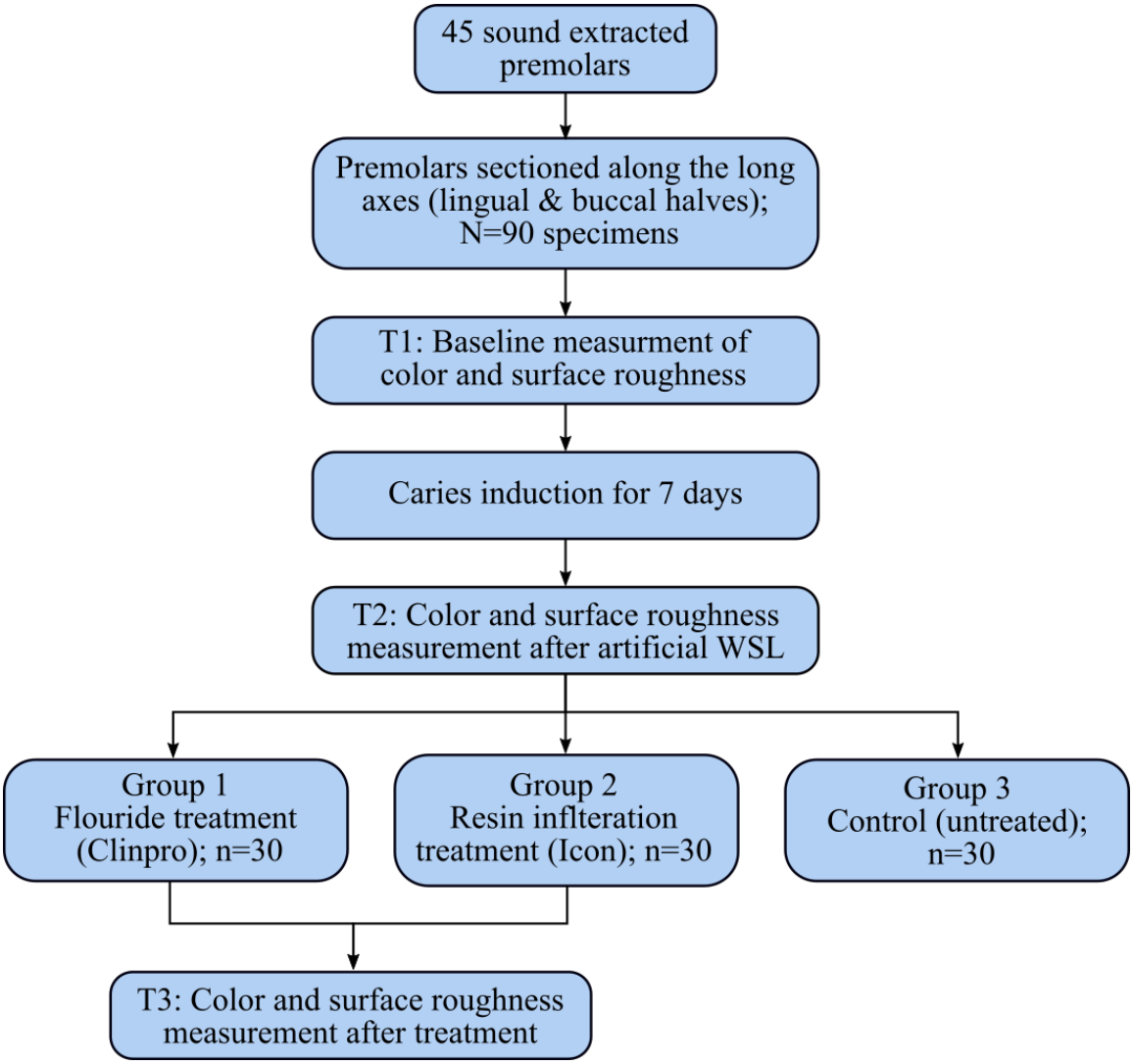


Figure 3: Flow chart of the Study design

4.2. Materials

A description of the types and the composition of the materials used in the study is illustrated in (Table1). The devices and equipment also shown in (Table 2).

Table 1: Materials used in the study

Brand	Composition	Manufacturer	Lot #
Demineralizing solution.	2.2 mM Calcium Chloride, 2.2 NaH2PO4, 0.05 M acetic acid with pH 4.4 adjusted with 1M KOH.	Chemical laboratory at faculty of Pharmacy, Mansoura University, Egypt.	Not available
ClinPro ™	5% sodium fluoride white varnish, tri-calcium phosphate.	3 M ESPE, Pymble, New South Wales, Australia.	N96b5009
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.	783531
Artificial saliva.	Contained 1.5mM CaCl2, 0.9mM NaH2PO4, 0.15M KCl (potassium chloride) at pH 7.0.	Chemical laboratory at faculty of Pharmacy, Mansoura University, Egypt.	Not available
Chemical-cure acrylic resin.		Acrostone manufacture, Cairo, Egypt.	Not available

4.3. Devices Used in the Study

A description of the types of the devices and equipment used in the study are shown in (Table 2).

Table 2: Devices used in the study

The equipment	Manufacture	Uses
Brass mold.		used for specimen mounting in acrylic block.
Portable Reflective spectrophotometer.	X-Rite, model RM200QC, Neu-Isenburg, Germany.	specimens color measurement.
USB Digital microscope with a built-in camera.	U500X Digital Microscope, Guangdong, China.	3D Surface Roughness Measurement.
Light cured machine.	Hilux Ledmax 5, Benlio_glu Dental Inc, Turkey.	for curing of resin infiltration.
Diamond bur.	KG Sorensen, SP industeria, Brazil.	for teeth suctioning.
Low-speed drill.	ARATHON, SAE YANG CO., Korea.	For mounting of the burs
Polishing flexible discs.	Sof-Lex, 3M ESPE, USA.	For polishing of enamel surface after the treatment with Icon.

4.4. Teeth Collection

The present study was carried out on extracted human premolars; teeth were obtained from patient requiring therapeutic extraction, from different public and private dental clinics. A total of 45 sound extracted human premolars were collected and used in this study.

4.5. Inclusion Criteria

Sound teeth, free of:

- Caries or white spot lesions WSLs.
- Developmental defects.
- Physical damage due to extraction.

4.6. Sample Preparation

4.6.1. Tooth cleaning

The teeth were thoroughly cleaned with ultrasonic scalar and slurry pumice to remove all soft-tissue remnants, calculus and plaque, and stored in normal saline at room temperature.

4.6.2. Tooth sectioning

All the teeth were sectioned along the long axes into two halves lingual and buccal halves to get 90 specimens.

As shown in Figure 4, the tooth root was cut with a diamond bur operated on low-speed drill under water coolant. Each crown half was embedded in a brass mold filled with chemical-cure acrylic resin in such way the enamel surface facing upward. The brass mold, as illustrated in Figure 5, compose of:

- Outer ring (20mm diameter and 22mm height) with lock screw for tightening.

- Inner split ring (18 mm diameter and 18 mm height).

Finally, enamel surfaces of the samples were polished with silicone carbide abrasive paper with different grits in ascending manner (400, 600, 800, 1000 and 1200 grits) under running water.

Figure 4: Premolar sectioned into buccal and lingual halves.

Figure 5: Non-assembled brass mold with inner split ring used for specimen mounting in acrylic block.

4.7. Testing Methods

4.7.1. Color assessment at baseline (T1)

The specimens' colors of all the groups were measured using a portable Reflective spectrophotometer illustrated in Figure 6. The aperture size was set to 4 mm and the specimens were exactly aligned with the device. A white background was selected, and measurements were made according to the CIE L*a*b* color space relative to the CIE standard illuminant D65.

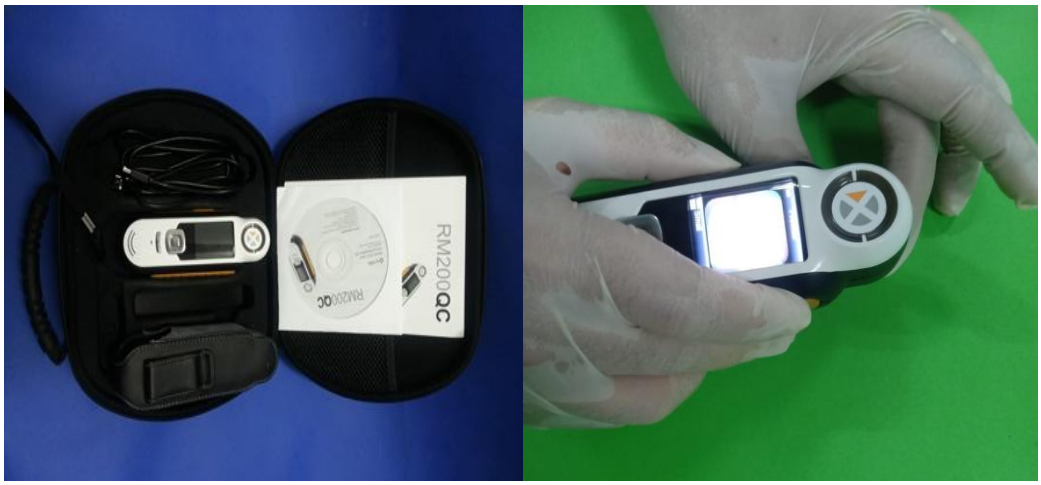


Figure 6: Reflective spectrophotometer used in the study.

4.7.2. Evaluation of roughness at baseline (T1)

The optical methods tend to fulfill the need for quantitative characterization of surface topography without contact (84). Specimens were photographed using USB Digital microscope with a built-in camera connected with an IBM compatible personal computer using a fixed magnification of 90X as shown in Figure 7. The images were recorded with

a resolution of 1280×1024 pixels per image. Digital microscope images were cropped to 350×400 pixels using Microsoft office picture manager to specify/standardize area of roughness measurement. The cropped images were analyzed using WSxM software (Version 5 develop 4.1, Nanotec, Electronica, SL) (85). Within the WSxM software, all limits, sizes, frames and measured parameters are expressed in pixels. Therefore, system calibration was done to convert the pixels into absolute real-world units. Calibration was made by comparing an object of known size (a ruler in this study) with a scale generated by the software. Subsequently, a 3D image of the surface profile of the specimens was created. Three 3D images were collected for each specimen, both in the central area and in the sides at an area of 10 µm × 10 µm WSxM software was used to calculate average roughness expressed in µm which can be assumed as a reliable indices of surface roughness (86).

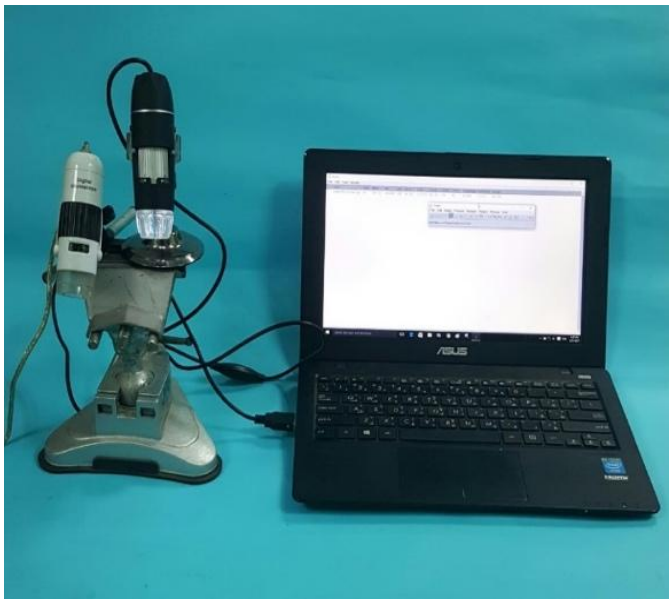


Figure 7: USB digital microscope

4.8.Caries induction

Artificial WSLs was induced on buccal and lingual enamel surface using a demineralizing solution. All specimens were immersed in demineralized solution for 7 days. The demineralizing solution, shown in Figure 8, composed of 2.2mM Calcium Chloride, 2.2 NaH_2PO_4, 0.05 M acetic acid with pH 4.4 adjusted with 1M KOH.

All the specimens were rinsed and stored in artificial saliva (Artificial saliva was prepared according to the formulation of Ten Cate and Duijsters) (87) which contained 1.5mM $CaCl_2$, 0.9mM NaH_2PO_4, 0.15M KCl (potassium chloride) at pH 7.0 after demineralization till the time of testing.

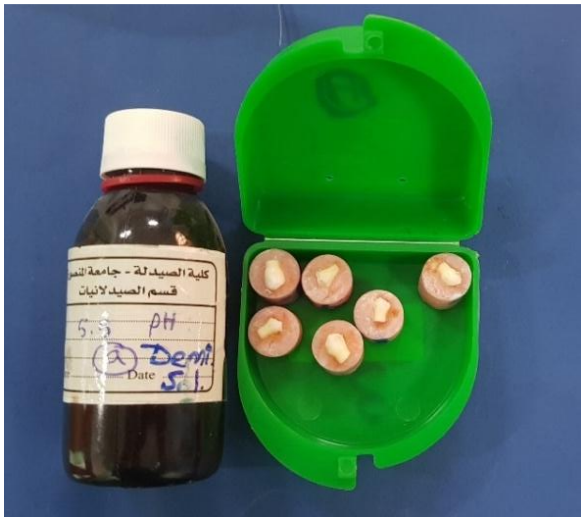


Figure 8: Demineralizing solution used for artificial WSLs induction.

4.9.Color and roughness evaluation after artificial WSLs (T2)

Directly after artificial WSLs (T2) color and roughness were evaluated in the same manner at baseline to quantify the change. The color changes (ΔE) of the specimens were evaluated using the following formula:

$$\Delta E_{CIELAB} = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{½}$$

Where: L* = lightness (0-100), a* = (change the color of the axis red/green) and b* = (color variation axis yellow/blue).

4.10.Grouping and Intervention

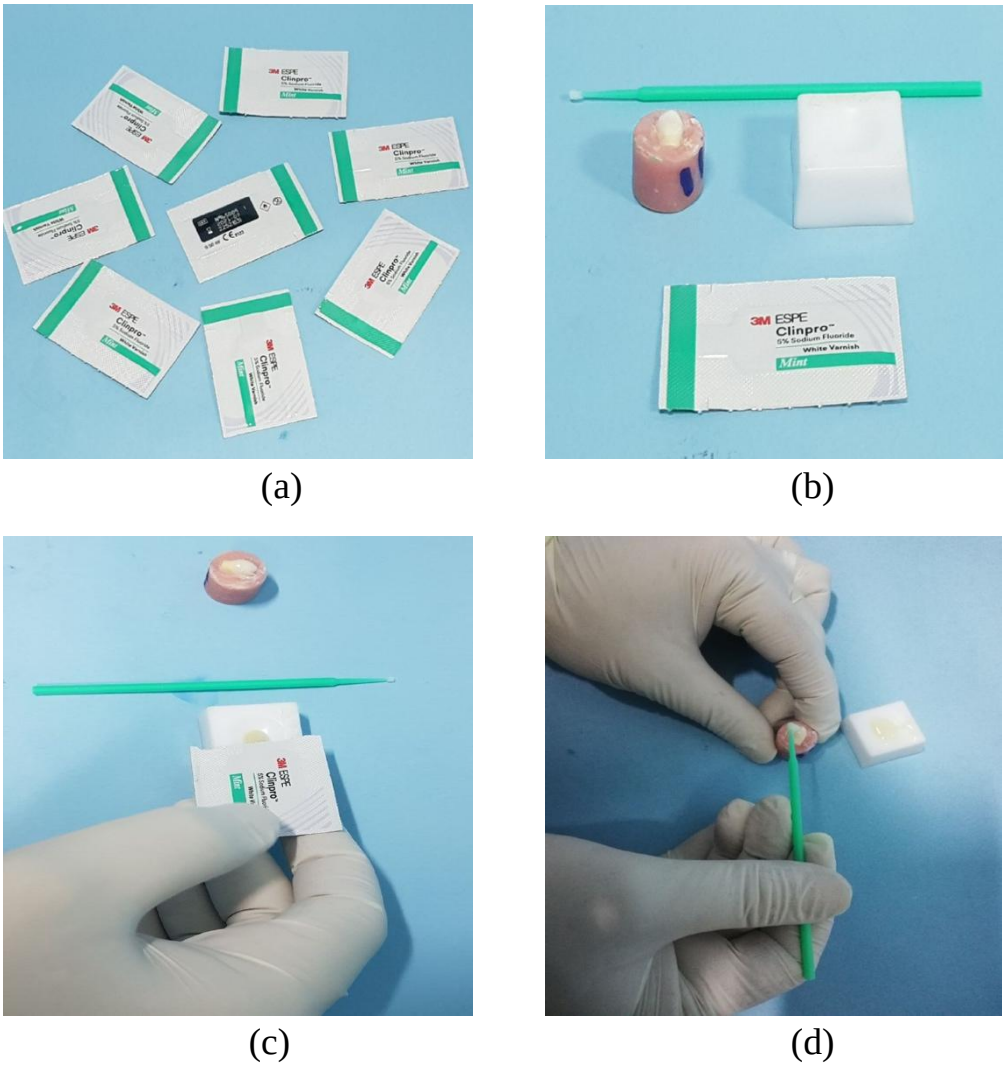
Specimens were devided randomly into three equal groups (n=30) according to the type of treatments.

- **Group 1** (n=30): was treated with fluoride varnish.
- **Group 2** (n=30): was treated by resin infiltration.
- **Group 3** (n=30): was used as a control group with no treatment.

4.11.Material application

4.11.1.Fluoride application (group 1)

Specimens were treated with NaF white varnish as shown in Figure 9 (a)-(d). A thin, uniform layer of the varnish was applied in a single stroke painting motion. The varnish was also left undisturbed for 20 seconds. After the daily fluoride treatment, the specimens were rinsed with deionized water and stored in artifitial saliva. The remining varnish was removed using a no. 15 scalpel blade followed by cleaning off the surface with cotton tip immersed in deionized water.



(a) (b)

(c) (d)

Figure 9: Sodium fluoride white varnish and the steps of its application. (a) The sodium fluoride (ClinPro). (b) Brush and dipping dish used for material application. (c) Putting the fluoride varnish in the dish. (d) Fluoride application using the brush

4.11.2. Resin infiltration application (group 2)

Specimens treated with resin infiltrated and stored in artificial saliva until the time of testing. Figure 10 presents the used resin infiltration icon kit.

Figure 10: Resin infiltration icon kit

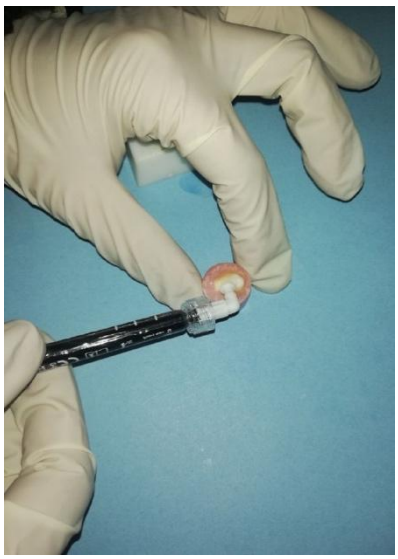
The infiltration procedure was performed according to the manufacturer's instructions in the following steps as shown in Figure 11 (a)-(c):

- Icon-Etch was applied for two minutes.
- Specimens were rinsed with water and air dried for 30 seconds.
- Icon-Dry was applied for 30 seconds and gently air dried.
- Icon-Infiltrant will be applied and left for 3 minutes. Excess resin was removed away with a cotton roll, then specimens light cured for 40 seconds. The resin infiltrant reapplied for 1 minute, then the specimen was light cured for another 40 seconds.

- Specimens tested enamel surfaces polished using polishing flexible discs for 20 seconds to remove any excess resin.

(a)

(b)



(c)

Figure 11: (a) Icon etch application. (b) Icon dry application. (c) Resin infiltrant application.

4.11.3. The control group (group 3)

This group left without treatment and stored in the artificial saliva.

4.12.Color and roughness evaluation after application of the treatment options (T3):

Directly after application of the treatment options (T3) color measurements and surface roughness were re-evaluated in the same manner at baseline to quantify the change.

The color changes (ΔE) of the specimens were evaluated using the following formula:

$$\Delta E_{CIELAB} = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})\ ½$$

Where: L* = lightness (0-100), a* = (change the color of the axis red/green) and b* = (color variation axis yellow/blue).

4.13. Statistical Analysis

Data analysis for this study was carried out using SPSS 25 (Social Package of Statistics Software). The data was first put in excel sheet, then uploaded on SPSS. The analysis started with exploration of data to identify normality and appropriate statistical tests. Mean and standard deviations of surface roughness and color changes were computed and presented using tables and graphs. The data of the study were investigated using independent sample t test and Mann Whitney u test, depending on the normality of the data. All statistical tests were conducted at significance level of 0.05.

Chapter 5

Results

5.1. Overview

The present study was set out to investigate the effect of resin infiltrate (Icon) and topical fluoride (ClinPro) on the roughness and color masking of initial enamel caries lesions (white spot lesion). Normality tests were first conducted to identify data distribution and appropriate statistical tests (Appendix 1). For roughness testing samples, data was not normally distributed and hence non-parametric tests were used. On the other hand, the data for color testing subgroups were normally distributed except for control group.

5.2. Effect of Demineralization on Surface Roughness

Figure 12 demonstrates the mean score of surface roughness of the intact enamel compared to demineralized enamel. The scores are slightly lower in demineralized enamel. However, the difference is negligible and is not statistically significant (p=0.260).

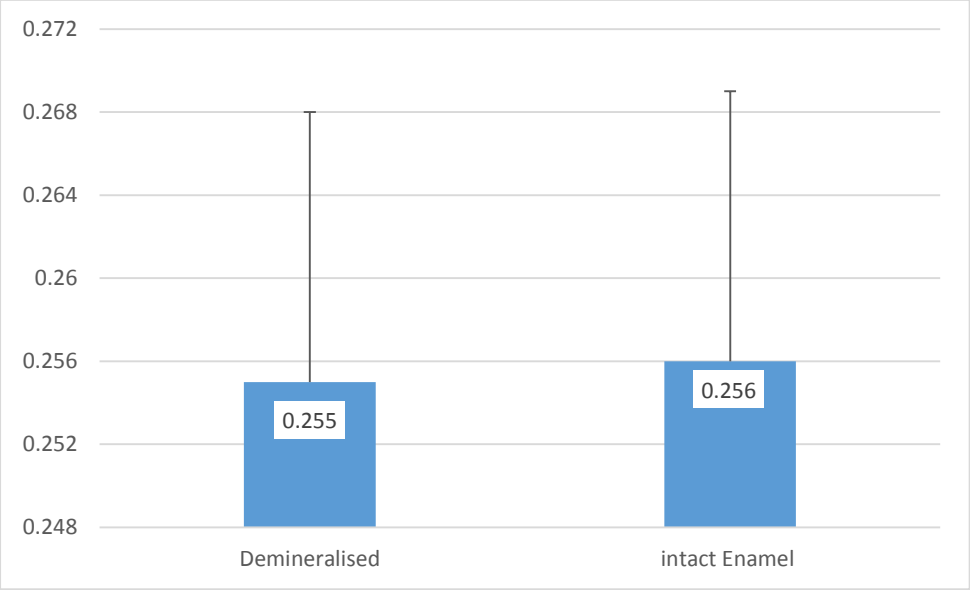


Figure 12: Comparison of mean scores of surface roughness in demineralized and intact enamel. Mann Whitney U test was used.

5.3. Effects on Roughness

Table 3 summarizes bivariate comparisons of surface roughness between demineralized enamel and control group, Icon-treated enamel and ClinPro-treated enamel. Surface roughness was almost equal in study groups with no statistically significant differences reported. However, control group demonstrated lower roughness than other study groups, though the difference was not statistically significant (Figure 13).

Microscopic and 3D images and the histogram showing description of topographical analysis of enamel surface at all stages of the testing (Figures.18-25).

Table 3: Comparison of surface roughness in study samples

Type	N	Mean	Std. Deviation	P value
ClinPro	21	0.2556	0.00228	0.755
Demineralized	21	0.2558	0.00133	
Icon	21	0.2559	0.00241	0.845
Demineralized	21	0.2558	0.00133	
Control	21	0.2541	0.00386	0.467
Demineralized	21	0.2558	0.00133	

Independent sample t test and Mann Whitney U test was used to compare groups.

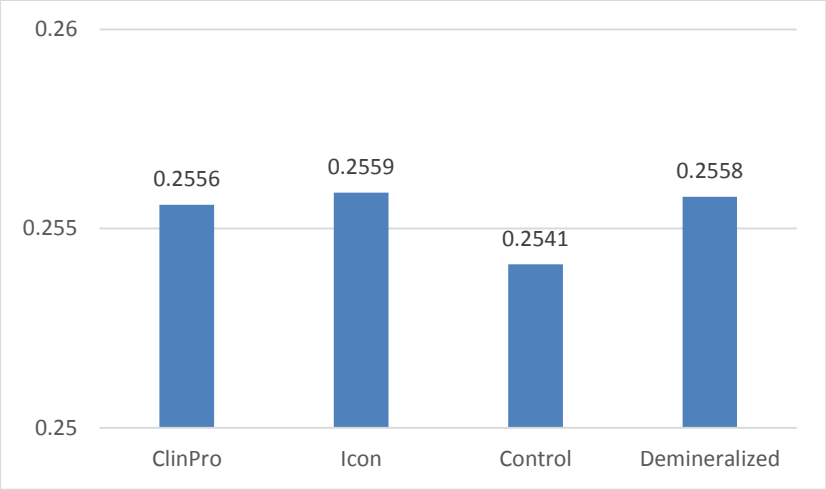


Figure 13: Comparison of mean scores of surface roughness.

5.4. Color Changes

Table 4 summarizes bivariate comparisons of color changes between demineralized enamel and control group (Figure 14), ClinPro-treated enamel (Figure 15), and Icon-treated enamel (Figure 16). Higher mean scores were observed in samples treated with ClinPro (7.10 ±2.47) and Icon (8.54±3.14) compared to demineralized enamel (4.62±1.44). However, the differences were not statistically significant (p=0.265) when demineralized enamel compared to intact enamel .

Table 4: Comparison of color changes in study samples

Subgroup	N	Mean	Std. Deviation	P value
Control	22	5.0088	2.21872	0.265
Demineralised	66	4.6163	1.43861	
ClinPro	22	7.1004	2.46867	0.001**
Demineralised	66	4.6163	1.43861	
Icon	22	8.5350	3.13544	0.000***
Demineralised	66	4.6163	1.43861	

Mann Whitney U test was used to compare groups. ** p≤0.01, *** p≤0.001.

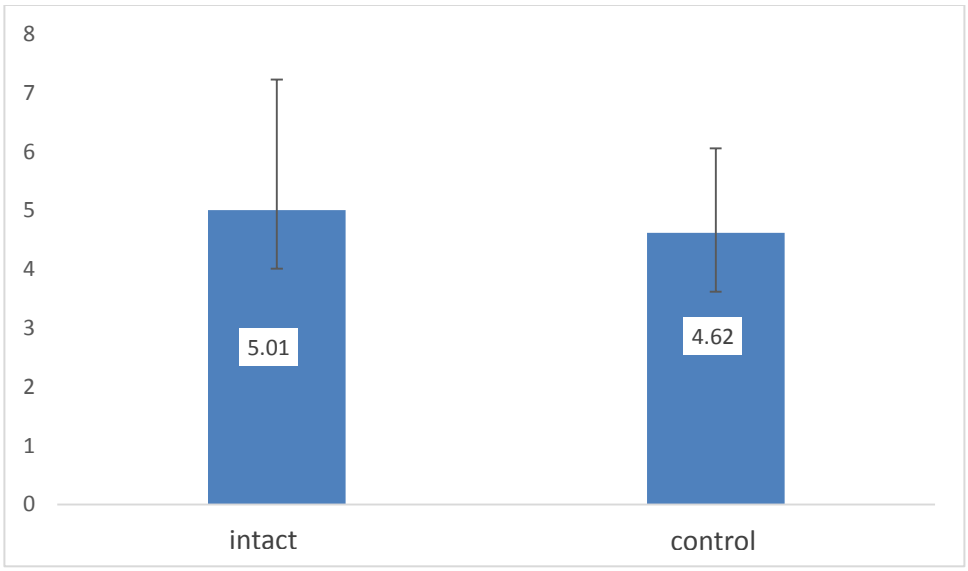


Figure 14: Comparison of color changes between demineralized enamel and intact enamel

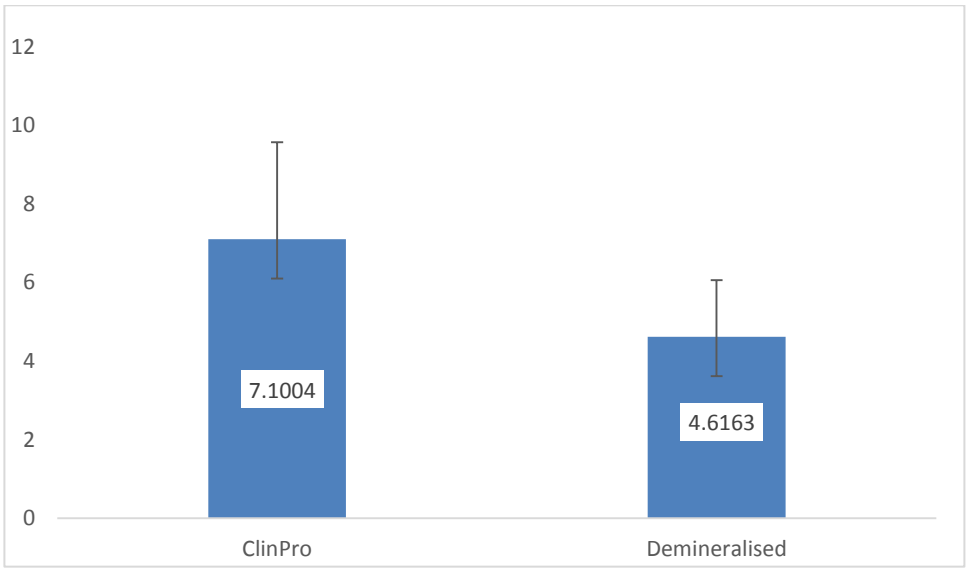


Figure 15: Comparison of color changes between demineralized enamel and ClinPro-treated enamel.

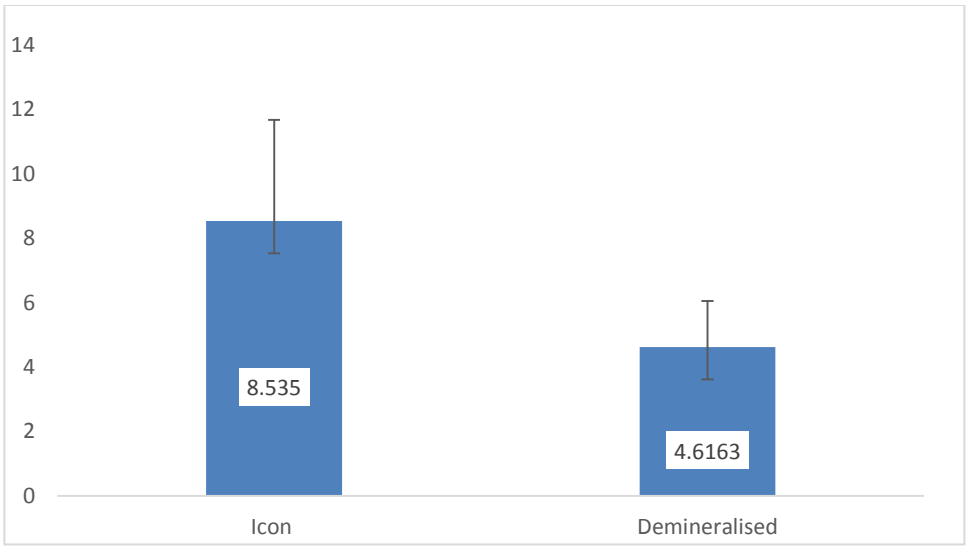


Figure 16: Comparison of color changes between demineralized enamel and Icon-treated enamel.

Comparison of intervention groups with control group suggested that Icon and ClinPro induced higher changes in enamel color than control group. Icon showed slightly higher color scores than that of ClinPro (Figure 17).

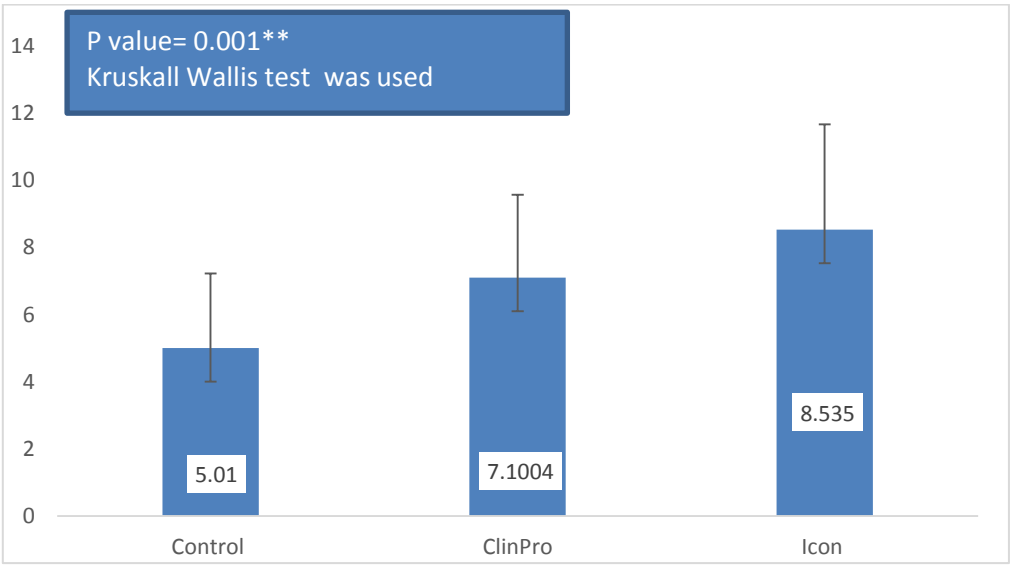
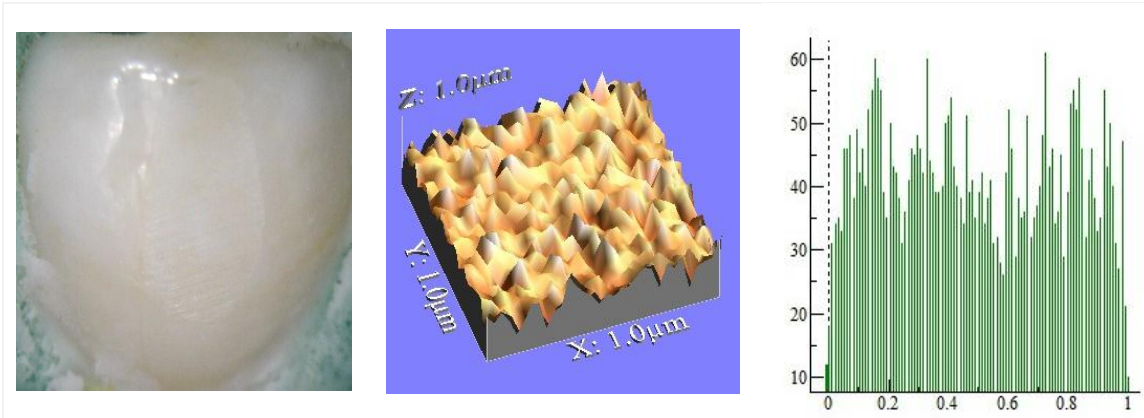
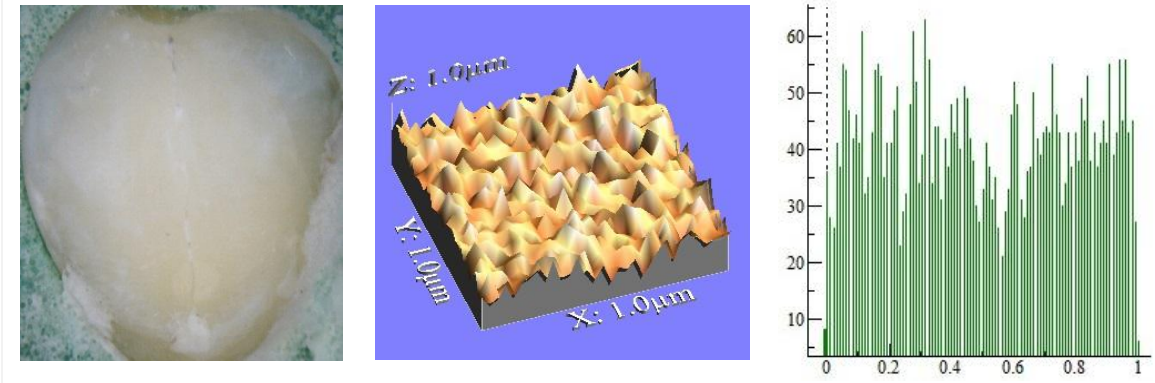


Figure 17: Comparison of color changes between demineralized enamel, ClinPro-treated enamel and Icon-treated enamel



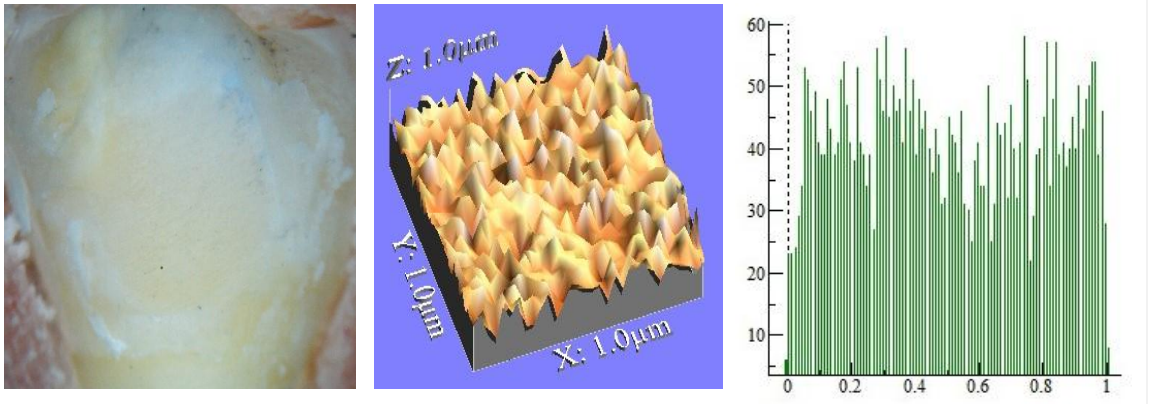
(a) (b) (c)

Figure 18: Control group baseline. (a) microscopic image of enmel surface at baseline; (b) 3D microscopic image of enamel surface at baseline; (c) Histogram showing topographical analysis of enamel surface.



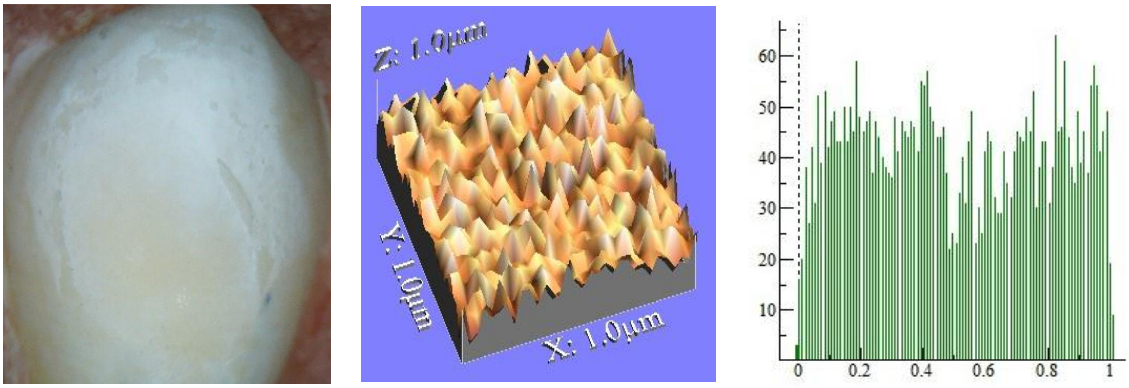
(a) (b) (c)

Figure 19: Control group after demineralization. (a) microscopic image of enmel surface after demineralization; (b) 3D microscopic image of enamel surface after demineralization; (c) Histogram showing topographical analysis of enamel surface.



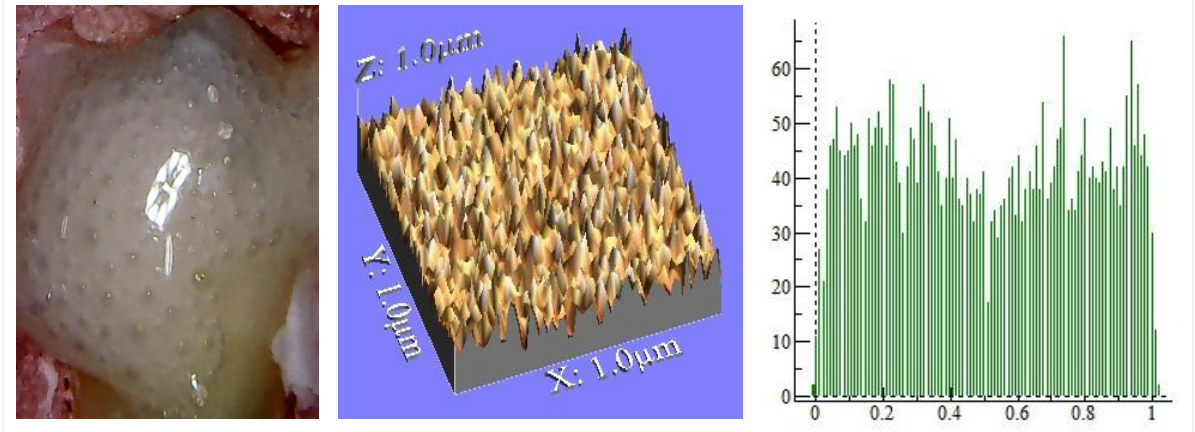
(a) (b) (c)

Figure 20: ClinPro group at baseline. (a) microscopic image of enmel surface at baseline; (b) 3D microscopic image of enamel surface at baseline demineralization; (c) Histogram showing topographical analysis of enamel surface.



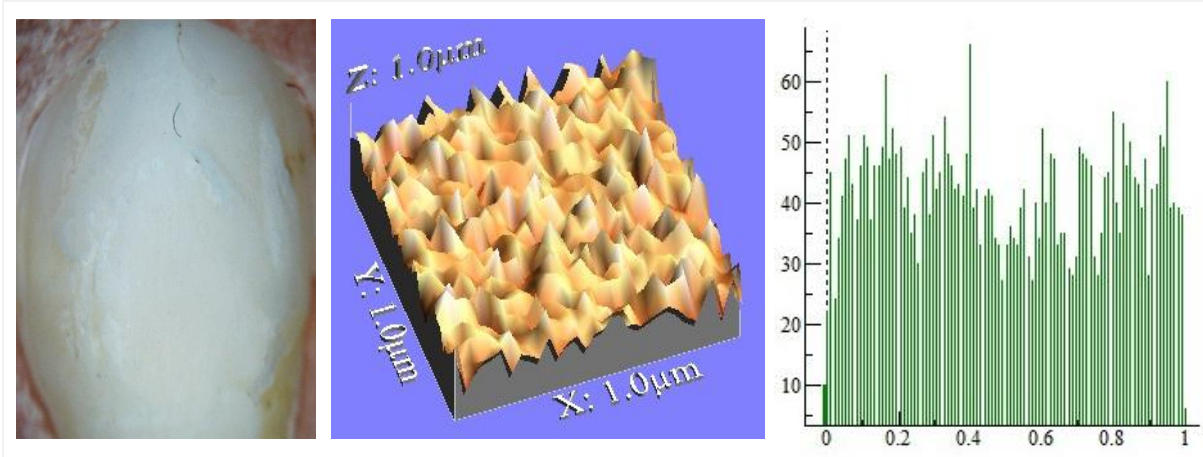
(a) (b) (c)

Figure 21: ClinPro group after demineralization. (a) microscopic image of enmel surface after demineralization; (b) 3D microscopic image of enamel surface after demineralization; (c) Histogram showing topographical analysis of enamel surface.



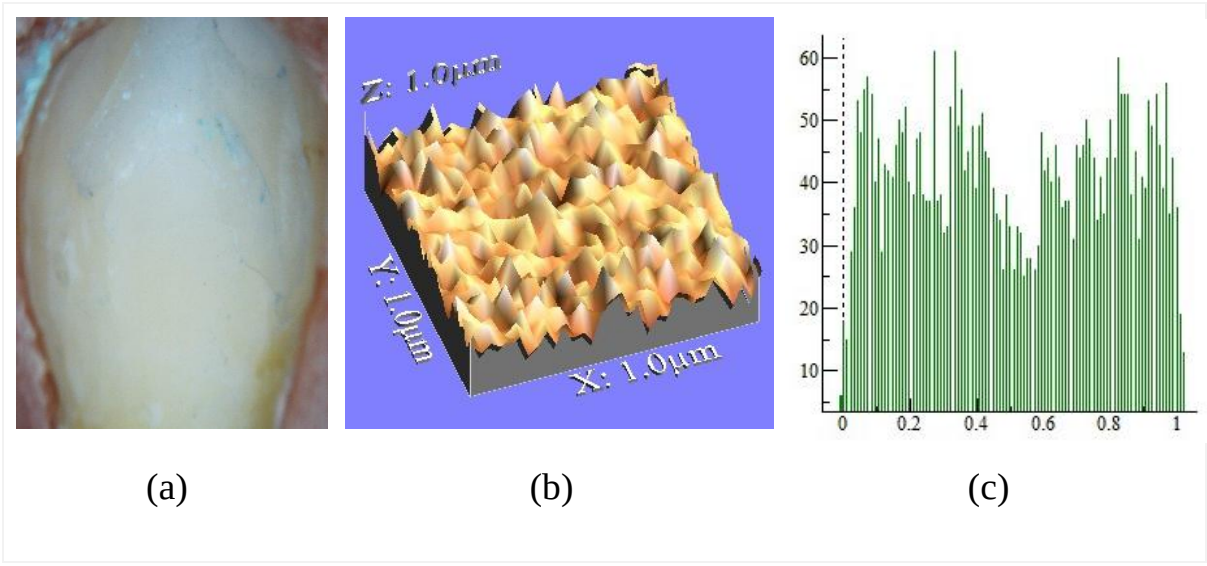
(a) (b) (c)

Figure 22: ClinPro group sample after application. (a) microscopic image of enmel surface after application; (b) 3D microscopic image of enamel surface after application; (c) Histogram showing topographical analysis of enamel surface.



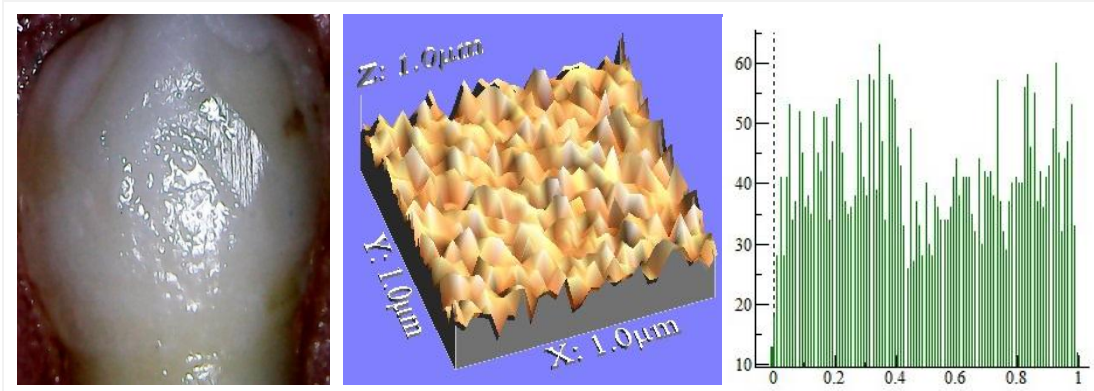
(a) (b) (c)

Figure 23: Icon group samples at baseline. (a) Microscopic image of enamel surface at baseline; (b) 3D microscopic image of enamel surface at baseline demineralization; (c) Histogram showing topographical analysis of enamel surface.



(a) (b) (c)

Figure 24: Icon group samples after demineralization. (a) Microscopic image of enamel surface after demineralization; (b) 3D microscopic image of enamel surface after demineralization; (c) Histogram showing topographical analysis of enamel surface.



(a) (b) (c)

Figure 25: Icon group samples after application. (a) Microscopic image of enamel surface after application; (b) 3D microscopic image of enamel surface after application; (c) Histogram showing topographical analysis of enamel surface.

Chapter 6

The Discussion

6.1. Overview

White spot lesions (WSLs) are the earliest visual signs of dental caries covered by an apparently intact enamel surface, which caused by the action of bacterial organic acids, the formed acids dissolve the tooth minerals, mainly calcium phosphate, in a process known as demineralization, the loss of the mineral creates porosities inside the lesion body that change the refractive index of the normally translucent enamel results in the typically whitish appearance of these lesions. Minimum invasive dentistry is a fast-growing trend in modern dentistry, mainly based on controlling the disease and then using minimally invasive techniques to restore the mouth to form, function and esthetics (9).

Great attention has been devoted to the non-invasive treatment of WSLs with the use of topical fluoride agents (e.g., toothpaste, fluoride containing mouth rinse, gel, and varnish) associated with diet and good oral hygiene to promote lesion remineralization. Fluoride plays a key role in the prevention and control of dental caries. However, this approach is not always successful, as it requires sufficient compliance of the patient and a change of harmful habits. However, caries resin infiltration represents a new concept in dentistry, such infiltration is effective in arresting smooth-surface enamel lesions and is an alternative approach to treat early caries lesions. This technique may bridge the gap between the non-invasive and minimally invasive treatment of WSLs, postponing the need for a restoration as long as possible (50).

So, for this reason the aim of this study was to compare the effect of the fluoride varnish (ClinPro^{TM}**)** and the resin infiltration (Icon, DMG) on

the color masking and surface roughness of induced caries enamel lesions in vitro.

The present study was carried out on extracted human premolars where a total of 45 sound teeth were used, after cleaning and rinsing all the teeth were sectioned along the long axes into two halves lingual and buccal halves to get 90 specimens, the specimens were divided randomly into three equal groups (n=30) according to the type of treatment:

- **Group 1** (n=30)**:** was treated with fluoride varnish.
- **Group 2** (n=30): was treated by resin infiltration.
- **Group 3** (n=30): was used as a control group with no treatment.

6.2. Color Assessment:

The color was measured three times at baseline (T1), directly after artificial WSLs (T2), directly after application of the treatment options (T3). The specimens' colors were measured using a portable Reflective spectrophotometer with a white background was selected and measurements were made according to the CIE L*a*b* color space relative to the CIE standard illuminant D65. The color changes (ΔE) of the specimens were evaluated using the following formula:

$$\Delta E_{CIELAB} = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{½}$$

Where: L* = lightness (0-100), a* = (change the color of the axis red/green) and b* = (color variation axis yellow/blue)

Going through the result of color evaluation it was reported that although there were no significant differences between the testing groups, higher score value was registered for Icon (8.5350), followed by Clinpro (7.1004), and compared to the control group which was (5.0088).

This indicated that, the resin infiltration of white spot lesions produced a positive effect in masking the color of teeth.

Our result was in agreement with **Torres et al., 2011** who performed a study that investigated the aesthetic effect of resin infiltration and fluoride on white spot lesions using a spectrophotometer. They found that resin infiltration was proven to be an effective treatment for masking white spot lesions (60).

Another study, matches with our result by **Yetkiner et al., 2014 who** studied the effects of infiltration, microabrasion and the fluoride in terms of improving the appearance of WSL, the calculation of the color components (L*, a*, and b*), was performed using a spectrophotometer. Based on the obtained results, infiltration and treatments performed better in diminishing the opaque WSL appearance compared with the fluoride treatment and control. Fluoride treatment improves the WSL appearance more than the clinical detectable limit, but the stability was not different than the effect of saliva remineralization (49).

Based on the obtained results, although the fluoride cannot be considered a masking agent, it is usually used as a remineralizing agent for white spot lesions. If an incipient lesion could be remineralized, its color would be altered, as the translucency of enamel occurs in function of its mineral content, as have been reported by **Brodbelt et al., 1981** (88). In previous study done by, **Yamazaki et al., 2007** postulated that, this remineralization seems to be superficial. The inner portion of the enamel lesion is more susceptible to demineralization, due to gradients in enamel solubility, with the inner enamel being more soluble relative to the outer enamel (89).

Jones & Fried 2006 reported that an increase in mineral volume from the fluoride enhanced remineralization can significantly decrease the optical reflectivity of artificial lesions. However, the findings showed that the reflectivity did not decrease significantly in the body of the underlying lesion after remineralization. It is important to note that the lesion body did not remineralize to the same level as the surface zone (90). Optical property experiments of enamel caries have shown that the change in scattering intensity depends on the mineral volume range (91). Thus, after a remineralizing treatment, although such lesions show a decrease in size in term of depth and width, they may remain clinically visible. This is because most of the detection signal comes from the body of the lesion, which cannot be completely remineralized. These results were reported by **Peters 2010** (92)**,** and **Kidd 2004** (93).

Furthermore, in agreement with this study, **Kim et al., 2013** explained that remineralization promoted by the fluoride provided a noticeable change in the color of the tooth. However, the whiteness in the remineralized enamel still remained under the short-term fluoride treatment, yielding a clinically discernable mismatch of color (94).

Regarding the previously mentioned studies from our results could be concluded that the ability of Icon for masking the color and the complete infiltrations of the enamel pores in the white spot lesions; the resin infiltration creates a diffusion barrier within the enamel lesion and enables the strengthening of the demineralized enamel structure, preventing cavity formation and the inability of fluoride of masking the color because most the remineralization effect occur on the surface of the lesion and most of the detection signal comes from the body of the lesion, thus our results support

the use of Icon as claim by manufacture as MIT of color masking of chalky white appearance of enamel.

6.3. Evaluation of Surface Roughness

In this study the optical methods tend to fulfill the need for quantitative characterization of surface topography without contact, Specimens were photographed using USB Digital microscope with a built-in camera connected with an IBM compatible personal computer using a fixed magnification of 90X. The images were recorded with a resolution of 1280×1024 pixels per image. Digital microscope images were cropped to 350×400 pixels using Microsoft office picture manager to specify/standardize area of roughness measurement. The cropped images were analyzed using WSxM software. The surface roughness was measured three times at baseline (T1), directly after artificial WSLs (T2), directly after application of the treatment options (T3).

Surface roughness was almost equal in the study groups with no statistically significant differences reported. However, the control group demonstrated lower roughness than other study groups, though the difference was not statistically significant.

Our results were in agreement with **Ulrich et al., 2015** who evaluated the effects of resin infiltration on surface roughness of non-cavitated lesions, etching with hydrochloric acid will increase the surface roughness of subsurface lesions, the resin infiltrant is not capable to fill up micro porosities, nor to sufficiently flatten the roughness of subsurface lesions to a clinically acceptable grade. Driven by capillary action, resin infiltrants are capable to penetrate deeply into the porous bed of an initial lesion, and this leads to a hybrid mixture of demineralized enamel prisms and a

circumfluent polymerized network of the resin infiltrant (95). Interestingly, it seems clear that the resin infiltrant will not form a smooth coat on the lesion surfaces but renders exposed enamel prism remnants uncoated (96).

These results were in accordance with the outcome of the present study revealing that the resin infiltrate is not capable to improve the baseline surface roughness of incipient enamel lesions.

Controversy**, Mohamed et al., 2018** performed a study showed that resin infiltration produced favorable esthetic results and improved surface roughness of the teeth. Color and surface roughness of each tooth were assessed at eight time points, Surface roughness were determined using Surface Texture Software Measurement (15). The demineralized surface layer hampers resin infiltration into the underlying subsurface of the WSL and has to be removed before resin infiltration, (97) (98), it has been shown that phosphoric acid does not adequately remove the demineralized surface layer. Whereas the 15% HCl for 90–120 second can adequately remove demineralized area enables resin infiltration into the body of the WSL, application of the resin infiltrates to the enamel surface had sealed the enamel porosities (99).

This result is similar to the one reported by **Taher et al., 2012** authors demonstrated that infiltrant application to the enamel surface may sealed the enamel porosities and the resulting product appeared smooth (59) .

On the other hand, in case of fluoride **Buzalaf et al., 2011** explained that the application of fluoride on enamel surface effectively protects the enamel crystals from dissolution by formation of fluoroapatite crystals instead of hydroxyapatite crystals that is much more resistant to acid dissolution of caries but when the coating of fluoride is partial, due to

insufficient application time or thin fluoride film, the uncoated parts of the enamel crystal will undergo dissolution leading to rough enamel surface (100).

According to the previously mentioned text regarding the surface roughness **Mohamed et al., 2018** concluded that the factors that may affect the improvement of color and surface roughness of the teeth after the treatment are: time of infiltrant and fluoride application, lesion depth, and duration of the WSL presence, so the techniques and time should be properly evaluated and control to gain the maximum effect (15).

Chapter 7

The Conclusions

According to the results of the present study, the concluded data that related to the comparison between Icon and fluoride indicated:

1- That resin infiltration produced favorable esthetic results compared to the fluoride therapy.
2- There were no significant differences reported regarding the surface roughness between all the groups.

7.1. Limitations and Strength:

The limitation of this study was in:

- The difficulty of teeth sample collection.
- The devices used to measure the color and the surface roughness were unavailable locally and the experiment has been done in Egypt.
- The high cost of the resin infiltration Icon material.

The strength point was studding and highlighting a new technique that change the old concept of treatment and management of the dental caries (DC).

7.2. Recommendations:

Further studies should be performed:

- For evaluation of color masking and surface roughness of fluoride and resin infiltration Clinically.

-To assess the interface between the enamel and the resin infiltration.

- To investigate the color stability and durability after the treatment with Icon in vivo.

Chapter 8:

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Appendix 1:

Test of Normality Results

Tests of Normality

	Type	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Rough_ClinPro	intervention	.202	21	.025	.935	21	.175
	control	.168	21	.125	.955	21	.414
Rough_Icon	intervention	.201	21	.027	.937	21	.186
	control	.168	21	.125	.955	21	.414
Rough_Control	intervention	.314	21	.000	.670	21	.000
	control	.168	21	.125	.955	21	.414

a. Lilliefors Significance Correction

Tests of Normality

	Type	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Mat_Control	intervention	.143	22	.200*	.958	22	.443
	control	.169	22	.104	.874	22	.009
Mat_ClinPro	intervention	.090	22	.200*	.975	22	.827
	control	.169	22	.104	.874	22	.009
Mat_Icon	intervention	.102	22	.200*	.945	22	.256
	control	.169	22	.104	.874	22	.009

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Appendix 2:

The Proposal

The Effect of Non-Invasive Treatment Techniques on the Color Masking and Surface Roughness of Induced Enamel Lesions

(In Vitro Study)

تأثير تقنيات العلاج الغير عدواني على اخفاء اللون وخشونة السطح لآفات المينا المستحثة

(دراسة مختبرية)

BY
Nada Ali Obead
(BDS, 2007)

Proposal

Submitted In Partial Fulfillment of Requirements for the Degree of Master of Science In Conservative and Endodontic

Supervisor: Prof. Nagat Bubteina
University of Benghazi
Faculty of Dentistry
Benghazi – Libya
2019

Introduction

Despite the fact that the prevalence of dental caries has decreased over the past decades, it still remains one of the most prevalent diseases worldwide (1).

Dental caries is defined as multifactorial, infectious oral disease characterized by localized demineralization of tooth structure caused by acids that is generated as a by-product of bacterial metabolism in dental plaque biofilms (2), (3).

Enamel demineralization and remineralization are considered as a dynamic and continuous process that occurs throughout the whole life of a tooth (4).

The earliest evidence of caries on smooth enamel surface is a white spot; these white spot lesions (WSLs) are chalky white opaque areas termed as non cavitated enamel caries lesions(3). These areas of enamel lose their translucency because of the extensive subsurface porosity caused by demineralization (4), (5).

Since light refraction through enamel is directly related to the level of mineralization, WSLs manifest themselves as white opacities visually (Derks *et al.*, 2004; Bergstr and Twetman, 2011). If the process is not interrupted and demineralization reversed, they might progress from demineralization to non-cavitated lesions, and finally to cavitated lesions (4).

Since the stage of white spot does not involve enamel cavitation, noninvasive treatment is indicated as with topical fluorides associated with diet and hygiene procedure orientation are recommended (6). However, the whitish appearance may remain, even if the lesion is arrested. This is because the remineralization of deeper lesions occurs only superficially, so

that the body of the lesion remains porous and therefore still whitish, as a permanent scar (6),(7).

The resin infiltration technique was recently introduced in dentistry to prevent further progression of enamel lesions. The objective is to fill the pores within the lesion body by capillary action, with a low-viscosity light-cured resin (8). This prevents further diffusion of bacteria, and establishes a barrier within the caries lesion, which can reinforce the enamel structure, avoiding or delaying cavitation and disruption of the surface (9).

An additional positive effect is the esthetic improvement of anterior teeth when white spot lesions are present. (10). this occurs due to the infiltration of the enamel porosities with the low viscosity resin, which alters the refractive index of the light and consequently the final tooth appearance. Since the refractive index of the infiltrant resin (1.51) is close to hydroxyapatite (1.62-1.65), its ability to mask white spot lesions was observed (11). Enamel white spot subsurface lesions compromise esthetics and precedes cavitation, there for must be halted.

So, in this study we will evaluate the resin infiltration technique and remineralization of enamel caries with fluoride solution on color masking and surface roughness of induced enamel caries.

The Aim of Study

The aim of this an in vitro study is to evaluate the effect of the resin infiltration technique and remineralization of induced enamel caries with fluoride solution on

1) Color masking of white spot lesions.
2) Surface roughness.

Materials and Methods

1 Materials

1) Fluoride solution.
2) Resin infiltration (Icon, DMG, Hamburg, Germany)

2 Method of the Study

2.1 Sample collection

The present study will be carried out on extracted human premolars; teeth will be obtained from patient requiring therapeutic extraction, from different public dental and private clinics.

2.1.1 Eligibility criteria of teeth

Inclusion criteria:

- Sound teeth that is free of caries or white spot lesions WSLs or developmental defects
- No physical damage due to extraction.

2.2 Sample preparation

2.2.1 Tooth cleaning

The teeth will be thoroughly cleaned with ultrasonic scalar and slurry pumice to remove all soft-tissue remnants, calculus and plaque and stored in normal saline at room temperature.

2.2.2 Caries induction

Artificial WSLs will be induced on buccal and lingual enamel surface of all the teeth using a demineralizing solution. All the samples will be rinsed and stored in artificial saliva after demineralization till the time of use.

2.2.3 Tooth sectioning

All the teeth will be sectioned along the long axes in to two halves mesial and distal halves.

2.3 Grouping and intervention

A total of 90 sound extracted human premolars will be collected and used in this study. The teeth will be classified randomly in to three equal groups according to the type of treatment.

Group 1: with a total number of (n=30) will be treated with fluoride varnish.

Group 2: with a total number of (n=30) will be treated by resin infiltration (Icon, DMG, Hamburg, Germany).

Group 3: with a total number of (n=30) will be used as a control group with no treatment.

2.4 Material application:

2.4.1 Fluoride application (group 1)

Specimens will be immersed daily in NaF solution. After the daily fluoride immersion, the specimens will be rinsed with deionized water and stored in artificial saliva.

2.4.2 Resin infiltration application (group 2)

Specimens will be resin infiltrated (Icon, DMG, Hamburg, Germany) and stored in artificial saliva. The infiltration procedure will be performed according to the manufacturer's instructions:

- Icon-Etch will be applied for two minutes.
- Specimens will be water rinsed and air dried for 30 seconds.

- Icon-Dry will be applied for 30 seconds and air dried.
- Icon-Infiltrant will be applied, and light cured for 40 seconds
- Specimens will be polished with aluminum oxide abrasive paper.

2.4.3 The control group (group 3)

With no treatment.

3 Testing Methods

3.1 Evaluation of colure change

The color values (L*,a*,b*) of each samples will be measured with a Reflective spectrophotometer .

3.2 Roughness Methodology

The optical methods tend to fulfill the need for quantitative characterization of surface topography without contact; Specimens will be photographed using USB Digital microscope with a built-in camera connected with a compatible personal computer. Digital microscope images will be cropped using Microsoft office picture manager to specify/standardize area of roughness measurement. The cropped images will be analyzed using software.

The software will be used to calculate average roughness expressed in μm which can be assumed as a reliable indices of surface roughness.

All the measurements will be done at:

1) Baseline (T1).
2) Directly after artificial WSLs (T2).
3) After application of the treatment options (T3).

Data Management and Analysis

The data will be collected, computed and analyzed using suitable statistical program.

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Appendix 3:

Cover and Abstract in Arabic Language

تأثير تقنيات العلاج الغير عدواني على اخفاء اللون وخشونة السطح لآفات المينا المستحثة

(دراسة مختبرية)

إعداد

ندى علي عبيد

المشرف

أ.د. نجاة بوبطينة

الملخص

هدف الدراسة: كان الهدف من هذه الدراسة في المختبر هو تقييم تأثير تقنية ارتشاح الراتنج وإعادة تمعدن تسوس المينا المستحث بمحلول الفلوريد على إخفاء اللون لآفات البقع البيضاء وخشونة السطح .**المواد والطرق :**مجموعه 45 سنًا سليمة خالية من التسوس أو آفات البقع البيضاء WSLsأو عيوب النمو .لا ضرر مادي بسبب الخلع. تم تقسيم جميع الأسنان على طول المحاور الطويلة إلى نصفين لساني وشدقي للحصول على 90 عينة، تم تصنيف العينات بشكل عشوائي إلى ثلاث مجموعات متساوية عدد 30 عينة في كل مجموعة وفقًا لنوع العلاج، المجموعة الاولى تم معالجته بورنيش الفلورايد .المجموعة الثانية عولجت عن طريق تسلل الراتنج Icon ، .المجموعة **الثالثة** تم استخدامها كمجموعة تحكم بدون علاج .تم غمر جميع العينات في محلول منزوع المعادن لمدة 7 أيام .تم قياس خشونة السطح واللون ثلاث مرات عند خط الأساس(T1)، مباشرة بعد WSLs الاصطناعية (T2) ، مباشرة بعد تطبيق خيارات المعالجة

(T3)، تم قياس الألوان باستخدام مقياس الطيف الضوئي العاكس المحمول ، قياس خشونة السطح ثلاثي الأبعاد باستخدام نظام رؤية بتقسيم الضوء. تم جمع البيانات وتحليلها إحصائيا . كانت خشونة السطح متساوية تقريبًا في مجموعات الدراسة مع عدم وجود فروق ذات دلالة إحصائية. أظهر Icon درجات ألوان أعلى قليلاً من تلك الخاصة بـClinPro. أعطى Icon نتائج جمالية إيجابية مقارنة بالعلاج بالفلورايد ولم يتم الإبلاغ عن فروق ذات دلالة إحصائية فيما يتعلق بخشونة السطح. **الكلمات المفتاحية:** WSL، رشح الراتنج، الفلورايد، اخفاء اللون، خشونة السطح.

تأثير تقنيات العلاج الغير عدواني على اخفاء اللون وخشونة السطح لآفات المينا المستحثة

(دراسة مختبرية)

قدمت من قبل:

ندى علي عبيد

تحت إشراف

أ.د. نجاة بوبطينة

قدمت هذه الرسالة استكمالا لمتطلبات الحصول على درجة الماجستير في العلاج التحفظي و علاج الجدور

جامعة بنغازي

كلية طب و جراحة الفم و الاسنان

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