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IN VITRO PREVENTION OF SECONDARY DEMINERALIZATION BY ICON® (INFILTRATION CONCEPT)

by

Atul Bidarkar

A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Dental Public Health in the Graduate College of The University of Iowa

July 2012

Thesis Supervisor: Professor James Wefel

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MASTER'S THESIS

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This is to certify that the Master's thesis of

Atul Bidarkar

has been approved by the Examining Committee for the thesis requirement of Master of Science degree in Dental Public Health at the July 2012 graduation.

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To my mother
Surekha. Bidarkar

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CHAPTER 1

INTRODUCTION

Dental caries is one of the most prevalent chronic diseases in the United States, affecting more children than asthma. According to current statistics, overall caries prevalence in the United States is declining among children and adolescents in the age group of 6-19 years. Despite the decline seen in the past several decades, caries remains a major public health challenge, as the caries prevalence rate in children and young adults remains high, affecting over 67% of individuals entering adulthood. Decay on the smooth surfaces, including the surfaces between teeth have benefited the most from the preventive effects of various fluoride agents, such as fluoride toothpaste and fluoridated water. Even with preventive measures such as fluoride and widespread use of sealants for many years, caries as a disease, particularly on pits and fissures has remained highly prevalent in the population. Caries on the pit and fissure of the occlusal, buccal and lingual surfaces of the teeth accounts for the large majority of caries experience in childhood and adolescence.

Management of pits and fissures for caries prevention has become a pertinent topic in contemporary dentistry and includes a variety of approaches, such as early diagnostics and remineralization of the enamel. The role of pit and fissure sealants has been significant in the prevention of caries on the pit and fissure surfaces of the teeth. Sealants are placed to prevent or retard the caries process by providing a physical barrier between the micro-organisms and pits and fissures of the teeth. Although sealants have been effective in the prevention of caries, there are challenges concerning their retention.

Sealants were first introduced to protect the pit and fissure surfaces in the 1960s by Cueto and Buonocore. Such sealants prevent the growth of bacteria that promote decay in pits and fissures of teeth and have helped to decrease the incidence of decay on the pit and fissure surfaces, but the occurrence of decay due to loss of sealant can be a problem. Effectiveness of sealants is linked very closely to their longevity. Failure of sealants is attributed to the fact that they can wear away over a period of time and also can be because of poor bonding between the sealant and the tooth surface. The decay preventive effectiveness for sealed pit and fissure surfaces ranges from about 81% after 2 years to 68% after 10 years, which reflects the decreased retention over time. Thus, when retained, the value of sealants in prevention of decay on the pit and fissure surfaces of the teeth is irrefutable.

The evidence for effectiveness of sealants in the management of dental caries has been questioned due to concerns about inadvertently sealing over caries. Sealing over caries involves application of a sealant material over a carious lesion confined to enamel. The concept of sealing over the carious lesion has not been widely accepted. However, data suggest that this is an option when the sealing occurs over a non-cavitated lesion rather than over a cavitated lesion, as the evidence supporting the sealing of non-cavitated lesions is stronger than for sealing cavitated lesions. Despite the effectiveness, sealants are not widely used for sealing over caries because of fear of misdiagnosis of a significant number of carious lesions on pits and fissures. However, the evidence suggests that sealing over caries confined to enamel decreases the bacterial count significantly. This procedure is thought to deprive the bacteria of a nutrient source and, therefore, limit viability and acid production.

Although sealants have been used successfully to prevent caries on the occlusal surface, studies have also been done to explore the effectiveness of adhesives, resin materials and sealants in infiltration of proximal smooth surface caries. These studies have focused on ways for the dental material to infiltrate the enamel as a means of overcoming the retention challenges. In these studies, the adhesives and sealants were bonded to the lesions to act as lesion barriers, and results showed a significant reduction in lesion progression. However, these studies also reported that the sealant only penetrated into the lesion superficially, as opposed to infiltration to the depth of the lesion. The reason behind this was that the etching was done with 37% phosphoric acid, and it could not remove the surface layer. Since the penetration ability of the resin material was limited, the development of an infiltration material with less viscosity was needed. The advantages of infiltration are that the resin material penetrates to the lesion base, arresting the lesion from further progression, providing mechanical support to the tooth material, and also improving the esthetics of the enamel.

ICON® (Infiltration Concept) was developed to address the retention issues and enhanced decay prevention on the smooth surfaces, but not the pit and fissure surfaces. It is a micro-invasive technique that fills the pores of an incipient lesion via capillary action. This creates a barrier that blocks the further diffusion of the bacteria and, hence, lesion development. No drilling and anesthesia is required and the natural anatomy of the tooth form is preserved. Thus, the sealant is only a mechanical barrier between the tooth structure and the oral environment compared to ICON®, which infiltrates the lesion and prevents further progression of the lesion. This property of infiltration should also make

the bacteria inactive, if present, and thus stop caries progression. As a result, one would not have to worry about sealing over caries.

Infiltration Concept (ICON®) is a relatively new resin product which is used in the treatment of incipient lesions. The material is commercially available in two kits; one is used to treat white spot lesions on the proximal surfaces of the tooth, and the other kit is used to treat all the other smooth surfaces. The product consists of hydrochloric acid as etchant, alcohol used as a dehydrating agent and the resin material as an infiltrant. The etchant used in the ICON® system is 15% hydrochloric acid, which is different than that used with the sealants (37% phosphoric acid). The ICON® product is said to replace demineralized enamel, once it has been etched, with a resin that fills the porosities created by demineralization. This process is claimed to resolve the opaque appearance of white spot lesions, as well as provide a less soluble filling material. It can be used on inter-proximal surfaces, as well as buccal or lingual smooth surfaces. However, the published literature has shown that no previous studies have been done to compare the effectiveness of ICON® in prevention of secondary demineralization, with sealant material.

This study was a preliminary study to see if the ICON® material could be considered as an alternative to the use of sealant material, and to see if it could be considered as equally or more effective in comparison to sealants. Thus, the purpose of this in vitro study was to assess the effectiveness of the ICON® in prevention of caries on the smooth surfaces in comparison with resin-based sealant. The study was initially planned on the pit and fissure surfaces, and because the ICON® material is a relatively new material, and was designed to use for smooth surfaces, the study will be done on the

smooth surface of the teeth. The results from this study on smooth surface of the teeth will help us evaluate the effectiveness of ICON® in comparison to the sealants and provide future directions for its use on the pit and fissure surfaces.

CHAPTER 2

LITERATURE REVIEW

Introduction

The review of literature begins with discussion of the structure of the tooth, mainly enamel and the dentin which leads into a discussion of the patho-physiology and histological features of enamel caries. The preventive modalities for enamel caries are then explained briefly.

The history and development of different types of bonding agents and how they led to development of various preventive materials such as sealants and Preventive Resins Restoration (PRR) is described. The history and development of the PRR and various types of sealants used in dentistry leads into discussion about the Infiltration Concept (ICON®), which was introduced to the field of dentistry recently and has been used as a secondary preventive material for treatment of early white spot carious lesions.

Structure of the tooth

Enamel

Physical characteristics of enamel

The enamel is the outer-most covering of the tooth structure which forms a protective covering of variable thickness over the entire crown of the tooth[24]. It attains a maximum thickness of about 2 to 2.5 mm on the cusps of the human molars and the pre-molars, thinning down to almost a knife edge at the cervical region of the tooth. Once the enamel has completed its formation, the final contour and shape of the cusps of the teeth are developed[24].

The enamel is the hardest calcified tissue in the body because of its high mineral content and crystalline structure arrangement[24]. The main function of enamel is to form a resistant covering of the tooth structure, rendering it suitable for masticatory purposes. Enamel is a calcified tissue, but its structure and hardness render it brittle, as is apparent when it loses its foundation of dentin.

The permeability of the enamel is another important physical property[24]. With the help of the radioactive tracers, it has been found that enamel can act in a sense like a semi-permeable membrane, permitting the passage of certain molecules like urea[24]. The color of the tooth varies from yellowish white to grayish white, which is determined by the translucency of enamel and the underlying dentin. The yellowish teeth have thin translucent enamel, reflecting the yellowish color of the dentin is visible, and grayish white teeth have thick and opaque enamel. This translucency is mainly attributed to the degree of the calcification of the enamel structure and its homogeneity[24].

Chemical properties of the enamel

The enamel consists of 96% inorganic material and 4% organic material and water. The inorganic material is similar to apatite[24]. Calcium-deficient carbonate-containing apatite is the largest mineral constituent that is present as a crystalline lattice framework and making up about 92% of the mineral content by volume[25].

Structure of the enamel

The enamel is composed of millions of enamel rods and prisms, which constitute for the primary structural component of the enamel, as well as the rod sheaths and a

cementing inter-rod substance in some of its areas[24]. The number of enamel rods has been estimated to be from as many as 5 million in lower incisors to 12 million in the upper first molar. The rods run somewhat tortuously from the dentino-enamel junction toward the surface of the tooth. Therefore, the length of the rods is greater than the thickness of the enamel, because of the wavy course and the oblique direction of the rods. In the cuspal area, which is the thickest part of the enamel, the rods are longer than those in the cervical area of the tooth[24].

The diameter of the rods, when measured with light microscopy is about 4 microns, which varies since the outer surface of the enamel is greater than the dentinal surface where the enamel rods originate. The rods generally have a clear crystalline appearance, permitting the light to pass through them. They appear to be hexagonal, when viewed in cross-section under the light microscope, but sometimes they appear round or oval in shape[24].

Dentin

The dentin provides the bulk of the tooth structure and is characterized as hard tissue, with tubules throughout the thickness of the tooth[24]. It determines the shape of the tooth, including the roots, since the formation of the dentin begins slightly before the enamel. The dentin, as a living tissue, contains within it processes of specialized cells called the odontoblasts. Although the odontoblasts are along the pulpal surface of the dentin, the cells morphologically belong to the dentin as they produce the dentinal tissue, as well as the odontoblastic process[24]. Physically and chemically the dentinal tissue

resembles the human bone, except for the difference that osteoblasts exist within the bone[24].

Physical and chemical properties of dentin

The dentin is light yellowish in color in young individuals and becomes darker with age[24]. Unlike the enamel, which is hard and brittle, the dentin is visco-elastic and subject to deformation. So it is harder than the bone, but softer than the enamel. The lower content of minerals in the dentin renders it more radiolucent than the enamel, which consists of 35% of organic matter and 65% of inorganic matter. The organic substance contains the collagenous fibrils and ground substance of muco-polysaccharides. The inorganic component is the hydroxy-apatite, and each unit of this hydroxy-apatite crystal consists of several thousand small unit cells whose basic composition is $Ca_{10}(PO_4)_6(Ca(OH)_2)$. The crystals here are plate-shaped and much smaller when compared to the hydroxyapatite crystals in the enamel[24]. The dentin also contains small amounts of phosphates, carbonates and sulphates[24].

Structure of the dentin

In the matrix of the dentin, the collagen fibers are arranged in a random network[24]. As the dentin calcifies, the hydroxyapatite crystals mask the individual collagen fibers[24].

The bodies of the odontoblastic cells that produce the dentinal tissue are lined along the pulpal surface of the dentin, and only the cytoplasmic processes are included within the dentinal tubules[24]. Each of the cells gives rise to one process which traverses along the pre-dentin and the calcified dentin within a single tubule and terminates at the

junction with enamel or the cementum, which is a bony structure covering the root of the tooth. The tubules are a normal characteristic of the dentin and are found throughout the normal dentin[24].

Dentinal tubules: The course of the dentinal tubules has a gentle curve along the crown and in root of the tooth, where it is S-shaped[24]. Starting at the right angles from the surface of pulp, the first convexity of the S shape is directed toward the apex of the tooth. The tubules end perpendicular to the dentino-enamel and dentino-cemental junction. The tubules are almost straight near the root tip along the incisal edges and the cusps. Along their entire length, the tubules exhibit minute and relatively regular secondary curvatures that are sinusoidal in shape. The thickness of the dentin ranges from 3 millimeters to 100 millimeters or even more[24].

Peritubular dentin: Peritubular dentin is that part of dentin that immediately surrounds the dentinal tubules[24]. This is the dentin that forms the wall of the tubules in all but the dentin near the pulp. It is highly mineralized when compared to inter-tubular dentin and twice as thick in outer dentin, when compared to the inner dentin. Studies with x-rays and electron microscopy show the increased mineral content of the peri-tubular dentin[24].

Inter-tubular dentin: The inter-tubular dentin constitutes the main body of the dentin which is located within the dentin tubules or specifically between the zones of the peri-tubular dentin[24]. The inter-tubular dentin is retained even after the decalcification; like it is with the bone and cementum matrix, as it is highly mineralized, but the peri-tubular dentin is not. About one-half of volume of the organic matrix of the inter-tubular dentin is collagen fibers, which are randomly distributed around the tubules. The fibrils range from 0.2 to 0.5 microns in diameter and exhibit a cross banding at 64 microns intervals. The

hydroxyapatite crystals are about 0.1 micro meters in length and are oriented parallel to the collagen fibers[24].

Pre-dentin: This dentin is located adjacent to the pulpal tissue and is 2-6 microns wide. It is the first dentin formed and is not mineralized[24]. With the process of mineralization of the collagen fibers at the pre-dentin and dentin junction, the pre-dentin becomes dentin and a new layer of pre-dentin is formed circumferentially[24].

Summary

The above section describes the tooth structure, and the physical and chemical properties within the enamel and dentin. It describes some developmental aspects of both the enamel and dentin and its mineral content.

Patho-physiology of Caries

Dental caries has been described as an infectious microbiologic disease of the teeth that results in localized dissolution and destruction of the calcified tissues[25]. The resultant damage caused by caries as a process is by demineralization and dissolution of the tooth structure that results from the highly localized drop in the pH at the plaque-tooth interface. The local pH drop occurs mainly due to the plaque communities with high concentration of Mutans Streptococci and Lactobacillus responsible for producing acids which cause the drop in pH of the saliva causing tooth demineralization. Exposure to sucrose and poor oral hygiene habits help bacteria to colonize, which in-turn metabolizes these nutrients to produce organic acids. However, a single such event is not sufficient to change the mineral content of the surface of the teeth, and many such

episodes are required over a longer duration to produce a characteristic carious lesion. The acid production from caries-active plaque can overcome the buffering capacity of the salivary bicarbonate, causing the pH to fall. Once the pH falls below the value of about 5.5, the tooth mineral starts dissolving and loses calcium and phosphate ions[25].

At pH values lower than about 5.5, such as 3.0 or 4.0 the enamel surface is etched and roughened and at a value of 5.0, the surface remains intact, while there is a subsurface loss of the mineral. This initial enamel caries/white spot lesion and is characterized by an intact enamel surface and subsurface demineralization[25]. The clinical appearance of this incipient lesion is characterized by subsurface porosity where the smooth intact surface becomes chalky white when dried and not detectable when hydrated. The drying of this incipient lesion with air removes the subsurface water, leaving air-filled voids, making the area appear opaque and white. These incipient white spot lesions can be reversed with the process of remineralization[25].

Cavitation of the lesion occurs when the subsurface demineralization is so extensive that the enamel structure collapses. This cavitation is not reversible and is associated with destruction of the tooth structure[25].

To summarize, diet, bacteria and the host factors form a triad involved in the formation of a carious lesions. All the factors are dependent on each other, and have to be balanced for optimal oral health. Any imbalance might cause the local pH to drop, compromising the tooth structure.

Histopathology of Caries

Enamel Caries

The enamel structure is composed of hydroxyapatite crystals which are organized into long rods. Each of these rods starts at the dentino-enamel junction and extends into the crown in a wavy fashion[25]. During tooth formation, the mineralization of these rods is somewhat discontinuous and is characterized by highs and lows in the activity level. The periods of low activity create a rest line within the enamel rods. These rest lines, in combination with the rest lines of the neighboring rods, form the striae of retzius which are characterized by relatively higher organic content[25]. The spaces in the striae and the inherent spaces in the prism boundaries provide sufficient porosity for the movement of hydrogen ions and water. Thus, the enamel structure acts as a molecular sieve, by allowing the passage of these small ions and blocking the larger ions. This sieve-like behavior explains the pulpal response before the penetration of the bacteria, because the movement of the ions can result in the acid dissolution of the dentin underlying the enamel before the actual cavitation of the enamel[25]. This attack at the outer ends of the dentinal tubules initiates the pulpal response by unknown mechanisms. The lateral spread of the enamel lesions is because of the greater permeability of the striae in the enamel surface[25].

Physical and Microscopic Features of Incipient caries

The amount of time that it takes to develop a carious lesion may be months or even years. Dental caries is simply not a continual and cumulative loss of mineral, but a dynamic process that involves a series of demineralization and remineralization events.

Demineralization is dissolution of the calcium and phosphate ions from the hydroxyl-apatite/tooth structure into the plaque and saliva. Conversely, the process of remineralization involves the deposition of the calcium and phosphate and other ions from the saliva into the previously demineralized tooth structure. Both these processes can occur simultaneously in the oral cavity, but the lesion formation results only when the rate of demineralization exceeds the rate of remineralization[31].

There are two distinct stages that have been described about the process of lesion formation, with the initial stage being incipient lesion formation, followed by frank cavitation within the enamel[31]. The incipient carious lesion is characterized by the appearance of an area of opacity which is usually described as a white spot lesion. The process at a microscopic level is well established at this earliest stage of lesion formation and is recognizable by an intact surface zone of the enamel. This intact zone is much more porous than the sound enamel and can be retarded, arrested and reversed at any time before clinical cavitation has occurred[31].

The early incipient lesion usually presents itself as a subsurface demineralization with an intact surface layer[31]. The thickness of the surface layer ranges from 10-30 microns, but microscopically the pores extend through the mature enamel to the point where the subsurface demineralization has occurred where the main body of the lesion is located[31].

The incipient carious lesion has been divided into four zones by Silverstone, namely[31]:

a. Translucent zone: the translucent zone is seen in 50% of the carious lesions. This zone is the advancing front of the lesion with slight demineralization.

b. Dark zone: the dark zone is seen in a majority of incipient/white spot lesions. It is the zone of active remineralization.
c. Body of the lesion: this zone is on the periphery of the dark zone
d. Surface zone: This is the outermost zone. It is also the zone of remineralization.

Alterations Seen at Ultra-Structural Level in an Incipient Lesion

When seen at the ultra-structural level with an electron microscope, the incipient enamel lesion presents a ragged profile, with pores extending into the structure of enamel[31]. The initial attack of the acid may be on the rods, between the rods, or can be both[31]. Micro-channels have been observed in the surface zone of an incipient lesion[31]. These micro-channels allow the ingress of acids to the subsurface region, which usually results in the removal of calcium and phosphorus that are a part of the crystal[31].
In the body of the lesion, the appearance of progressive demineralization occurs along the striae of Retzius. Once the lesion reaches the dentino-enamel junction (DEJ), lateral spread occurs along the DEJ, which causes the undermining of enamel. Eventually the surface layer breaks down and this process progresses inwards[31].

The above section mainly describes the structure of the enamel at a macroscopic level, which helps to understand how the process of dental caries is initiated. The physical and microscopic properties describe the different zones of the white spot lesion. The alterations seen at the electron microscopic level is briefly described.

Quantitative Light-Induced Fluorescence (QLF)

The caries process is now better understood to be a dynamic one that is affected by various factors. In particular, it is clear that the process of remineralization and demineralization is cyclical and can affect mineral content in either direction[34]. White spot lesions are very common and may progress to frank cavitation. Thus, caries diagnosis at early stages is important, but has been a challenge for clinicians[34]. Quantitative Light Fluorescence is a visible light system that helps in detecting carious lesions at an early stage and also can be used to monitor them over a period of time[35]. The QLF system uses two forms of fluorescent lights (blue and green) to detect whether the lesion is active[35]. It works on the principle of auto-fluorescence of the mineral content of the enamel. When there is an increase in the porosity due to sub-surface demineralization light scatters as it enters the tooth structure, resulting in the loss of fluorescence[35]. When the tooth is illuminated with a violet-blue light of a wavelength of 290-450 nm from the camera hand-piece, changes in the enamel fluorescence can be detected. The images made are saved on the computer software and processed for analysis[35].

The QLF equipment consists of a light box that contains a xenon bulb and a hand piece. The light is passed to the hand piece via a light guide, and the live images are displayed on the computer and additional pictures of individual teeth can be taken and stored[35].

With QLF, live fluorescent images are captured by a camera, transferred into a computer and stored in an image database. Using the images stored in the database,

measures such as mineral content, lesion depth and lesion size can be monitored with high precision.

Once the images are captured and stored in the computer, they are ready for quantitative assessment of the demineralization of the tooth[35]. This procedure involves proprietary software using a patch of sound tooth to define areas of sound enamel around the lesion of interest. Following this procedure, QLF software in the computer uses the pixel values of the sound enamel to reconstruct the surface of the tooth and subtracts those pixels which are considered to include the lesion[35]. Once these pixels have been assigned as sound or lesion, the software then automatically calculates the average loss of fluorescence in the lesion which is called *delta F/*ΔF. The multiplication of the delta F value by the area of the lesion in square millimeters (mm^2) gives the *delta Q*[35].

Polarized Light Microscopy (PLM)

A polarized light microscope consists of a light microscope with a polarizer and an analyzer, placed perpendicular to each other[44]. Both polarizer and analyzer transmit light oscillation only in one plane and they are made up of sheet of Polaroid.

Polarized Light Microscopy helps to selectively visualize anisotropic structures (presence of anisotropy indicates polarity and order); the microscopy helps to visualize these objects with good optics and proper alignment under which they appear bright and shining[44]. Anisotropic objects exhibit a number of properties, one of which is birefringence. Birefringence is defined as characteristics of an object to transmit plane polarized light at different velocities at different angles[44]. Birefringent objects are seen as shining bodies under the polarized light microscopy. The birefringence property has a

sign. If the refractive index (RI) of the polarized ray which runs parallel to the length of the fiber is greater than the ray polarized in the place perpendicular to the axis, the fiber exhibits positive birefringence[44].

In dental tissues, polarized light microscopy plays an important role in studying a number of diseases, but it mainly helps in understanding caries. In modern times, it was noted by a number of authors that dental caries is associated with changes in birefringence[44], but the birefringence of the carious enamel is lower than that of the normal enamel tissue. It was also noted by Hoppe in 1862[50] that enamel is more birefringent than is dentin. In a study published in 1961, Gustafon and Gustafon[51] found that carious lesions are surrounded by a zone of increased birefringence.

Bonding in Dentistry

Bonding of various materials to the enamel and dentin is one of the most significant advances in the field of dentistry[1]. Many advances have been made in this field since its evolution in the 1950s. Etching techniques for bonding to enamel have revolutionized the practice of dentistry. Although bonding to the dentin has been a challenge, ongoing advances are improving the reliability of dentin bonding agents[2].

History of dentinal bonding agents

It is important to understand the history of bonding agents, as bonding has become a cornerstone of modern dentistry[1]. In the past 50 years of dentistry, there has been an evolution in the development of bonding agents. New concepts of restorative dental practice and adhesive dentistry have been developed during this period. Principles

of adhesive dentistry date back to 1955 when a scientist named Buonocore used the techniques of industrial bonding and postulated that acids could be used for surface treatment before application a of restorative material[1]. Several possibilities were explored to obtain bonding between filling material and the tooth structure. This exploration included the development of resin materials which possessed adhesive properties, modification of available materials, development of adhesive material between the tooth and the filling, and lastly, chemical treatment of the tooth to produce a new surface to which the materials might adhere[1]. Later, Buonocore suggested that the formation of the resin tags was the phenomenon behind the adhesion of resin to the acid etched enamel, and this idea of micromechanical bonding is mostly widely accepted today.

Retention of composite fillings or composite cements is the primary aim of dental adhesives. Development of etching procedures to enamel was another major effort in bringing about bonding of materials to the tooth structure. As time passed, various acids, including phosphoric acid, with different etching times were tested for etching of the enamel[3]. The ability to bond to enamel is reliable and widely accepted today. Bonding to the dentin has been more elusive than to the enamel. The fact that the enamel contains less protein than does the dentin, and that pores of the dentin contain fluid which impedes the bonding to the dentin[5], may help to explain why dentin bonding is more challenging. In addition to withstanding mechanical forces, and in particular shrinkage stress from the lining composite, a good adhesive also should be able to prevent leakage along the restoration's margins. Adhesive systems are commonly used with esthetic resin restorative materials, and the major shortcoming of the earlier acrylic and other filling materials is the lack of adhesion to the tooth structure[1]. Acid treatment is one of the most

commonly used methods to achieve adhesion of bonded restorations to the tooth structure[1]. Buonocore found that resins could be bonded to the enamel of the tooth that was conditioned with 85% phosphoric acid for a period of 30 seconds. He also proposed potential uses of this new bonding technique for various restorative purposes and as pit and fissure sealants[2].

Buonocore et al[2] suggested the formation of resin tags as the primary attachment mechanism of resin to phosphoric acid-etched enamel. The acid etching mechanism removes about 10 micrometers (um) of enamel and creates a porous layer of 5-50 um deep to allow for the resin penetration into the porous layer[2]. Etching increases the surface area of the enamel substrate by exposing the organic material of the enamel, which can then serve as a network to which the resins can adhere[2]. In other words, etching creates spaces deep along the inter-prismatic areas to which the resin can penetrate[3]. When the low viscosity resin is applied to the etched area, it flows into the micro-porosity channels of this layer and polymerizes to form a micromechanical bond with the enamel surface[2]. Conventional adhesive systems consist of three components: an acid conditioner, a primer and an adhesive resin[4].

Bonding mechanisms

"Adhesion is been defined as the attraction between molecules of different materials at the interface"[45].

In bonding between the restorative material and the tooth structure many factors must be considered, including adhesive properties, the use of adhesive interface and the

alteration of the tooth surface to promote adhesion[1]. Adhesion can be categorized by three different mechanisms:

1. Chemical adhesion, which includes covalent, ionic and metallic bonds,
2. Physical adhesion, which includes secondary valence forces such as Vander Waals forces, hydrogen bonds and London dispersion forces, and
3. Mechanical adhesion which is defined as penetration of one material into a different material[46].

Components of bonding agents

The dentin bonding agents usually consists of:

1. Conditioner/Etchant: The conditioner is used to prepare the dentin surface and, if it is used, it is applied before the primer. The conditioners are usually washed off before the application of the primer[4]. Most systems use phosphoric acid, citric acid, nitric acid or oxalic acid as an etchant.
2. Primer: The primers are the bi-functional molecules, of which one functional group contains a hydrophilic component to facilitate wetting and adhesion to the dentine and the opposite end is the hydrophobic group which readily bonds to the bonding resin[4]. The chemical names of the types of primers that are used are HEMA (Hydroxy-Ethyl Methacrylate), PMDN (Pyromellitic diethyl-methacrylate), NPG-GMA (N-phenylglycine glycidyl-methacrylate), NTG-GMA (N-tolylglycine glycidyl-methacrylate), PENTA (Phosphonated penta-acrylate ester), and glutaraldehyde.

3. Adhesives: The role of the adhesives is to chemically bond to the primers and provide an increase in additional micro-mechanical and macro-mechanical surface retention[4]. The chemical names of types of adhesives that bond to the primed dentinal surface are Bis-GMA (Bisphenol-glycidyl methacrylate), UDMA (Urethane-dimethacrylate), TEGMA (Triethylene glycol-methacrylate), and 4-META (4-Methacryloxy-ethyl trimellitate anhydride).

Mechanism of adhesion to enamel

Adhesion to the enamel surface is achieved through the acid etching procedure. Buonocore introduced the concept of acid etching in the year 1955[2]. Etching of the enamel is achieved through removal of the inter-prismatic mineral crystals by acid treatment resulting in an increase in the surface energy, the surface area, and creation of micro-porosities on the surface of the enamel into which the bonding agents can flow, resulting in reliable enamel bond strengths[2, 3].

There are three types of etching patterns. The type 1 etching pattern is the most common type, and it involves preferential removal of enamel prism cores, but the prism peripheries remain intact[2]. The type 2 etching pattern is the reverse of the type 1, in which the peripheries are removed and the core remains intact. The type 3 etching pattern is less distinct and includes areas resembling both of the other patterns and also etching patterns that appear unrelated to the prism morphology[2].

Summary

The above section mainly describes the history of bonding in dentistry, which led to the development of various dental materials like the composites and cements. It then describes concept of acid etching has evolved after the development of various developments in etching techniques and its improvement in achieving adhesion between the tooth structure and the restorative material. It then describes the chemical composition of the various agents used in bonding, and a mechanical type to the tooth structure is achieved.

Sealants

Sealants are coatings applied to the pits and fissures of mainly molar teeth which prevent the growth of bacteria that promote decay in the pit and fissure surfaces of molar teeth[32].

As described by Simonsen, 'the term pit and fissure sealant is used to describe a material that is introduced into the occlusal pit and fissures of caries susceptible teeth, thus forming a micro-mechanically bonded, protective layer cutting access of caries producing bacteria from their source of nutrients'[12].

History

Physically occluding the pits and fissures dates back to as early as 1923. Hyatt suggested that a technique called prophylactic odontotomy, which involved a minimal operative procedure of restoring sound fissures with amalgam[33]. The idea was not fully accepted, which led to a widespread use of Blacks' extension for prevention cavity

preparation[19]. The prophylactic odontotomy was done on sound teeth to prevent them from having cavities, whereas Black's principle was applied on teeth with caries. In the pre-fluoride era, various chemicals (zinc chloride, ammoniacal silver nitrate) were painted on the tooth surface, but none proved to be successful[19]. After the advent of fluoride into the practice of dentistry, an interest developed for preventive materials specifically for pit and fissure surfaces[19].

The breakthrough came in 1955 with the development of the acid etch technique by Buonocore[15]. Cyanoacrylates were used as sealant materials in the 1960s, but their use did not last long. In the late 1960's, the BIS-GMA (product of Bisphenol A and glycidyl methacrylate with methyl methacrylate monomer) was developed and it proved to be successful. The American Dental Association (ADA) issued a provisional acceptance for the first Bis-GMA material in 1972 and gave it full acceptance in 1976. The most widely used sealant materials used today are Bis-GMA resins and urethane-based resins. The Bis-GMA formulation later became a basis for development of a number of other products[16].

Sealants were introduced in the 1960's for the protection of pits and fissures of the occlusal surfaces and the buccal and lingual pits from dental caries[32]. The main function of the sealants was to prevent the growth of bacteria in these pits and fissures, which promote decay in the teeth. Currently, there are two main types of sealants available in the market, the resin-based sealants or composites and the glass ionomer sealants[32].

According to the mechanism involved in the polymerization and the material content, the resin sealants have been divided into different generations. The first

generation products were activated with an ultraviolet light, the second and third generations were auto-polymerized and visible-light activated, respectively, and finally the fourth generation contained fluorides[32].

The glass ionomer sealant is the second main type of sealant material which was introduced in 1974 by McLean and Wilson[32]. Glass ionomer sealants contain fluoride and are thought to prevent caries through the release of fluoride present in them. However, a problem with the glass ionomer cements has been inadequate retention, and results of studies about their relative effectiveness have so far been conflicting[32]. Later in the 1990's compomers which were a combination of composites and glass ionomer, cements were introduced into the market.

Rationale for use of sealants as preventive material

The battle against decay in the pits and fissures has a long and interesting history, which includes the preventive innovations such as early physical blocking of fissures with zinc phosphate cements, mechanical fissure eradication, prophylactic odontotomy, and chemical treatment with silver nitrate[15]. At the time when the acid etch bonding technique was first described in 1955 by Buonocore, it was a new technology and a logical step in the use of bonding in prevention of pit and fissure decay[15]. New methods of caries prevention have focused on the pit and fissure surfaces, as the smooth surfaces have become less susceptible to decay[15] with the advent of fluoride. The disproportional occurrence of the caries on the pit and fissure surfaces continues to date, with these surfaces accounting for approximately 91% of dental decay[15]. The high risk of pit and

fissure caries on almost all the molars has driven the dental profession to see sealants as a highly advantageous procedure[15].

Dental sealants have become an integral part of contemporary restorative dentistry[14]. The advent of the acid etch technique, availability of new restorative material and a better understanding of the caries process has helped clinicians make better recommendations[14].

Types of sealants

The resin-based sealants are divided into categories based on their polymerization as auto-polymerizable, which polymerize by themselves/ without any source for activation, photo-polymerizable; which polymerize after exposure to a source of light of a particular wavelength, and the combination of both[17].

Another category of material is a composite that incorporates particles of glass-hardened glass ionomer cements within its mass. The two other types of glass ionomer are those with light-polymerized liquid and those modified with inclusion of metal[18].

1. Glass ionomer cements: The glass ionomer cements were developed in the year 1974 and reported by Wilson and Kent[18]. Glass Ionomer cements are materials made up of calcium or strontium alumino-fluorosilicate glass powder (base) combined with water soluble polymer (acid) [18]. The term ‘glass ionomer cement’ is applied to materials that involve an acid-base reaction as a part of the setting reaction. The components when mixed together undergo a setting reaction involving a reaction of neutralization of the acid groups by the powdered solid base[18]. As the cements contain fluoride ions,

significant amounts of fluoride ions are released during this reaction, without diminution of physical properties of the hardened cement[19, 32].

2. Resin based sealants: The development of resin-based sealants has progressed through generations, with first generation activated with ultra-violet light, through the second and third generations which were auto-polymerized and visible light activated, respectively, and finally to the fourth generation sealants which contained fluoride[19.]

There were many studies done to study the effectiveness of the sealants over a period of time. The studies done were either to compare sealants (both resin and glass ionomer) with no sealants, or glass Ionomer with resin sealants.

Review of sealant studies

Sealant materials were developed in the 1960s and have been used widely by clinicians since then. Several studies were done that looked at the effectiveness of sealants in prevention of dental caries. The studies compared sealants vs. no sealants (control group), resin-based sealants vs. glass ionomer sealants. The studies included both long term and short term studies.

For the present project, only long term sealant studies will be reviewed. The review will compare resin-based sealants vs. no sealant, and resin-based sealants with glass ionomer sealants.

Resin sealant vs. no sealant

One of the longest studies followed to evaluate the effectiveness of sealant material was done by Simonsen et al[12]. The study was done to compare the sealant

application in the treatment group to a control group with no sealant application. The results of the study showed that, at the end of 15 years, carious or restored teeth made up 31% of surface in the sealed group, compared to 83% in the unsealed group[12].

The Cochrane review of pits and fissure sealants, published in the year 2009, reviewed many studies that compared sealant as a treatment group and no treatment as the control[32]. In the treatment group, the sealants were mainly applied on sound permanent molars in children aged 5-10 years. The results of five split-mouth studies comparing resin-based sealant with the control were all highly significant in providing protection from caries. The mean reduction in caries rate ranged from 87% at 12 months to 60% at 48-54 months[32]. Another study included in the Cochrane review that did a follow up for nine years found significantly more caries in the control group. At the end of nine years, 27% of sealed surfaces were decayed, compared to 77% of decay in the unsealed surfaces[32].

Glass ionomer sealants vs. no sealants

Studies were done to compare the effectiveness of glass ionomer sealants in comparison to the control (no sealant group). According to the Cochrane review[32], a 24 month study done by Songpaisan, the results found no statistically significant difference between the two groups[32].

Glass ionomer vs. resin-based sealants

Several long term and short term studies have been documented for comparisons done between glass ionomer sealants and resin-based sealants. The two groups in the

studies received respective treatment with respective sealants i.e. with a resin-based sealant and a glass ionomer sealant.

A study done by Forss et al[47], compared the retention rates of glass ionomer sealant (GIC, Fuji III) with a resin-based sealant (Delton). On the sealed occlusal surfaces, 10% of the GIC and 45% of the resin-based sealants were totally retained, and 9% of GIC and 20% of resin-based sealants were partially present[47]. Twenty-three percent of the occlusal surfaces filled with GIC, and 16% filled with the resin-based sealant were carious or filled. The results concluded that there was no statistically significant difference between the two materials.

A study done by Raadal et al[48], compared the effectiveness of resin-reinforced glass ionomer sealant with a resin sealant, over a 3 year period[48]. The glass ionomer sealants and resin sealant were applied in pits and fissures of newly erupted permanent first molars. The study design used here was a split mouth experimental design. The results found that the resin-based sealants were almost totally retained (97%) after a period of 3 years with no caries. In the glass ionomer treatment group, the sealant was lost with a complete retention in only 9% of the teeth. Caries were found in 7.4% of the sites in the glass ionomer group. It was concluded that resin-based sealants were superior to glass ionomer sealants[48].

A study done by Beiruti et al[49] compared the caries preventive effect of resin-based sealant with a glass ionomer over a period of 60 months[49]. Forty-six boys and 57 girls were divided into two groups. The sealants were each placed in 180 fully erupted first molars, in their respective treatment groups. The results found that, at the end of 5 years, 86% of the resin-based sealants and 88% of the glass ionomer sealants did not

survive. It was also concluded that the caries preventive effect of glass ionomer was 3-5 times higher than the resin sealant. Therefore, the retention rate of both the types of sealant was low, but, the glass ionomer was more protective compared to the resin based sealant.

The previous section briefly discussed about the sealant material, their history, types of sealants and the review articles that describe the success of the sealant in comparison to no sealants and compare the resin-based sealants with the glass ionomer sealants.

The results suggest that the resin-based sealants are protective in comparison to no sealant, whereas, when the glass ionomer is compared to the no sealant group, there was no statistical difference between them. When a comparison was done between the glass ionomer and the resin-based sealant, some studies concluded that the resin-based sealants were superior, while some concluded that the glass ionomer were superior, and some concluded saying no statistical significant difference was found between both the sealant materials.

Preventive Resin Restorations (PRR)

The predilection for caries of the pits and fissures of the occlusal surfaces has been known for a long time[6]. Arthur, in 1867, said that decay in pits and fissures were inevitable and that obliteration of them could prevent its occurrence[6]. Application of solutions like silver nitrate solution, zinc chloride and potassium ferro-cyanate solution and obliteration of pits and fissures with black copper cement and silver amalgam were once advocated for preventing pit and fissure caries[6].

Preventive measures like fluoride therapy and application of sealants in the pit and fissures of the occlusal surfaces of the posterior teeth gave a new thrust to preventive dentistry for children in the 1970s[6]. The scope of dentistry not only includes primary prevention, but also secondary prevention. Thus, on occurrence of dental caries in the pit and fissures, there should be an emphasis on secondary prevention or the preservation of sound structure of the tooth. Preventive resin restorations are a class of restoration that focuses toward preservation of sound structure of the tooth.

A preventive resin restorations (PRR) consists of a composite filling preparation with the addition of an occlusal sealant applied to the un-decayed pits and fissures. Preventive restorative resins were developed after sealants in the early 1970's and have been discussed in the literature since the year 1977[7, 8].

Before the advent of sealants into the field of dentistry, clinicians believed in the concept of extension for prevention[9]. Extension for prevention was proposed to eliminate the frequent multiple restorations on the occlusal surface[9]. Restoration of a small area of caries would be extended by restoration of all the susceptible fissures[9] and thus, healthy tooth structure would be removed to prevent the occurrence of decay on those surfaces.

Amalgam was widely used by clinicians for class 1 fillings and also in lesions that did not involve all the pits and fissures. They removed the carious part, along with the entire pits and fissures of the tooth, creating a class I cavity[7]. Thin amalgam restorations were not strong enough to withstand the occlusal forces unless the cavities or preparations extended into the dentin and, thus, more tooth structure was removed. With the advent of etching and bonding techniques into dentistry, the PRR technique was widely accepted[9]. The term PRR was used because it was both restorative and preventive

and the amount of tooth reduction was minimal compared to class I cavity preparations for amalgam. This procedure removed only the diseased portion of the tooth and resin material was used to restore the tooth[7].

Types of PRR

According to the clinical studies done in the past, preventive resin restorations have been used differently on posterior teeth [9]. Three types of PRR's have been classified looking at the use on the molars, and also the development of wear resistant composites for posterior use[9]. The type 1 PRR is used in case of minimal decay and the presence of decay is questionable. The carious tissue is removed with the help of a small round bur and restored entirely with a sealant material. Once the small cavity preparation is filled with a sealant material, rest of the sealant material on the pits and fissures can be applied[9]. The type 2 PRR is used when caries is confined to a small area but has extended into the dentin. Careful examination is to be done to see if caries have spread along the dentino-enamel junction, if so; the cavity preparation is completed with the removal of adjacent pits and fissures. A protective layer of calcium hydroxide/glass ionomer cement is placed on the exposed dentin with thin intermediate coating of dentin bonding agent, then a composite material is placed into the cavity. Once the tooth is restored with a composite resin, rest of the pits and fissures can be restored with a sealant material to prevent further occurrence of caries[9]. The type 3 PRR is very similar to the type 2, as the restorative material is used only to fill the cavity preparation and the pit and fissure sealant is used to seal the rest of the pits and fissures which makes the sealant an integral part of the preparation[9].

The sealants are used for the purpose of primary prevention from dental caries. The preventive resin restoration is a combination of composite and a sealant that is placed over the lesions and seals the rest of the pits and fissures. The review of sealant studies in the previous sections explains various issues, like the loss/failure of sealant that can lead to the occurrence of secondary caries. In a preventive resin restoration, the loss of the sealant material on the restoration can led to secondary caries. So, the major drawback of the preventive resin restoration is the sealant material.

According to a recent published study by Simonsen[55], preventive resin restorations have been in use for the past 35 years[55]. The concept and types of remineralization therapies have advanced since then. The type 1 PRR, which uses the sealant material for filling the small cavity prepared along the pits and fissures to preserve the anatomy, can be remineralized with the current therapies and revisions can be made in the classifications[55]. The type 2 and type 3 are both used in dentistry, mainly the type 3 where the cavity is prepared and restored with a composite and the remaining pits and fissures are coated with a sealant material.

The PRR ideally suits small carious lesion in a tooth confined to the enamel that would otherwise lose a considerable amount of tooth structure if the extension for prevention philosophy of conventional treatment was followed[9].

The success of the PRR depends mainly on the success of the sealant that covers the preventive restorative material. The occurrence of secondary caries due to the loss of sealant has been controversial. Fiegal et al[15] reported that PRRs had a good record, but were susceptible to failure if the overlying sealant failed[15]. A number of clinical studies have been done to assess the effectiveness of the preventive resin restoration in

comparison to amalgam and other sealants. A systematic review table[10] (Appendix: Table1) has been produced, and all the studies show a favorable outcome, though many of them report a total or partial loss of the sealant as major problem[10]. The review table which was published by McComb[10] briefly describes the outcomes of clinical studies done with preventive resin restorations. A brief outcome of each study is described further.

Summary of studies in the systematic review

A study done by Raadal[58] compared the effectiveness of sealing and filling with composite resin (Concise® diluted with concise enamel bond®), to only sealing. The study included 896 permanent first molars from 281 children, and fissure sealants were placed on 647 teeth and preventive composite filling in 249 teeth. The follow-up results at the end of 30 months showed that, in the sealing group the success rate was 94% after 6 months, falling to 75% after 30 months. In the composite group the success after 6 and 30 months were 93% and 83.5% respectively, which means that the composite fillings showed a better rate of success. The main reason for the failure in the composite group was loss of sealant or the filling material.

A three year study of preventive resin restoration published by Simonsen[60] compared three types of treatments in newly erupted molars among 6-8 year olds, with questionable caries[60]. Group A consisted of teeth that were prepared; none of which exceeded the size of a round bur (no.1) and treatment was done with white sealant. Group B consisted of teeth with either single or multiple preparations in the tooth with the preparation greater than the size of a no.1 bur but less than the size of no.2 bur, after

which the teeth were treated with diluted resin (mixture of filled and unfilled resin). In group C, preparations were included that were bigger than the size of a bur no.2 and the treatment was done with filled resin after the application of unfilled resin[60]. The results of the study showed that after a follow up of three years, 99% of the subjects in group A had fully retained sealants with no caries. In the group B the success rate was 97.3% and no caries, and 71% with no caries in the group C.

A clinical trial done by Welbury et al compared the efficacy of an amalgam restoration along with a composite restoration and fissure sealant only, in the management of occlusal caries[61]. One hundred and fifty pairs of restorations were placed in a 103 patients and a follow up was done over a 5 year time period. A total of 19 restorations failed, 11 of which were the amalgam and 8 were composite restorations. In all the remaining amalgam restorations, there was some anatomical deterioration. Of the remaining composite restorations, seven had clinical wear, and five had marginal staining.

The authors concluded that there was no statistically significant difference in median survival rates of amalgam and a composite resin restorations[61].

A study done by Granath et al[62] did a clinical evaluation of preventive and class I composite restorations[62]. Preventive resin restorations in 87 permanent teeth, 35 occlusal composite restorations in primary molars and 13 in pre-molars were followed for 2 years. The evaluation was done by assessing the anatomic form of restoration, marginal adaptation, caries, color match, marginal discoloration, and surface structure. In the preventive resin restorations on permanent molars and occlusal composites on primary

molars, none had a success rate lower than 93%. In the third category, which is on the pre-molars, the success rate was 100%[62].

The results concluded that composites on the occlusal surfaces offered a promising future for operative dentistry.

A study conducted at the University of Minnesota by Roth et al[63], measured the longevity and clinical success rate of preventive resin restorations[63]. The study was a retrospective study which audited the charts of the patients with one or more PRR's placed during their visit to the dental clinics at the university. The record of treatment was reviewed and teeth were examined. One hundred restorations were examined in 64 patients. Of all the restorations, 34% restorations received an intermediate bonding agent, and information on the rest was not available. All the teeth were restored with P3O or P5O composite except four teeth, which were restored with Silux™. The sealant material used to seal all the teeth was 3M Concise ™ or Helioseal. ANOVA was used to analyze the relationship between the sealant and age of the restoration. The mean values showed that there was a greater loss of sealant in the older restoration but was not statistically significant with a p value of 0.47[63]. The author suggested that PRRs were promising in this study and recommended their use by clinicians.

A three year study that compared preventive resin restoration with amalgam was done at the University of Tennessee, by Cloyd et al[64]. The study compared class I amalgam restorations with preventive resin restorations[64]. Seventy-four PRR's and 52 amalgam restorations were placed in molar teeth of 38 patients. Disperse-alloy (Johnson and Johnson) was used as amalgam alloy and P-50, a composite material (filled) and white sealants, both manufactured by 3M™, were used as PRR materials. Evaluation of

the fillings was done after every 6 months for the first 2 years and then at the end of three years. The results showed that during the three year period, none of the amalgam restorations failed. The PRR results showed that, during the first 6 months there were 2 PRR failures and at the one year there were 6 failures[64]. The failures in the PRR were because of the improper sealant retention. No additional failures were reported after that till the end of three years.

A study done by Stadtler[65] analyzed the 5 year survival rates of fissure sealants and fissure restorations. Sealants were applied on sound teeth with no caries and fissure restorations (extended sealants) were applied on teeth with questionable caries. Five hundred and thirty-four teeth were included in the study, of which 242 had sealants placed and 292 were treated with fissure restorations[65]. Contingency tables and survival analysis were done, and the survival rates for both were calculated using the Wilcoxon and log-rank test. The results showed that the survival rate (no total loss of sealants or fissure restorations over the 5 year period) was 94%. The probability of no partial loss of both the materials over a 5 year period was 77%[65]. According to the author, the PRR technique was recommended, but periodic checks and repairs of the restorations would have to be done.

A study done by Gray and Paterson[66], conducted a field trial that assessed glass ionomer restorations and resin sealants at the end of one year[66]. One hundred and twenty-eight teeth were included in the study. Glass ionomer restorations were provided to the teeth because the cavities just extended into the dentin and resin sealant material was placed over the restorations[66]. Patients were recalled after 6 months and one year. At the

end of one year, 98 restorations were reviewed. All the glass ionomer restorations were intact in all the examined teeth, but only 12 sealants were completely retained.

One of the longest studies that were conducted on preventive resin restoration was done by Houpt et al[67]. The subjects were followed for nine years. Three hundred and thirty-two class I restorations were placed in 240 permanent molar teeth in 114 children, aged 6-14 years. All the teeth that were included had an incipient lesion. After the caries removal, the base of the cavity was covered for pulpal protection with dycal (calcium hydroxide cement), and an auto-polymerizable, composite resin (Miradapt, Johnson and Johnson dental) was applied for cavity obturation. Once the composite was set and excess removed a sealant material (Delton, Johnson and Johnson dental) was applied. Tinted sealant was used on half the teeth and rest of them had a clear sealant material applied on them[67]. The cavity that had minimal preparations had the sealant material as restoration. The restorations were further examined at 6 months, 1, 1.5, 2, 3, 4, 5, 6, 6.5 and nine years. At the end of nine years, 79 restorations in 28 subjects were available for assessment. In 43 (54%), the sealant was intact and retained, in 20 (25%), the sealant was partially lost and 16 (20%) had completely lost the sealant. In 19 (25%) of the subjects had sealant loss along with caries[67]. The results concluded that composite restorations were excellent given such long term results.

A clinical retrospective study conducted by King et al[68], evaluated the performance of preventive resin restorations placed in a hospital environment[68]. Records for the patients that received the PRR within the period of 1990 to 1993 were retrieved in a hospital in Hong Kong. The inclusion criterion for the study was that the restoration had to be in the mouth for a minimum 6 months. Records were obtained for the treatment

procedure, type of composite, sealant and any sealant repairs done. Five hundred and thirty-two restorations in 351 patients had been included. The clinical performance was determined with a US public health services rating system.

The results showed that there was no marginal discoloration in 425 (80%) of the PPR's, 95 were discolored without penetration (18%), and discoloration with penetration along the margins occurred in 5 (1%) of the PRR's[68]. The marginal adaptation was fully sealed in 151 (28%) of the PRR's, while it was open in 2 (.5%) of the PRR's. Five hundred and twenty (98%) of the PRR's did not have any caries, and 12 restoration had secondary caries. The study concluded that the PRR's are an excellent option as a minimally invasive procedure as well as for the long term survival property.

A study done by Kilpatrick et al[69] evaluated the clinical performance of light cured glass ionomer sealant with a composite sealant restoration/preventive resin restoration[69]. The Glass Ionomer sealant and the PRR were placed in this split mouth study. Sixty seven patients with 80 restorations were recruited initially, out of which 9 patients with 14 restorations failed for appointments after the restorations were placed. Of the remaining 58, 25 were male and 33 were female and they had a total of 66 restorations. The restorations were followed for 27 months at an interval of every 6 months. Minimal cavity preparations were made along the enamel and dentin if necessary for both of the materials. For the resin restoration a base of calcium hydroxide cement was given before the composite was placed. The sealant material was placed above the composite restoration. The glass ionomer cement was placed directly over the cavity preparation without any base and extended as a sealant[69]. The success of the sealant material and the composite restoration was evaluated with the amount of sealant loss or

occurrence of caries. A binomial test and McNemar's chi square test were used to analyze the rates of failure between the two types of restorations, and survival analysis was also done. The results showed that over the 27 months of the trial, 26 of composite restorations and 24 of the glass ionomer restorations survived completely. In a total of four cases there were indications of failure of the underlying restoration. One was from the composite restorations and the other three were from the glass ionomer resotations[69]. Three of the composite restorations required sealant repairs, compared to 10 of the glass ionomer group. The study concluded that there was no significant difference in the durability of composite restorations compared to glass ionomer restoration, but the sealant retention was better in the composite group.

A study that was done at the University of Iowa College of Dentistry, evaluated the effectiveness of preventive resin restorations in pediatric patients[70]. Patient information, type of treatment, any further treatments, and the related dates, were retrieved from the patient management system[70]. This retrospective records review found that 5185 PRR's had been placed in the permanent teeth of pediatric patients in the age range of 6-18 years. Out of 5185 PRRs, 4314 had not required any replacements, while 323 required only sealant replacement. A follow up for 6.5 years was done with the teeth that did not require any treatment. The results found that molars required a statistically significant increase in size of the restoration after the placement of PRR compared to the pre-molars. Reasons for the increase in the size of restoration included failure to replace the sealants, smooth surface caries at a distant coronal site, restoration in a partially erupted teeth and placement of the PRR where the teeth were hypoplastic[70]. The results

concluded that PRRs are very effective when the restorations are placed in a proper manner and examined at regular intervals.

Mertz-Fairhurst et al[71] in 1998 published a 10 year follow up of a study which evaluate the effectiveness of treating frank cavitated lesions; sealed composite restorations placed over a carious lesion and placement of ultraconservative, localized sealed amalgam restorations without an extension for prevention. These two treatments were then compared to traditional unsealed class I amalgam restoration[71]. The study design was four-celled study design. This design allowed comparing the four groups of restorations. One hundred twenty three patients aged 8-52 years were recruited for the study. All of patients in this split-mouth study received either a localized sealed amalgam as group 3 (77), which was paired with bonded and sealed composite restorations as group 1 (77) and an unsealed amalgam as group 4 (79) paired with bonded and sealed composite restorations as group 2(79). All soft and demineralized tissue was removed for both the types of restorations, and hard and stained areas were left. The treatment assignment was statistically randomized for each group. The combination of these two composite was used to assess the clinical longevity and to monitor caries progression under the sealed composite restoration[71]. Group 1 and 3 and group 2 and 4 compared directly in same patients. Different patients in group 1 were compared with different patients in group 2; similarly different patients in group 3 were compared with different patients in group 4. Of the 156 pairs at the baseline, 85 pairs (54%) were evaluated at year 10[71]. The results showed that, 16% of the composite restorations and 25% of the amalgam with sealants, exhibited complete sealant retention. Fifty-four percent of the composite and 57% of the amalgam with sealants exhibited partial retention. Caries

occurred in the margins of only one composite restoration and one amalgam restoration with sealants, but seven of the amalgam restoration without sealants had developed secondary caries. There were three sealant failures in the composite group compared to two in the amalgam with the sealant group. The results indicated that both the types of sealed restorations exhibited superior clinical performance and longevity compared with unsealed amalgam restorations[71].

Summary

The success of the PRR mainly depends on the success of the restoration which depends on the overlying sealant material. Failure of a sealant material either led to failure of the PRR or secondary caries (caries adjacent to the restoration). Although PRR is a successful method of treating lesions in the enamel, periodic examinations have to done to check the sealant material overlying the restoration; which attributes to the success of the PRR.

Infiltration Studies

The ICON® (infiltration concept), developed recently, works using a micro-invasive technique. The material is infiltrated into the porosity of the tooth structure and stops the further progression of the lesions.

After the advent of bonding agents and development of sealants, some studies on infiltration of early carious lesions were conducted. However, the research on these kinds of studies is very limited.

The earliest of the studies done on the concept of infiltration dated back to 1976 when Robinson et al[42] published their work. They investigated the concept of infiltrating a carious lesion using an adhesive compound called resorcinol formaldehyde[42]. An attempt was made to develop an infiltration material with certain properties such as having low viscosity, and being bactericidal, hydrophilic, mechanically supportive and cosmetically accpetable[42]. Resorcinol formaldehyde was the material that matched the properties and was selected for the experiment. Extracted human molar teeth with white spot lesions were infiltrated with the resin solution (resorcinol formaldehyde) and polymerized. The penetration of the resin greatly increased when etched with HCl (1 N) for 5-10 seconds. The teeth were further sectioned and examined under transmitted and reflected light. A different method (experiment) was used to determine the space volume within the lesion before and after the treatment[42]. Standard white spot lesions were created on pre-molars using 10% gelatin gel which was acidified to a pH of 4.5[42]. Chlor-naphthalene was applied on to the surface of the lesion, and gradually the material penetrated into the porosity. Once the infiltration into the porosity was complete, the excess material was wiped away and the teeth were immersed into 10ml of spectrophotometric hexane. The chlor-naphthalene diffused from the lesion pores into the hexane. The mass of the chlor-napthnalene was calculated. The demineralized panels were then treated with the resin and the volume determined. The difference between the two volumes was used to determine the volume of resin was occupied the pores[42]. The results showed that 60±10% of the lesions pore volume had been occupied by the resin. The histological section also showed that the resin evenly occluded around 60% of the lesion. The effect of resin on subsequent demineralization showed that in the control

group (no treatment) the pore volume of the lesion increased by 300%, as compared to the treatment group[42].

An in-vitro study that was comparable to previous one was done by Robinson et al[37], was published in 2001 and assessed resorcinol formaldehyde in comparison to other commercially available resins including Scotch bond ™ (3M Dental products), Gluma® 2000 (Bayer Dental, Germany), All-bond (Bisco Inc., Ill), Amalgam bond (Parkell Biomaterials, NY) and Cyanoacrylates[37]. The study was done using extracted human teeth (mainly on molars and pre-molars) by creating artificial lesions using acidified gelatin gel, and the pH was adjusted to 4.5 using lactic acid, and the time of exposure was 6 weeks[37]. Each group had a sample size of five. The pore volume of the lesions was assessed in a similar way as in the previous study. All the resins were applied to teeth in their respective groups for 3 applications, and pore volume was assessed[37]. Sections were made to assess the extent of infiltration. The treated teeth were subjected to secondary demineralization and were exposed to 5% gelatin gel with a pH adjusted to 4.5, for seven days. After the secondary demineralization the pore volume was re-determined. Analysis was done using two-way ANOVA. The results showed that all the polymers reduced the available pore volume to chlornaphthalene (used to measure the pore volume), significantly after the first and second application[37]. After the third application only resorcinol-formaldehyde and scotch-bond further reduced the pore volume significantly. After the secondary acid attack, the pore volume in the control group (no treatment) increased significantly when compared to the treatment groups[37]. The study concluded that the resin material had the potential to infiltrate into lesions and protect them from a secondary acid attacks[37].

A study done by Rodda[43], published in 1983, assessed the impregnation of dental resins in artificial carious lesions[43]. The study was an in-vitro study, and was done using extracted human third molar teeth. Artificial white spot lesions were created on the lingual and the buccal surface of the teeth. A longitudinal control section was cut from the center of each lesion and the space was filled with wax. The exposed enamel was etched with HCl for 60 seconds. The 4 lesions in each tooth were treated with four resin materials; methyl Methacrylate (Sevitron^{TM}), and three different Diacrylates that were chemically and light activated. The chemically activated resin was Delton^{R} fissure sealant (Johnson and Johnson dental products) and Adaptic Bonding agent^{TM} (Johnson and Johnson dental products). The light activated were Nuva seal^{TM} (L.D Caulk Company) and Visio-bond ^{TM} (Espe, Seefield, Germany). Quantitative assessment for the depth of penetration was done using polarized light microscopy. The methyl methacrylate failed to penetrate any lesions. All of the other resins infiltrated into the body of the lesion, but the penetration depths varied[43]. Sevitron failed to penetrate into the lesion, whereas Adaptic bonding agent and Delton sealant were able to penetrate completely to the base of the lesion[43]. NuvaSeal penetrated to approximately half of the lesion while partial penetration was observed with Visio-bond as well.

A study done by Gray et al[20] assessed the extent to which carious lesions can be infiltrated with a polymerizable resins[20]. The in-vitro study was done on extracted human pre-molars. The roots of the teeth were resected and the crown was cut into two halves vertically. Two lesions were created on each half (buccal and lingual) by exposing the teeth for 6 weeks to 15% gelatin gel with pH at 4.5. Lesions were treated using ethanol before the resin application. Surface treatment was done using Scotch Bond^{TM} and Seal &

Protect [TM, 20]. The groups of both resins were further subdivided using an etching time of 0, 5 and 10 seconds. The number of applications with zero as etching time was one. The etching times of five and ten seconds had three resin applications.

Electron microscopy was done to determine the extent of infiltration, and two-way ANOVA was used to test for differences in penetration due to surface treatment regimen within each group of results. The results showed that as the time of etch and number of applications of resins increased the percentage penetration of the resin material also increased. The study concluded that the current available bonding resins had a potential to be used for infiltration technique[20].

Goepferd et al[50] tested the resistance of white spot lesions to acid attack when etched with phosphoric acid and sealed with a resin material[50]. This in-vitro study compared the resistance of resin treated white spot lesions with control lesion where no treatment was done. Fifteen caries free teeth were painted with nail varnish leaving a small window (on buccal and lingual surfaces) for artificial lesion creation. Lesions were created using 15% acidified gel with the pH adjusted to 4.0[50]. The white spot lesions were divided into zones: baseline lesion, re-exposed lesion and sealed lesion by applying varnish in the middle third of the original lesion. One of the windows on each surface was enlarged, and the surrounding enamel was etched with 37% phosphoric acid. The lesions and the surrounding etched enamel were treated with unfilled, light-cured resin material. The enamel around the treated area was further exposed. The control lesion on the other half of the tooth was untouched[50]. The teeth were re-exposed to acidified gel for an additional six weeks. The teeth were sectioned bucco-lingually and then studied under polarized light microscopy[50]. The results showed that the resin treated area progressed

less when compared to the baseline (mean lesion depth=67 microns) and progressed lesions (mean lesion depth=100 microns), but the new lesions around the resin treated (mean lesion depth=57 microns) areas progressed less than the treated areas (mean lesion depth=60 microns) explaining the resin tags that are formed around the treated area[50].

Another study done by Garcia-Godoy[51] et al, assessed the caries progression of white spot lesions sealed with an unfilled resin[51]. The in-vitro study used extracted human molars for the experiment. Two small rectangular windows (5mm×2mm) on the mesial and distal side of the crown were left open and rest of the tooth was painted with a varnish for lesion creation[51]. The teeth were immersed in acidified gel using lactic acid with the pH adjusted to 4.2. Out of the two windows created, on one of the lesion, half of the area was covered with a varnish to maintain the areas of the initial lesion and the other half was left open. The second window was etched with 37% phosphoric acid and then treated with Prisma Universal Bond 2 adhesive™. After the treatment the teeth were subject to secondary demineralization in the gel for 40 days until frank caries was induced in the non-treated area. Statistical analysis was done using ANOVA and Duncan's Multiple Range test. The results showed that the mean depth of the initial control lesion covered under the varnish progressed to a depth of 366 microns, where the frank caries depth was 746 microns. The treated areas depth after the secondary acid attack was 298 microns[51]. The study demonstrated the effectiveness of sealing a white spot lesion with an unfilled resin to prevent from further demineralization[51].

All the above described studies were done in-vitro. There were some studies to assess the effectiveness of the infiltration technique in a clinical setting.

One such study was done by Clarisse Abuchaim et al[54], at the University Of Oeste De Santa Catarina Brazil, and evaluated the effectiveness of sealing active proximal caries lesions with an adhesive system after one year[54]. Subjects were recruited from those coming to the dental school for treatment purposes. The age range of the subjects was 18-40 years old[54]. Around 230 individuals were examined for the study, out of which 45 were included in the study, with 13 in the control (no treatment) and 32 in experimental group. Two from the control group, one from the experimental were excluded due to difficulties in radiographic interpretation. The inclusion criteria were that the individuals had to be healthy, and bite-wing radiographs had to show at-least one proximal carious lesion. Patients in both the groups were advised to use fluoridated paste along with flossing. The patients in the treatment groups received treatment with an adhesive (OptiBond Solo, Kerr, Orange, CA, USA) after etching with 35% phosphoric acid for 20 seconds. Excess material was removed using dental floss and light polymerized for 20 seconds. After 6 months and at the end of one year the participants were called for follow up radiographs[54]. The caries progression rate was analyzed descriptively after one year, and the efficacy of sealing was evaluated using the McNemar's test[54]. At the end of one year, in the experimental group, 22% of the cases showed regression of the lesion, no change was seen in 61% and progression was seen in 16% of the cases. In the control group, regression, no change and progression was observed in 27%, 36% and 36% of the subjects, respectively.

Summary

Various infiltration materials (resins) showed promising results in terms of infiltration into the lesions, prevention from secondary acid attack. Although one clinical study demonstrated promising results, very few clinical studies have been done to assess the efficacy of such materials.

ICON® (Infiltration Concept)

ICON® is an abbreviation for Infiltration Concept, a new treatment concept introduced into the field of dentistry in 2008 for treating white spot lesions[20]. It is a micro-invasive technique used for the treatment of white spot carious lesions that occurs either on the buccal, lingual and proximal surface of the teeth.

The early enamel lesions are mainly characterized by loss of mineral beneath an intact surface layer, and such lesions appear as white spots because of the increased porosity within the body of the lesion[29]. Enamel lesions in the buccal area of the teeth are a frequent occurrence as a result of orthodontic treatment/fixed orthodontic appliances as these appliances make it difficult to clean these areas. This results in increased plaque accumulation and caries formation[29]. Such lesions are arrested or reversed through either remineralization through the precipitation of mineral ions dissolved in the saliva into the lesion or through other processes promoted by the various sources of fluorides such as water, dentifrice, fluoride varnish[20, 21].

Various minimally invasive approaches for the treatment of white spot lesions have been proposed in the past[22]. One of the procedures involved a combination of micro-abrasion and enamel remineralization, where the micro-abrasion was performed

using a paste containing silicon carbide micro-particles in soluble water paste and 6.6% hydrochloric acid[22]. A paste containing casein phospho-peptide amorphous calcium phosphate complexes (CCP-ACP) was used as the remineralization paste[22]. The major drawback of using this technique is the high amount of enamel that is eroded as a part of micro-abrasion. The infiltration technique has several advantages over the other techniques. First, deeper lesions can be improved by the infiltration techniques which aren't amenable to remineralization, and the esthetic improvement can be seen instantly. Secondly, the infiltration is much less invasive compared to restoring the tooth[29].

ICON® is a caries infiltration technique and an alternative therapeutic approach to prevent the further progression of enamel lesions. The aim of this treatment is to occlude the porosities within the body of the white spot lesion with a low viscosity light-cured resin that has been optimized for penetration into the porous enamel[29]. Infiltrants are light-curable resins that were developed for penetration into the porosities of a lesion, with low viscosity, low contact angles to the enamel, and a high surface tension. All of these properties are important for complete penetration into the porosity[29]. Before the infiltration of the resin, the enamel is conditioned using 15% hydrochloric acid gel. The infiltrant (resin) penetrates into the lesion driven by a capillary force. The resin material was developed after a several pilot studies done at the University of Kiel, Germany, that developed the ICON® infiltrant, for which they had experimented with various mixtures of resin materials such as TEGDMA, BisGMA and ethanol using various mixing ratios. A mixture of highest penetration coefficient was preferred as an infiltrant[30], so as to create a diffusion barrier within the lesion and not on the lesion surface. Once the resin

material is infiltrated, excess material was removed from the surface of the lesion using a cotton swab and the material infiltrated, and curing was done using an ultra-violet light.

A single ICON® package consists of an Infiltrant which is a composed of tetra-ethylene glycol dimethacrylate, additives and initiator, an acid conditioner to etch the enamel surface made of 15% hydrochloric acid, and ethanol. ICON® works on the principle of the light-scattering phenomenon[29]. The sound enamel has a refractive index of 1.62. The porosities of a white spot carious lesion are usually filled with either a watery medium or air, which have refractive indices of 1.33 and 1, respectively[29]. The whitish appearance of the lesion is because of the difference in the refractive index between the enamel crystals and the medium within causes the scattering of the light[29]. The micro-porosities that are caused by the lesion are infiltrated with the resin material which has a refractive index of 1.46, thus making the differences between the enamel and porosities negligible, so that the lesion appears similar to the surrounding enamel[29].

Of all the previous research done with resin materials' infiltration capacity and ability of those materials in prevention of secondary demineralization (secondary caries), some were not conclusive, some showed promising results, and some concluded saying that further research was required. To bridge this gap the development of ICON® material was done at the University of Kiel, Germany. There were a series of lab studies involved in the development of the ICON® material. The summary of the relevant studies is summarized further below. It should be noted that some of these studies were done on bovine teeth. These studies are not included in this literature review as the physical properties of bovine teeth are different than human teeth, so that the findings of those studies may not be relevant to human teeth.

Invitro Studies with ICON®

An in-vitro study[41] done by S.Paris et al evaluated the penetration co-efficient of four experimental resin materials. The aim of the experiment was to evaluate the effect of infiltrant composition and penetration co-efficient (PC cm/sec) on inhibition of progression of proximal caries lesions[41]. Four experimental resins varying in penetration co-efficient were used as positive controls, and untreated lesions served as negative controls. Eighty-four extracted human molars and premolars having proximal non-cavitated lesions were selected for the study. Standard protocol was followed for cleaning the teeth and examining them for any defects. Lesions were etched with 15% HCl, subsequently air dried and treated with experimental light-curing resins that differed in composition and penetration co-efficient (PC63, PC185, PC204, PC391)[41]. As a positive control (PosC) lesions were etched with 37% phosphoric acid and treated with an adhesive (Excite, Ivoclar, Vivadent) for 30 seconds. Subsequently a flow-able composite (Tetric Flow;Ivoclar Vivadent) was applied was applied to the surface as a covering coat and light cured. Caries lesions that were neither etched nor treated served as negative controls (NegC). All the teeth were exposed to a cycle of demineralization for 400 days in a gel solution with a pH of 4.95. The teeth were sectioned perpendicular to the lesions to yield sections with a thickness of 1300 microns. The lesions were ground further to obtain thinner sections for analysis[41].

Statistical analysis was done using the SPSS software. Differences in mineral loss before and after storage were analyzed by Wilcoxon test. The various groups were compared using the Kruskal-Wallis and Mann-Whitney tests[41]. The mineral loss in all 77 teeth was evaluated at baseline and did not differ significantly among the six groups. After the teeth

were stored in the demineralizing solution for 200 days and subsequently for 400 days, the negative control and PC63 and PC185 showed significantly higher demineralization compared to the baseline, and positive control and PC204 and PC391 did not progress significantly after 200 days, but after a time of 400 days, the percentage of mineral loss in PC294, PC391 and positive control was lower compared to all the other groups[41].

An in-vitro study was conducted by Lueckel et al[39] to assess at the penetration co-efficient of the ICON® resin material in comparison to a commercially available adhesive. Two groups of teeth were treated with either an adhesive and with the ICON® resin material[39]. Extracted human molars and pre-molars showing white spot lesions on the proximal surface of the teeth were used for the experiment. Standardized radiographs were taken of each tooth. Roots of the teeth were resected and the crown cut into halves along the lesion providing two halves of each lesion[39]. Lesions were checked with stereo microscope, and if the lesion extended into inner half of dentin, they were excluded. Corresponding halves with same lesion criteria were assigned to infiltration (experimental infiltrant which was ICON® in experimental stage) or adhesive (Excite, Vivadent). Standard procedure was followed to treat the teeth with the respective material[39]. Assessment was done using the confocal microscopy and transverse micro-radiography. The differences in lesion depths, penetration depths and percentage were analyzed using the Wilcoxin test corrected for multiple testing. The results showed that the mean maximum lesion depths for both the groups were comparable. Mean maximum penetration depth and penetration percentage was significantly higher for the infiltrant (ICON®) compared to the adhesive[39]

Penetration of the resin infiltrating material is hampered by the mineralized surface layer of the white spot lesion. This surface layer has to be removed by the etching procedure. A study was done to test and compare the efficacy of three different etching gels in removing the surface layer[36]. The etching gels that were used were 37% phosphoric acid and two experimental HCl gels for varying applications times[36]. Human molars and pre-molars with proximal white spot lesions were used for this in-vitro study. A total of 96 teeth were selected and cut into half and were allocated to twelve groups (n=8). The cut surface and one half of the lesion served as control. Subsequently, the lesions were treated with 37% phosphoric acid, 5% HCl or 15% HCl for 30, 60, 90, or 120 seconds. Confocal microscopy and microradiography were used to examine the specimens. Paired t-tests were done to assess the differences in sound and erosion samples within the groups. Comparison between the groups was done using one way ANOVA and Bonferroni post hoc test[36]. The results showed that the surface layer reduction was significant with 15% HCl when the etching time was 90 and 120 seconds as compared to those etched with the 37% H_3PO_4[36].

The study demonstrated that 15% hydrochloric acid was more effective than the 37% phosphoric acid in surface erosion and creating porosity to enable the infiltration.

The effect of resin infiltration in cavitated lesions is unknown. To test this, an in-vitro study was done by Paris et al[56]. The caries infiltration technique has mostly been tested on non-cavitated lesions, but this study assessed the infiltration on cavitated lesions on the proximal surface of pre-molars. The aim of this study was to evaluate infiltration patterns of proximal caries lesions differing in ICDAS (International Caries Detection and Assessment System) classification [56]. Extracted human pre-molar teeth were used for

the experiment. Two trained examiners were used to classify the lesions according to the ICDAS codes (inter-observer agreement was 0.80). The inclusion criteria were teeth with active lesions that had codes ICDAS codes 2, 3, 4 or 5. Lesions were infiltrated with the ICON® infiltrating material following the manufacturer's instructions (Acid etching with 15%HCl, rinsing with water, air drying, drying with ethanol, and infiltration with ICON®) [56]. Three sections, each of thickness .5-1mm were made from each lesion, mounted on a slide and polished. The sections that had deepest cavity extension were used for further analysis[56]. Dimensions of the image taken under the microscope (lesion depth LD, area of demineralized enamel LA_{demin}, cavity depth CD, cavity width CW, cavity area LA_{cav}, extent of the resin penetration, also called as infiltration depth InfD, infiltrated area in demineralized enamel $InfA_{demin,}$ and area of the cavity filled with infiltration $InfA_{cav}$,) were measured. In addition to these, the other variables that were calculated were area of the entire lesion, percentage infiltration of non cavitated lesion parts, percentage of the cavity infiltrated by infiltrant, and percentage infiltration of the entire lesion including the cavity. Shapiro-Wilk tests was used to test the assumption of normality, and Kruskal-Wallis test and Mann-Whitney U test were used to calculate differences in infiltration percentages of demineralization, cavity infiltration, ,infiltration of whole cavity, and depths of penetration for the different ICDAS codes.

The median lesion depth was 961 microns in the enamel, however, most of the lesions showed an increase in porosity in the dentin[56]. The resin infiltration penetrated deeply in all the demineralized parts, but no significant difference in percentage infiltration of demineralized enamel of various ICDAS codes was found[56]. The cavity depth, width and

area increased with an increase in ICDAS code. Lesions with ICDAS codes 3, 4, and 5, had no infiltration.

No significant difference with infiltration or filling was observed between lesions with various ICDAS codes[56]. The depth of infiltration in ICDAS 4 and 5 was significantly lower compared to code 2.

Clinical Studies with ICON®

After the ICON® infiltration material was developed and in-vitro studies assessed the effectiveness of the material, there were a few clinical studies done to assess the efficacy of the material. Below is description of few field trials that were conducted to test the ICON® material.

A clinical study done by Shin-Kim et al at the Department of Pediatric Dentistry, Pusan National University, evaluated the resin infiltration for masking labial enamel[52]. Specifically, the aim of this study was to clinically assess the effectiveness of the resin infiltration technique in masking white spot lesions[52]. Twenty-one subjects with white spot lesions on their maxillary anterior teeth were recruited into the study at the time of their regular dental visits. Twenty teeth with developmental defects of enamel from 12 children and eighteen teeth with post orthodontic decalcification from nine children were selected as subjects[52]. The lesions that were selected had to meet the criteria of being inactive lesions with smooth, hard, and shiny surfaces, white spot lesions with ICDAS code 2. Treatment was provided with the ICON® resin infiltration material by following the standard procedure. The lesions were etched with 15% HCl for 120 seconds. The etching gel was then washed away for 30 seconds and air dried. Ethanol was used to

further dry the lesions. Infiltration (ICON®) was applied and allowed to penetrate for three minutes. Excess material was wiped away using a cotton roll and the surface was light cured[52]. The process of infiltration was repeated with infiltration of the resin for one minute followed by light curing it, and polishing was done using polishing discs[52]. Standardized clinical photographs of the lesions were taken before treatment, immediately after treatment and one week after the treatment. Evaluation of the color changes was done using an image analyzing software. The degree of color changes (as evaluated by CIELAB, an international system for color measurements) was evaluated after one week of treatment, the teeth were categorized into three groups; T1: where the whitish appearance was completely masked, T2: where the whitish appearance of the lesion was partially masked and T3: the whitish appearance showed little change[52]. Intra-rater reliability was evaluated by asking the examiner to re-evaluate ten randomly selected images after an interval of 2 weeks (value: 0 .91, $P<0.05$). Statistical analysis used Wilcoxon's Signed rank test to determine whether statistically significances existed between the pre and post treatment color differences[52]. The results showed that, of the 20 teeth with developmental defects of enamel, 5 teeth (25%) were completely masked, and seven (35%) and eight (40%) were partially masked or remained unchanged, respectively. Among the 18 teeth with orthodontic white spots, 11 teeth (61%) were completely masked, 6 teeth (33%) were partially masked and one tooth (6%) remained unchanged[52]. The conclusion of the study was that the color changes were dramatic in some compared to others, but that longer duration studies of color changes are required to assess differences.

An eighteen month split-mouth study done by Martignon et al, looked at the efficacy of sealing proximal early active lesions[53]. The study used conventional and subtraction radiography as an assessment technique. The outcome variable was caries progression after 18 months. Subjects were selected from those coming to dental clinics in Copenhagen, Denmark and Bogota, Columbia. The individuals included had to have at least two proximal lesions apparent on bitewing radiographs. Eighty-two subjects, 43 Danish and 39 Columbian individuals were recruited into the study. Lesions that were restricted to the outer half of the enamel, inner half of the enamel, and outer third of the dentin were selected for the study. Lesions deeper than the outer third of dentin were recommended for operative procedure and excluded[53]. Another inclusion criterion was having bleeding on probing, adjacent to the lesion. For each individual, the two lesions were randomly assigned to be either the control lesion (no treatment), or the treatment lesion. Two appointments were made for the subjects. During the first appointment, caries risk assessment was done, and radiographs were taken. The analysis with the cariogram showed that majority of Columbian and Danish (approximately 61%) subjects were under intermediate risk category and approximately 30% were high risk[53]. At the second appointment, 72 lesions received treatment with the Gluma one Bond Adhesive (Heraeus Kulzer). However, 10 subjects from the Danish group were treated with concise sealant (3M ESPE). Instructions were given to floss all proximal lesions three times a week[53]. Eighteen months later the patients were recalled for follow up radiographs of both the test and the control lesions. Statistical analysis was done using the chi-square test and McNemar's test.

There were 10 (12.2%) patients who dropped out of the study (six Columbians, four from Denmark) [53]. Two subjects had restorations on the lesions (one had on both test and control, and the other on test lesion), so they were excluded from the final analyses. The final data from the subtraction method showed that 30 (43%) of the test, and 59 (84%) control lesions had progressed ($p<0.001$, 95% CI: 28-56%).

This study showed that, compared to the control lesions, the test lesions performed better. The authors concluded that sealing early proximal lesions was very feasible[53].

Ekstrand et al[57], did a clinical split mouth study that aimed to assess the efficacy of resin-infiltrated caries lesions covered by fluoride varnish compared to fluoride varnish only[57] after one year. Children with two or more superficial proximal lesions on the deciduous molars were included. The lesions were detected using bitewing radiography[57]. Two clinicians helped to assess the ICDAS codes of the lesions at the beginning of the study. The target population consisted of 5-8 year old children, and the total sample size for the study was 48. Two lesions in each of these 48 children were randomly allocated to one of the two treatments, resin infiltration along with fluoride varnish (2.26%) versus fluoride varnish only. The resin infiltration treatment was given with the ICON® resin infiltration. Standard manufacturer's instructions were followed for the resin infiltration[57]. The proximal surfaces were cleaned with floss and a rubber dam was applied. The adjacent tooth was protected using a plastic/metal strip and treatment with ICON® was accomplished according to the manufacturer's instructions. A coat of fluoride varnish was applied to both the treated lesions and the control lesions[57]. The children were examined after six months and another coat of varnish was applied. After one year the lesions were clinically examined by the same two examiners that assessed

the lesions at the baseline. In addition radiographs were taken after one year using the same equipment and methods as done at baseline[57].

Of the 48 children at the baseline, three children had exfoliated either the test or control or both the teeth[57], three of them moved to a different area, so six children had left the study leaving a sample size of 42. In three of these 42 children, radiographs could not be obtained so the radiographic examination was done on 39 children. The sign tests was used to assess whether there was difference between the baseline and test group, in median ICDAS score and median depth of the lesion. McNemar's test was used to assess the proportion of progressed lesion. The results showed that after one year, based on ICDAS score, 31% of the test lesions and 67% of the control lesions had progressed. Radiographically, 23% of the test and 62% of the control lesions had progresses[57]. The authors concluded that fluoride varnish along with resin infiltration showed promising results[57].

A radiographic study done by Paris et al [40], evaluated the progression of lesion after infiltration. The study was a randomized split-mouth placebo-controlled trial. The aim of this experiment was to assess whether resin infiltration into the proximal lesions was more effective than non-operative measures alone with respect to the progression inhibition[40]. Twenty-nine pairs of proximal caries lesions from 22 young adults were included in the study, with lesions having to be in the inner half of the enamel and outer third of the dentin. Radiographs were taken at baseline for evaluation, out of 61 screened individuals 22 met the inclusion criteria, and informed consent was obtained before inclusion into the study. ICON® infiltration was performed on the test group and placebo was used for the control group. Manufacturer's instructions were followed for the

treatment with ICON® and water was used as treatment in the control group (placebo) [40]. Oral hygiene methods such as brushing and flossing were suggested for all subjects as part of the treatment. After 18 months, a radiographic assessment was done for both groups. Digital subtraction radiography (standardized bite wings) was used to compare the progression of the lesion by a pair-wise comparison. Statistical analysis was done using the McNemar's test[40]. The results showed that in the ICON® treated lesions, 7% of the lesions showed progression, while in the placebo 37% lesions showed progression.

Summary

Sealants provide a physical barrier between the pits and fissures and the oral environment and prevent dental caries. Bunocore's revolutionary work with different adhesives led to the development of sealant material in 1974. The two most commonly used sealants are resin based, and glass ionomer sealants. Several studies have demonstrated the success of sealants. Some studies concluded that resin based sealants to be superior to the glass ionomer cements based on their retention and longevity. Failure of the sealant material has been attributed to decreased retention, and may lead to secondary caries. The benefit of using sealants on already cavitated lesions is questionable, and preventive resin restorations (PRR) were developed for these situations

PRR consists of a tooth preparation with a composite filling, with a sealant on the top. However, the success of PRR is again attributed to the sealant materials, failure of which can cause secondary caries. Clinical studies done on the PRR have documented the success of using PRR material. The studies that have failed were attributed to the sealant

failure. Thus, alternate treatments which do not rely on surface retention have been explored to overcome these shortcomings.

One such approach is the concept of infiltration which has been studied since the early 1970's. These early studies have provided the ground-work for evolution of infiltration material by helping to develop the materials and methodology for treating cavitated and non-cavitated lesions with infiltration resins. These studies have concluded that resin infiltration materials are promising in prevention and treatment of dental caries. Early work on the infiltration resins, led to the development of ICON® at the University of Kiel, Germany. ICON® is a resin infiltration technique that is used for the treatment of white spot lesion on the smooth surface of the tooth. The treatment kit consists of 15% hydrochloric acid (as opposed to 37% phosphoric acid in sealants) as an etching material, methanol (used as a drying agent), and the resin for infiltration. Several in-vitro studies were done to develop the ICON® infiltration material.

Recent clinical studies with the ICON® infiltration technique were done to test the physical properties of the material to see the color changes after the infiltration and to assess the progression of the lesion after treatment. These studies concluded that ICON® material is effective in preventing the progression of the caries lesion, in comparison to no treatment. Radiographic studies have also been done to monitor the progression of the lesion. The clinical studies that were conducted using the ICON® showed promising results. However, longer studies with more subjects are required.

No studies were done to compare ICON® material with a sealant material. The present study was an in-vitro study done to compare the ICON® material with a resin based sealant material, in prevention of secondary demineralization.

Rationale for the study

This study was done to assess the effectiveness of the ICON® (infiltration concept) material in prevention of secondary demineralization of the tooth structure, in comparison with sealant material. The study was initially planned on the pit and fissure surfaces, but to better understand the material, its properties and infiltration the study was done on the smooth surface.

The ICON® material was designed to be used on the smooth surfaces and acts on the principle of infiltration into white spot lesions on the tooth surface. When the treated and infiltrated lesion undergoes attack at the margins of the restoration, it is believed that the porosities within the lesion are infiltrated and therefore, further demineralization is prevented. Not only should the material be acid resistant, but the bacteria should be prevented from traveling within the restored area, because the material has infiltrated throughout the porosity.

The infiltration concept was compared to a standard sealant, which also must form a strong seal to be beneficial. Sealants are a mechanical barrier placed on the pit and fissure surfaces of the teeth which prevent the tooth from decay by preventing the bacteria to colonize in pit and fissure surfaces. For the bacteria that are already present in the pit and fissures, the sealants prevent them from acid production by cutting them from the source of carbohydrates. Sealants are usually recommended on sound tooth structures without any decay, although some studies recommend placement of sealants on non-cavitated enamel lesions. In the case of sealing a non cavitated carious lesion, a secondary caries attack at the margin of a sealant could possibly spread beneath the

sealant and make the lesion active again. Since the ICON® material works on the concept of infiltration, retention is not a concern and secondary caries risk is greatly reduced.

CHAPTER 3

MATERIALS AND METHODS

Overview

This chapter describes the laboratory procedures that were involved in data collection, data management and analysis of the data. It describes the steps involved in tooth collection along with disinfection, lesion creation (tooth preparation and initial demineralization), treatment with ICON® and sealant materials and finally secondary demineralization. It also describes the pilot studies that were done to arrive at the final methods for the thesis project.

Research Question

The research question and the hypotheses involved are presented below:

- Is the resin based-material ICON®-Infiltration Concept effective in the prevention of secondary demineralization of smooth surface enamel in comparison to a resin-based sealant material?

Hypotheses

- There is no significant difference in the ΔF among three treatment groups at each time point.
- There is no significant difference in in the ΔF between the ICON® (infiltration concept) and Sealant groups at the visit 3.
- There is no significant difference in the values of (ΔF3-ΔF4) among three treatment groups.

Operational Definitions

ICON®: ICON® (Infiltration Concept) is a relatively new treatment material introduced in the field of dentistry in 2008 and used for treating white spot lesions and was marketed by DMG® America in the United States. The ICON® kit consists of an infiltrant, which is a resin material (composed of tetra-ethylene glycol di-methacrylate, additives, initiator), an acid conditioner to etch the enamel surface made of 15% hydrochloric acid and ethanol.

Demineralization: It is the loss of minerals or mineral salts from the tooth, especially through disease, as in the loss of calcium. This can be measured with quantitative light fluorescence.

Resins: A synthetic compound, usually acrylic based, to which consists of high percentage of inert filler material and principally used as a restorative material in dentistry.

Sealant: A resin-based preventive material that is applied to and adheres to the pit and fissure surfaces of teeth to prevent access to pits and fissures where plaque, food, and bacteria usually become trapped.

QLF (Quantitative light-induced fluorescence): QLF is a dental diagnostic tool for *in-vivo* and *in-vitro* quantitative assessment of dental caries lesions, dental plaque, bacteria, calculus, staining, and tooth whitening. With QLF, real-time, fluorescent images are analyzed using software to calculate the loss of mineral and they can be stored in an image database.

Polarized light microscopy (PLM): It is a type of optical microscopy which involves transmission of polarized light through a thin section of the tissue. In this work, the tissue consists of tooth sections.

Institutional Review Board

The present study using extracted human teeth is exempt under the criteria of the Institutional Review Board (IRB) of The University of Iowa, so no human subjects approval was required.

Laboratory Procedures

As demonstrated in Chapter 2, no published studies have evaluated the effectiveness of the infiltration concept (ICON®)/ infiltration technique in preventing secondary demineralization of smooth surface lesions, in comparison with sealant material. This study involved the comparison of a resin-based sealant material with infiltrant concept (ICON®), which infiltrates into the white spot lesion. The study was an *in-vitro* laboratory study which compared the sealing effectiveness of ICON® to that of a resin-based sealant on smooth surface lesions, when challenged by a secondary acid attack or demineralization. The dependent variable for this experiment was the amount of demineralization, or ΔF (which is loss of mineral content within the tooth and a loss of fluorescence) at each time point, and the difference in the mean ΔF3- ΔF4. The independent variables were the types of treatment used, which were the resin-based sealant and the ICON® material.

Pilot studies

Pilot studies were done to better understand the ICON® material, including the process of curing and infiltration, and evaluation of sections of the teeth under PLM. The studies also assessed the methods of lesion creation, infiltration of the ICON® material into the lesions, and methods of sectioning for polarized light microscopy.

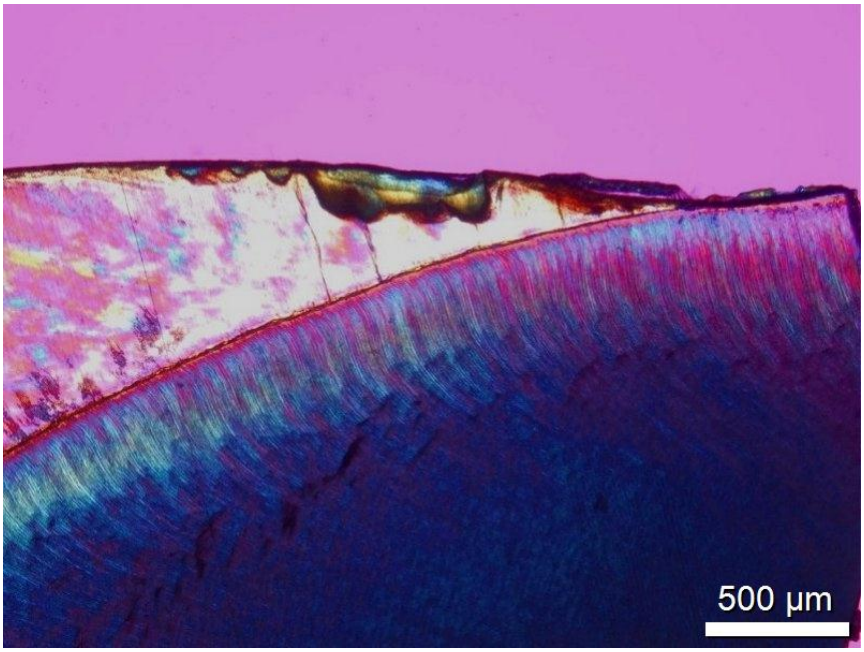
The literature reported the use of ICON® on the smooth and inter-proximal surfaces. Several pilot studies were done to look at the feasibility of using the material on the occlusal surface, although the occlusal morphology can often be problematic. It was not known if predictable infiltration was possible on the occlusal surface, whether it could be measured, and if polarized light microscopy (PLM) sections could be obtained for evaluation. Thus, the use of ICON® in the laboratory was a new effort that required many initial trials and patience to find the right combination of measurement techniques, lesion parameters, and tooth surface which could be evaluated in a meaningful fashion.

Thus, to determine the final materials and methods, an extensive series of pilot studies was done to better understand the material and to design the final study protocol.

Study 1: Five extracted molar teeth were selected with natural white spot lesions on the buccal surface of the teeth. An attempt was made to treat those lesions with ICON® material, following the directions suggested by the manufacturer's instructions, but it became clear that a single etching of the lesions for three minutes was not sufficient to break the surface layer (a mineralized layer on a white spot lesion) to allow for penetration of the infiltrant. The lesions were deep, so no significant difference was observed after the ICON® treatment, when viewed with the naked eye, as the lesion color did not change. A review of the scientific literature on ICON® suggested that increased

etching time would facilitate the rupture of the surface layer of the enamel and allow the penetration of the infiltrant into the lesion. Thus, we followed the procedure of etching the lesion twice for three minutes each before we could facilitate a proper infiltration of the resin material into the lesion. No significant difference was observed in the lesion appearance after infiltration of the material, when viewed with the naked eye, as the material is supposed to reduce the white opaque appearance of the lesion. When the teeth were sectioned and viewed using polarized light microscopy, it was observed that there was infiltration of the material into the outer one third of the lesion (Figure 1). The study demonstrated the need for an increase in the time and number of etches.

Figure 1: Smooth surface lesion treated with ICON®, showing infiltration. The lesions were etched using 15% HCl, twice, for three minutes.



Study 2 evaluated the occlusal surfaces. We used a small number (5) of mandibular molar teeth which had less complicated pit and fissure anatomy, but also had either some demineralization present as a white spot lesion or some discoloration along the pits and fissures on the occlusal surface.

The molars were etched twice with the 15% hydrochloric acid for three minutes each and infiltrated with ICON® resin onto the occlusal surfaces of the molars. After the etching procedure, the teeth were washed under running water for 30 seconds and dried with moisture free air for 45 seconds. Ethanol was also used to further facilitate drying of the lesions. To evaluate the infiltration of the ICON® material into the occlusal surface we sectioned the teeth along the bucco-lingual direction. However, we failed to obtain any intact sections of enamel on the pits and fissures since the cusp tips chipped off, leaving a flat occlusal surface. There was a thin layer of enamel present in the pits and on the cusps. (Figures 2 and Figure 3).

Figure 2: Occlusal section treated with ICON® that was distorted while cutting

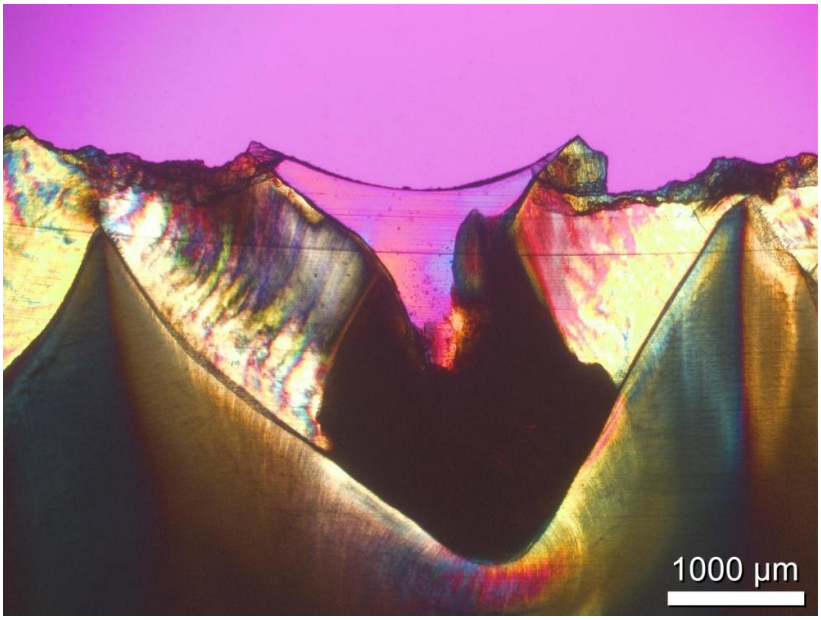
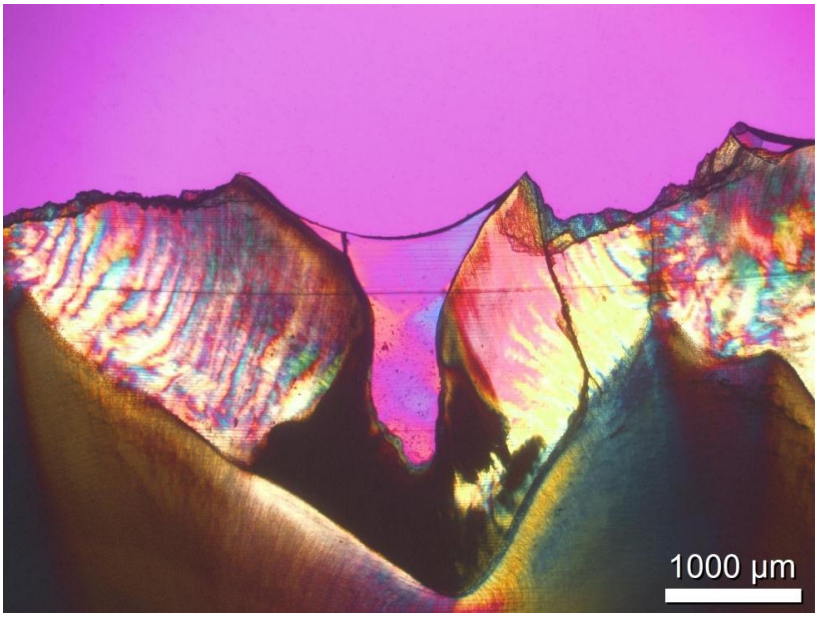


Figure 3: Occlusal section treated with ICON® that was distorted while cutting



Following this, it was decided to infiltrate the resin material on the occlusal surface with one half of the tooth treated and the other half left as a control. Then sectioning started in a mesio-distal direction from the middle of the tooth and we continued the cut toward either the buccal or the lingual surface. This was done to see if we could differentiate the treated side from the control (no treatment) under the polarized light microscopy.

Thus, for *Study 3*, five molar teeth were selected. The teeth had white spot lesions on the occlusal surface, which were treated with ICON®. The treatment was done according to the manufacturer's instructions. During the sectioning, the tooth structure did not hold together, and again we failed to get any sections. Studies 2 and 3 informed us that additional techniques would be required to obtain sections from ICON® treated teeth.

In *Study 4*, during the etching and curing of the material on the occlusal surface of the tooth, we decided that we would leave some excess resin material to support the enamel and facilitate making sections that could be interpreted. So, we selected five molar teeth that had discoloration and white spots on the pit and the fissure surfaces of the teeth. For these teeth, we etched twice for three minutes each and further washed and air dried. Infiltration was done with alcohol supplied by the manufacturer to dry the excess water inside the lesion and to facilitate the infiltration of the resin material. Infiltration was done with the ICON® resin supplied, and the material was allowed to penetrate into the lesion for three minutes, after which the excess was removed and polymerized with a UV light. A second application was done for two minutes and excess material removed and polymerized with UV light. When the sections were subsequently

made, they were undistorted, with good visibility of the enamel along the occlusal surface of the tooth. However, due to the occlusal morphology, most (approximately 90%) of the sections were also chipped and broken and, therefore, not usable. However, there was no significant amount (infiltration limited to the surface only) of resin infiltration on the occlusal surface of the enamel when viewed under a polarized light microscope. The discolorations seen along the pits and fissures of the teeth selected were arrested carious lesions. The infiltrated resin material flowed along the walls of the pits and fissures sealing them properly like a sealant, but it was not feasible to distinguish the infiltrated areas from the normal enamel on the occlusal surface with natural lesions. The examples of sections for this study are shown in Figures 4 and 5.

Figure 4: Example of a section of a tooth treated with ICON®

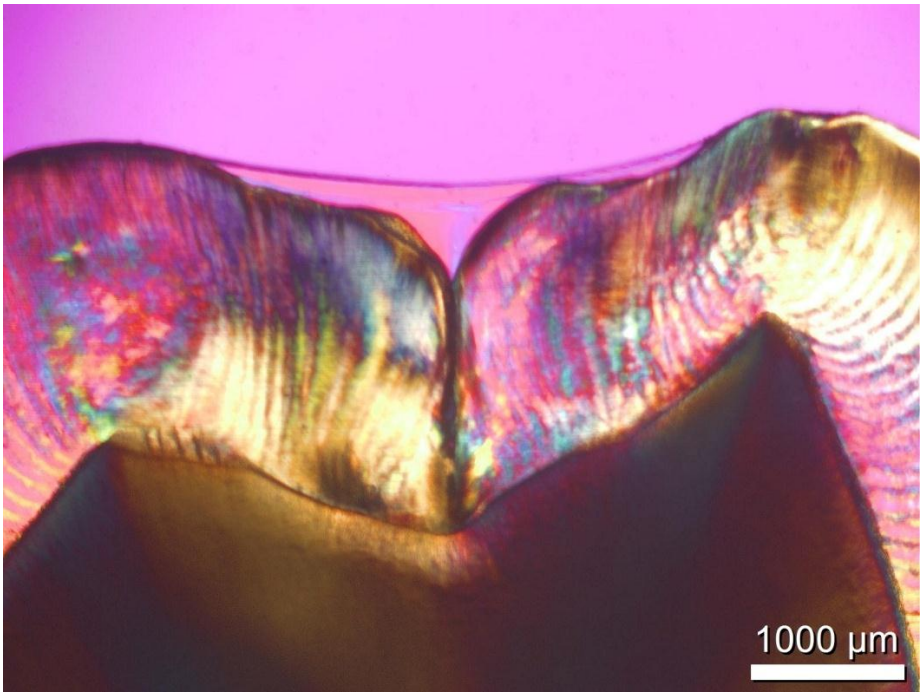
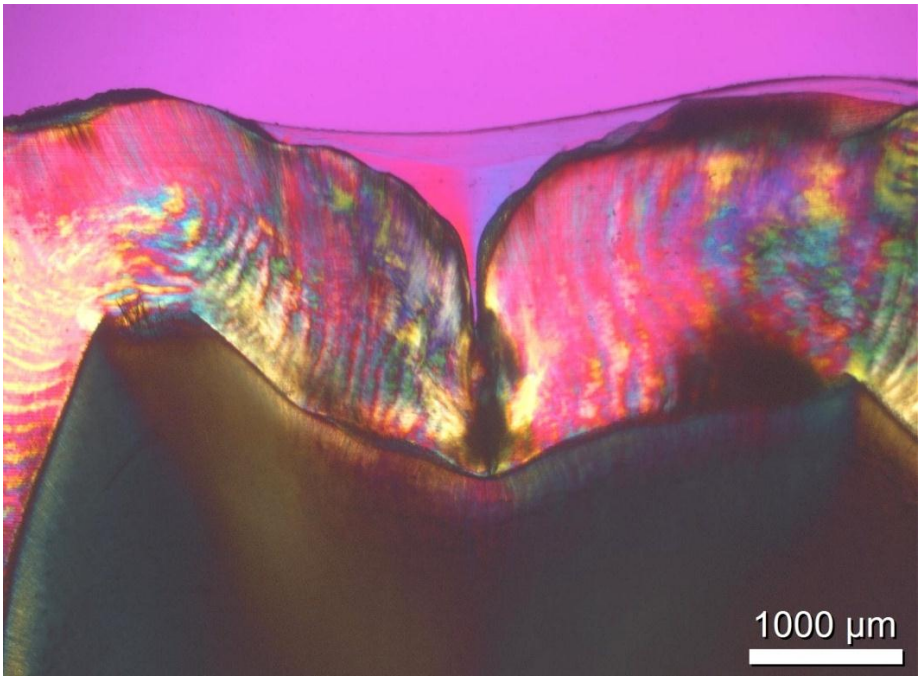


Figure 5: Example of a section of a tooth treated with ICON®



The next step (*Study 5*) evaluated the extent of infiltration into the lesion. We collected five mandibular molar teeth with white spot lesions along the pits and fissures of the occlusal surface. We wanted to see if the material infiltrated to the base of the lesions. The teeth were thoroughly cleaned of debris and any other human tissue and were treated with ICON® according to the manufacturer's instructions, as described earlier. Assessment for this study was done with the quantitative light fluorescence technique (QLF), before making any sections for the polarized light microscopy. The examples of QLF images obtained are displayed in Figure F. Change in the fluorescence was calculated using the QLF technique. Although the ICON® infiltration technique was effective in reduction of ΔF values on the occlusal lesions after treatment, the ΔF values increased (change of mineral) increased after secondary demineralization, suggesting that

the resin infiltration was not effective in prevention of secondary demineralization. With the images captured on the QLF machine, it was difficult to interpret the infiltration of the resin material on the occlusal surface due to poor differentiation of the infiltration material from the tooth structure.

After the data collection, some ICON® material was left on the occlusal surface to support the enamel while sectioning the tooth. Sections were made using the Silverstone-Taylor thin section microtome to examine lesions under polarized light. Upon examination, it was not possible to interpret if the material had infiltrated and, thus, we were not able to draw conclusions about the infiltration of the material into the lesions. An example of the PLM sections for this study is shown in Figure 7.

Figure 6: From left to right: Natural caries on occlusal surface, Lesion treated with ICON®, Lesion after the secondary acid attack.

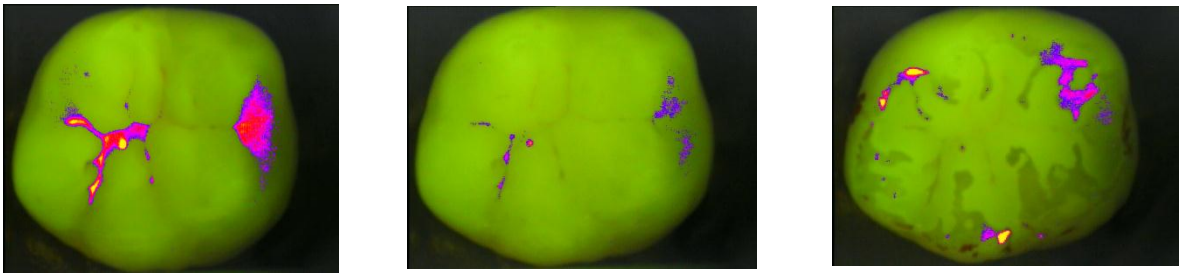
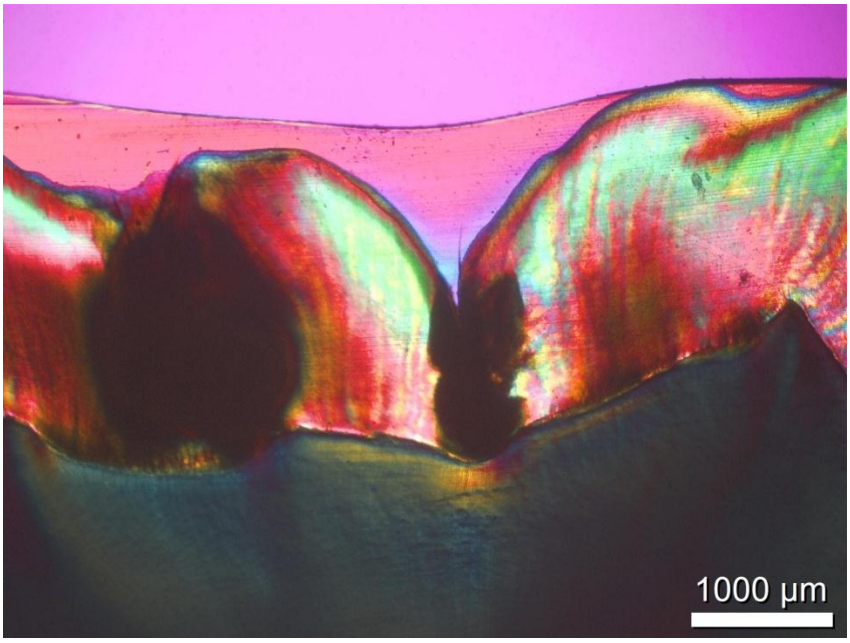


Figure 7: Example of section after treatment with the ICON® material on the occlusal surface.



The morphology of the occlusal surface made it difficult to assess and interpret the infiltration when thick sections were used. Some of the larger lesions which extended into the dentin would not consistently infiltrate (dentinal fluid could have been the problem).

Thus, Studies 4 and 5 suggested that it was not possible to assess infiltration of ICON® on occlusal surfaces.

For all the above reasons, the decision was made to focus this thesis on the smooth surface, instead of the occlusal, in order to better evaluate and study the material. It was apparent that PLM could only be used at one point in time, since it is a destructive technique, and a method to follow tissue changes during the ICON® treatment procedure,

after every step, would be required. For this reason, the QLF technique was chosen as the main evaluation technique for this study. One reason for not deciding on doing this earlier was the initial desire to study the occlusal surface, if possible, but the QLF technique is not suitable for this surface.

Since the ICON® infiltration technique was designed for infiltration into the porosity of the white spot lesions, assumptions were made that the infiltration required wide pore volumes. The lesions created in the lab using an acid-containing gel demonstrated wide pores with increased pore volume, when compared to the usual acid-buffer created lesion.

For *study 6,* teeth were selected for creating white spot lesions on the smooth surface of mandibular molars, and two different methods of demineralization were evaluated. An acid-containing gel and acid-buffer demineralization solution with a pH of 4.5 were the two methods used to create the lesions. The time taken to create lesions with the acid-containing gel was slightly longer than the time required for acid-buffer solution (approximately 24 hours longer). The gel lesions were also shallower when compared to the acid-buffer lesions. The shallow lesions were used to see if the infiltration penetrated to the base of the lesions. After the infiltration of ICON®, the appearance of the lesions using both methods was not significantly different when compared by the polarized light microscopy, as the material did not infiltrate to the base of the lesion in either type of lesion. According to the PLM sections, ICON® material infiltrated to significant depth of the lesion.

An example of gel lesions treated with ICON® is shown in Figure 8 and an example of acid buffer lesions, treated with ICON® is shown in Figure 9:

Figure 8: Example of lesion created with an acid gel and treated with ICON®

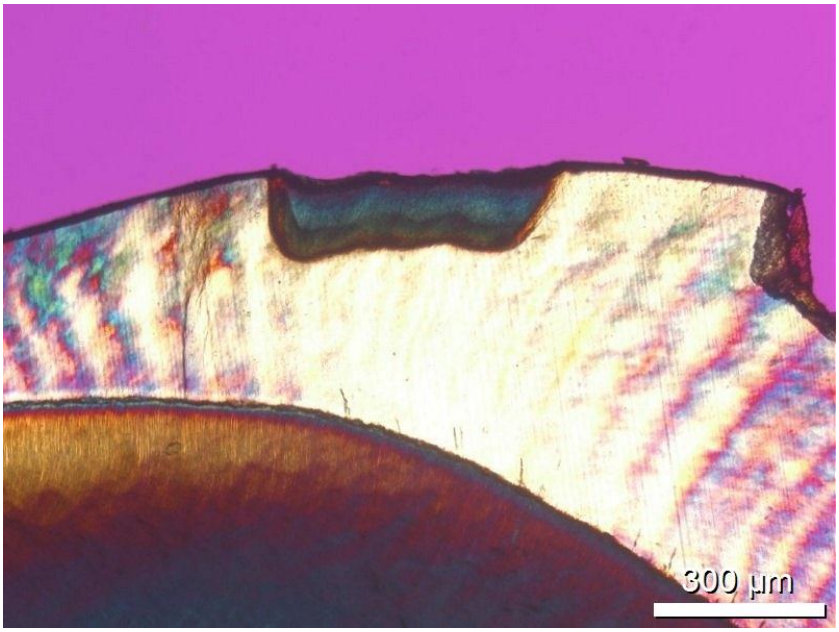
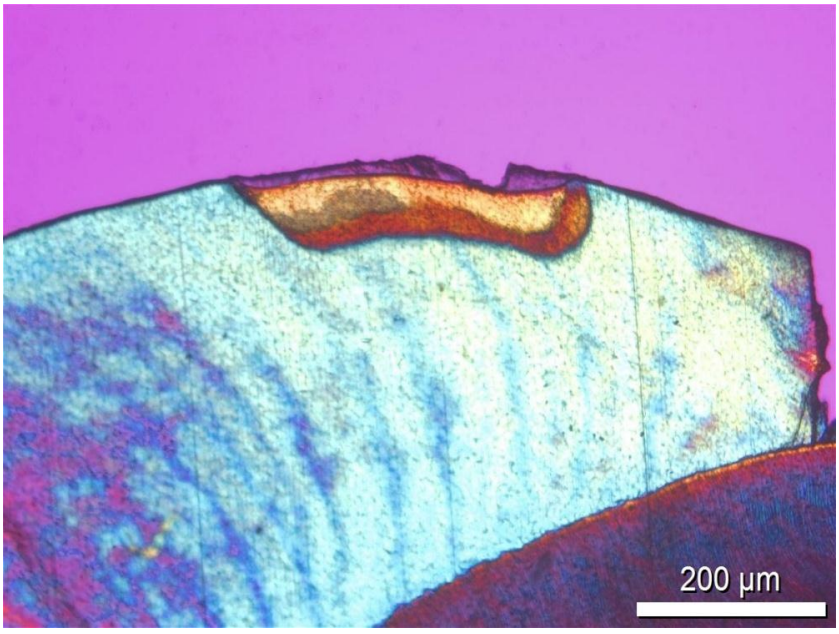
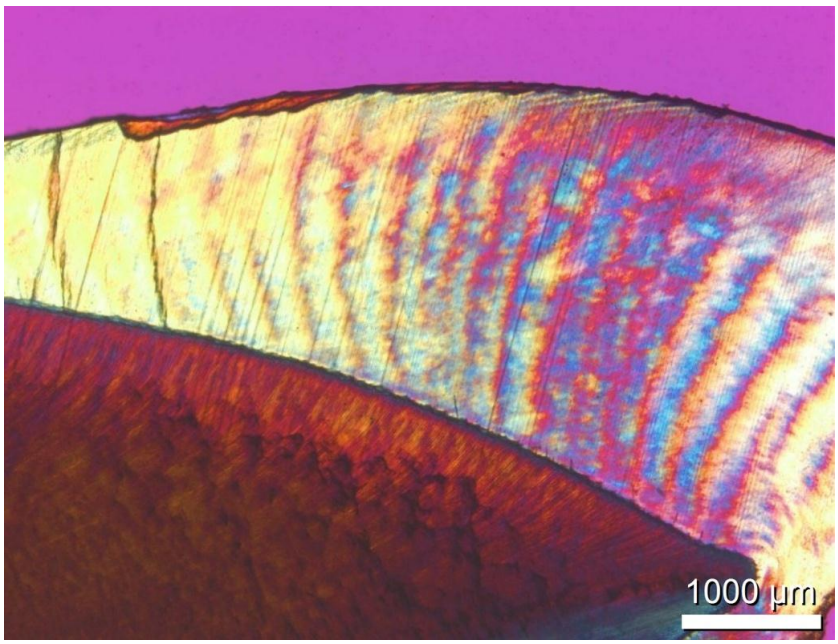


Figure 9: Example of lesion created with acid buffer solution and treated with ICON®



To overcome the problems faced in the previous study, where the infiltration material did not reach the base of the lesion, new shallow lesions were created using an acid-buffer demineralization solution (without fluoride) as a new pilot *Study 7*. The main reason for doing this was to obtain adequate pore size to permit the infiltration to the base of the lesion. For this step, two molar teeth were used to create lesions on the buccal surface of the teeth. The manufacturer's instructions were followed to infiltrate the material and additional sections were made and examined using polarized light microscopy. The results were promising using this method to create lesions, as significant infiltration was seen in the lesions, when sectioned. Example of the lesion is shown in the Figure 10.

Figure 10: Example of section showing infiltration into the lesion created by acid buffer solution and treated with ICON®.



All of the above pilot studies on the smooth surface involved polarized light microscopy as a method of assessment for determining the penetration of the material within the lesions. Because there was limited material penetration into the lesion, it was decided that a Quantitative Light Induced Fluorescence (QLF) technique would be used to evaluate the potential of the material to infiltrate the lesions, and thus, monitor the material's effectiveness in preventing secondary decay.

To test this technique (*Study 8*), five molar teeth were selected and lesion formation on the buccal surface of the teeth was done with an acid-buffer demineralization solution without any fluoride. The teeth were treated with ICON® material and then demineralized again to look at the effectiveness of ICON® in preventing secondary decay. QLF analysis was done after every step starting from baseline and following initial demineralization, treatment, and secondary demineralization. The QLF data showed that the ICON® material was able to prevent the treated lesion from further acid attack. Figure 11 shows the QLF images of a tooth from sound to secondary demineralization.

Results:

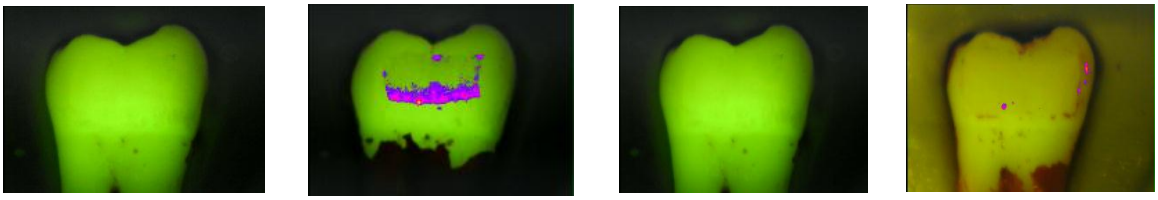


Figure 11: ΔF values for the above lesions from left to right: -6.66 (sound), -8.93 (initial demineralization), -6 (treatment), and -11 (secondary demineralization).

A similar type of study (*Study 9*) was performed on the smooth surfaces and a comparison was made between ICON® and control (no treatment), to determine the preventive effect on secondary demineralization. Both the control (no treatment) group and ICON® groups had five teeth each. Lesions were created on the buccal surface of the molar teeth and treatment in the ICON® group was done etching with 15% HCl, twice for three minutes each. Drying was done with moisture-free air and then further drying was done with ethanol. Treatment was done with ICON® resin infiltration material for three minutes, then light-polymerized and infiltrated for one minute before the final light polymerization. QLF measurements were taken after every step; at baseline, after initial demineralization, after treatment (no treatment for control group) and after secondary demineralization.

After the data collection was completed, the power analysis was performed using nQuery advisor 7.0 to calculate the sample size using two sided t-test.

A two-sample t-test was used to compare the two treatment groups after each step of treatment. Since the control group did not receive any treatment, values from initial demineralization were carried forward as treatment and comparison was made. The analysis revealed that, the comparisons of ΔF between the two groups had a statistically significant difference after the treatment step ($p<0.0001$) and secondary demineralization step ($p= 0.02$). Results: The descriptive statistics for ΔF are shown in the table 9 below.

Two sample t-test formula[73]:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{(s_1^2 / n_1 + s_2^2 / n_2)}}$$

Table 1: Descriptive statistics for pilot study 9.

Control Group

Variable	Sample size (N)	Mean	Standard deviation	Minimum	Maximum	Median
Baseline	5	-3.46	3.16	-5.98	0	-5.65
Initial Demineralization	5	-10.75	3.35	-16.30	-8.12	-9.70
Treatment	5	-10.75	3.35	-16.30	-8.12	-9.70
Final Demineralization	5	-28.98	5.61	-34.50	-22.60	-30.30

ICON® Group

Variable	Sample size (N)	Mean	Standard deviation	Minimum	Maximum	Median
Baseline	5	-6.09	3.59	-9.14	0	-6.85
Initial Demineralization	5	-12.19	3.38	-15.60	-7.81	-12.90
Treatment	5	-5.29	2.99	-7.08	0	-6.72
Final Demineralization	5	-9.82	1.87	-12.20	-7.95	-9.67

Comparison of ΔF after treatment between control and ICON®:

Based on the two-sample t-test, the data showed that there was statistically significant difference between control and ICON® groups after the treatment/time point 3 ($p<0.0001$). The two-sample t-test indicated that mean ΔF observed in control group was significantly greater than that observed in ICON® (mean ΔF: -10.75 vs. -5.29, respectively).

Comparison of ΔF between control group and ICON® group after time point 4 (secondary demineralization): Based on the two-sample t-test, there was a statistically significant difference between control group and ICON® group after secondary demineralization/time point 4 (p=0.02). The mean ΔF values after the visit 4 were -29.98 for the control group and -9.82 for the ICON® group.

With an effect size of 5.04, the results of pilot study showed that a sample of five in each group was sufficient to detect a statistically significant difference between the two groups of control and ICON® after secondary demineralization. The sample size of five has a power of 99% (for, after the secondary demineralization) to detect a difference in means of -19.16. For after the treatment step, with an effect size of 1.71, a sample size of five has 65%, seven will have 80%, and nine will have a 90% of power to detect a difference in means of -5.46.

Our final project was planned to assess the effectiveness of ICON® material in comparison to the sealants in prevention of secondary demineralization on smooth surfaces. Since the pilot study detected a statistically significant difference between the ICON® and control groups after treatment and secondary demineralization with a sample size of five, the required sample size for the final experiment can be five or more.

Collectively, the pilot studies (in which we encountered problems such as, difficulty in interpretation of lesion with infiltration, analysis of the occlusal surface data, and the loss of lesions during sectioning), led to the final project design that compared ICON® and resin-based sealants on smooth surface caries, using QLF to evaluate.

Final Experimental Conditions

The final study was an *in-vitro* laboratory study done using extracted human molar teeth. It compared the difference in effectiveness of ICON® and resin-based sealant material (Delton®) in prevention of secondary demineralization. A control group, without any treatment, was also included to see if the treatment groups would prevent secondary demineralization better than the control group. The ICON® resin infiltration material used was marketed by DMG® America in the United States. A resin based sealant material (Delton®) was used for a treating the teeth in sealant group.

The sample size of 78 teeth was decided for the final study with 26 teeth in each group. According to the final pilot study, with two groups of control and ICON®, at a α level of 0.05, with an effect size of 5.04, a sample size of five was required to get a statistically significant difference after treatment with a power of 99% after secondary demineralization and a sample size of nine was required to have a power of 90%. The sealant group was added to the study for having a comparison with the ICON® group, and also the addition of sealant group increases the sample size and thus power of the study. Also, the decision was made based on previous published in-vitro studies, which typically used 15-20 samples per group.

Specimen Preparation

For the final experiment, 110 extracted permanent human molar teeth were used as specimens and were obtained from the Dows Institute for Dental Research. Because we anticipated that not all the teeth would demineralize (because of the amount of fluoride they are exposed to until they are extracted) equally after exposure to the acids

for a specific period of time, 110 teeth were selected for the initial step of demineralization.

The teeth were previously collected from the Oral and Maxillofacial surgery department a month before the actual experiment started. The teeth were cleaned of debris and blood, and disinfected by placing them in 10% buffered formalin solution for 2 weeks. They were then cleared of any leftover residues with a prophylaxis brush and pumice powder (with no fluoride). The teeth were then stored in distilled water with thymol crystals at a temperature of 4° C for one week, until the final experiment began. The teeth used for this experiment were mandibular and maxillary 1st and 2nd molars and were extracted for either periodontitis or for orthodontic purposes. No third molars were included in the study because of uneven surfaces. Sound teeth were selected for the final experiment without significant decay and restoration on occlusal surface, no decay on mesial, distal, buccal and lingual surfaces. To make sure, the teeth were then examined under a light microscope for cracks and enamel defects, such as hypoplasia, white spots, and teeth with such defects were excluded from the sample. All of the teeth were selected based on having an occlusal-gingival width large enough for creating lesions, so the infiltration of the material could be interpreted, and also because, after the final step of the experiment (secondary demineralization), there would be expected to be adequate margins left occlusally and gingivally. This allowed us to obtain sections with normal enamel on either side of the lesions.

Once the selection of teeth was done for the final experiment, molds were prepared for all the individual teeth using the Exaflex® putty material (vinyl-polysiloxane impression material), and numbered from 1 to 110 (as shown in figure 12). The molds

helped to assure proper placement of the teeth under the QLF hand-piece for measuring the mineral content (change of fluorescence) of the teeth. The molds also helped to place the teeth in the same orientation under the QLF hand-piece after each step. A single baseline QLF measurement for ΔF was taken for each of the 110 teeth.

Figure 12: Example of the teeth placed on the molds that were prepared with polyvinyl siloxane material for positioning of the teeth during QLF measurements.

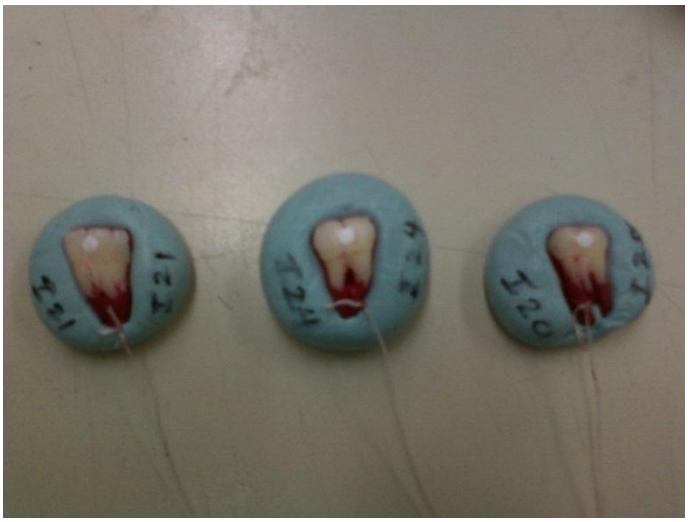
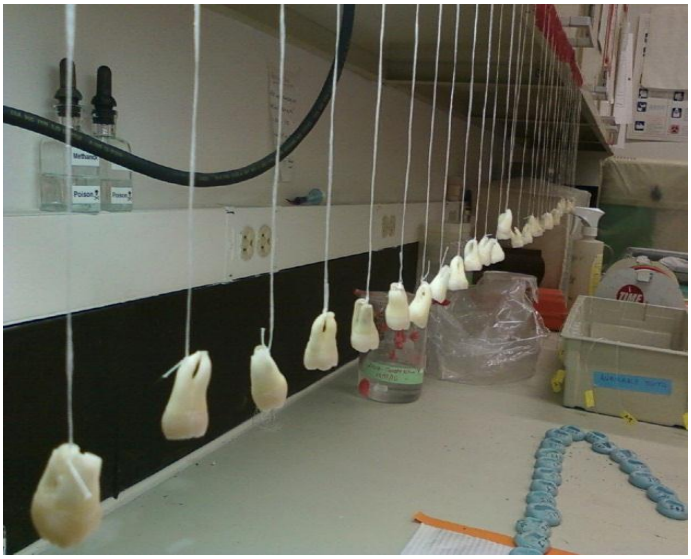


Figure 13: Example of cleaned teeth hanging with floss, ready for the initial demineralization.



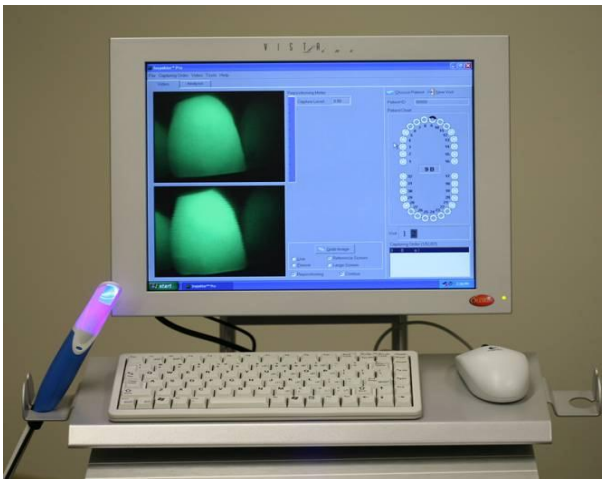
Methodologies used in Thesis

QLF Measurements

QLF is a dental diagnostic tool used for quantitative assessment of dental carious lesions, dental plaque, calculus etc., both in-vivo and in-vitro[35]. The QLF machine has a portable intra-oral camera connected to a computer, as shown in figures 3 and 4, with which a longitudinal assessment of the caries activity can be done in-vivo, or loss/change in mineral content can be done in-vitro[35]. For this study, a quantitative assessment of the teeth was done using the QLF machine software and associated. As described earlier in the review of literature, once the images are capture and stored in the computer, a quantitative assessment of the tooth can be done. It involves the use of the software to define area of sound enamel around the lesion of interest. The software assigns pixels to the sound area and the lesion, and automatically calculates the average loss of fluorescence in the lesion which is called as *delta F/*ΔF. The multiplication of the delta F

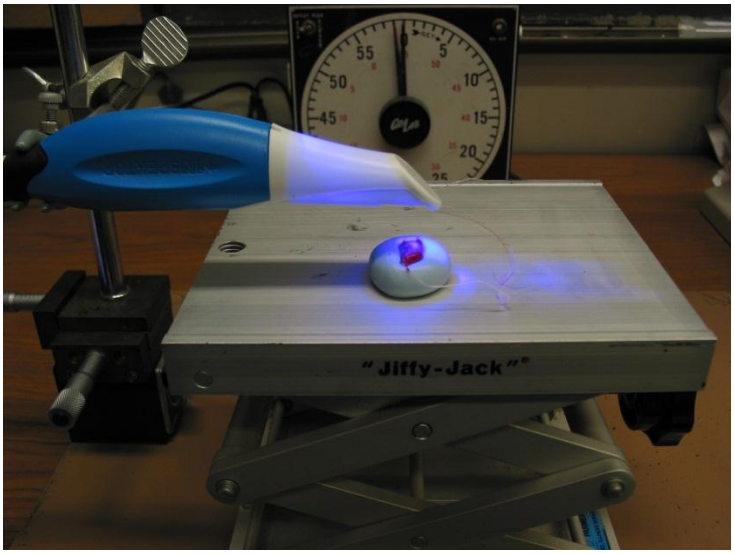
value by the area of the lesion in square millimeters (mm^2) gives the *delta* Q^{35}. A picture of the machine is shown in Figure 14.

Figure 14: Quantitative Light Fluorescence Machine.



Fluorescence of the teeth was measured at baseline and at each time point. For the control group, ΔF value was measured at baseline, after initial demineralization and after the secondary demineralization. The mean ΔF values was lowest for the baseline (lowest in negative value), and increased after the initial demineralization (higher in negative values), decreased after the treatment and increased again after the secondary demineralization. For both treatment groups, the ΔF value was measured at baseline, after initial demineralization, after treatment and after secondary demineralization. The treatment groups had an extra treatment step. The QLF hand-piece, shown in the picture below (figure 14), was used to measure the ΔF (change in fluorescence) at time point.

Figure 15: An example of tooth placed under the QLF hand-piece to record the mineral at baseline, after initial demineralization, treatment and secondary demineralization.



Polarized Light Microscopy (PLM)

The polarized light microscopy was discussed in detail in the review of literature. A polarized light microscope consists of a light microscope with a polarizer and an analyzer, placed perpendicular to each other[44]. Both polarizer and analyzer transmit light oscillation only in one plane and they are made up of sheet of polaroid.

Polarized Light Microscopy helps to selectively visualize anisotropic structures (presence of anisotropy indicates polarity and order); the microscopy helps to visualize these objects with good optics and proper alignment under which they appear bright and shining[44]. Anisotropic objects exhibit a number of properties, one of which is birefringence. Birefringence is defined as characteristics of an object to transmit plane polarized light at different velocities at different angles[44]. Birefringent objects are seen as

shining bodies under the polarized light microscopy. The birefringence property has a sign. If the refractive index (RI) of the polarized ray which runs parallel to the length of the fiber is greater than the ray polarized in the place perpendicular to the axis, the fiber exhibits positive birefringence[44].

Initial Demineralization and Lesion Creation

The first cycle of demineralization was used to create initial lesions. This step was carried out with all the teeth before they were systematically assigned to their respective treatment groups. The demineralization was carried out in the acid-buffer demineralization solution without fluoride. The detailed protocol is described below.

Once the teeth were taken out of the storage media (4% thymol) and thoroughly cleaned, they were painted with an acid-resistant nail varnish leaving a circle with a diameter of 2.5 mm in the center of the buccal surface of each tooth. These standardized circles were made by using a custom-made round sticker obtained using adhesive hole punch. The sticker was then pasted on the tooth surface, after removing the backing paper covering the adhesive surface, and the surface was painted with a nail varnish. Once the varnish dried, the sticker was peeled off using tweezers to leave a small uncovered circle at the center of the buccal surface of the tooth.

This established circle seen at the center of the tooth was then ready to be demineralized using the demineralizing solution. The acid-buffer demineralization solution was a standard demineralization solution that has been used for creating artificial carious lesions. The solution contained 1.2 mM calcium chloride, 2.2 mM potassium meta-phosphate, and 50 mM acetate buffer. All the teeth were suspended in the acid buffer demineralizing solution with a pH of 4.5 for 72 hours. After 72 hours, all the teeth

were taken out of the solution, and the teeth that had well-established lesions (chalky white appearance) were selected and assigned to their respective groups. On this basis, after the initial demineralization, a total of 78 teeth were selected for the experiment. In a systematic order, the first 26 were assigned to control group, the next 26 to the sealant group and the last 26 to the ICON® group. Floss was tied at the root ends of the teeth, and the ends of the floss were color-coded with a sticker, enabling us to identify the group assignments.

Once the teeth were assigned to the three groups, the nail varnish around the lesions was scraped off using a surgical blade, and QLF measurements were taken for the initial demineralization cycle, as shown in figure 16. The procedure for the QLF measurements was described in an earlier section.

Figure 16: Example of teeth suspended in demineralization solution.

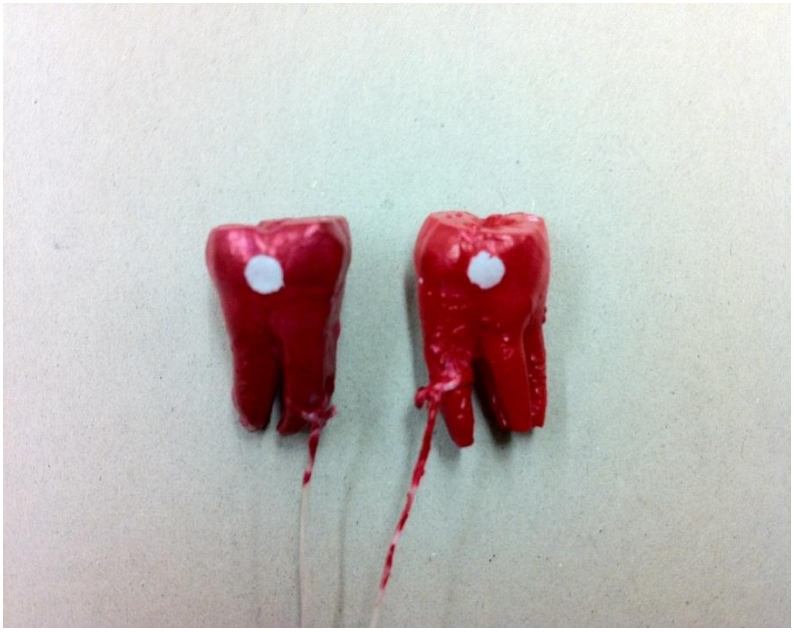
Treatment Cycle

As will be described in in detail in the next section, the teeth in the sealant group were treated with the resin-based sealant, and the ICON® group treated with the ICON® resin material.

Control Group

The control group did not receive any treatment and served a comparison group for the other two treatment groups.

Figure 17*:* The figure below shows examples of the artificially-created lesions. The white circular areas are the lesions. The varnish was applied to localize the lesion to a small circle.



Sealant Group

The teeth in the sealant group were treated with a resin-based sealant material. Delton® light-cured pit and fissure sealant (clear) material was used for treatment (figure 18). After lesion creation and the QLF measurements, the teeth assigned to the sealant group were treated with the sealant material as described below. All the teeth assigned to this group were treated in at least 2 sessions over 2 days.

The procedure for the sealant application was done in a standard way according to the manufacturer's directions and standard clinical protocol, and involved a step by step process. The first step involved the application of acid for acid-etching that was supplied with the sealant material (37% phosphoric acid, supplied as a liquid). The acid-etching technique involved the application of the phosphoric acid with a brush to each lesion created. Each tooth was then allowed to etch for 30 seconds and the etchant was then was rinsed under running water in the sink for 15 seconds and dried thoroughly with moisture free air for 30 seconds, before the sealant material was applied. The etching procedure erodes a very thin layer of enamel from the surface of the tooth and creates porosity in the enamel for the process of infiltration; this prepares the tooth for a better bond between the resin-based sealant and the enamel.

After thorough drying of the tooth surface, the resin-based sealant material was dispensed in a dappen dish and applied using micro-brush, according to the instructions provided with the package. Excess material was removed from the tooth surface using a cotton swab and the material was light-polymerized. The polymerization was done for 30-45 seconds using a LED light of a frequency indicated by the manufacturer (450 NM).

The same light that was used throughout the pilot studies was used for all sealant applications in the final experiment.

All the 26 teeth assigned to this group were treated in a similar fashion under similar conditions at room temperature and humidity.

Figure 18: Delton® sealant material was used in the sealant group

ICON® treatment group

The demineralized teeth that were assigned to the ICON® group received treatment with the Infiltration Concept material (ICON®). The treatment kit is shown in the Figure 19. Each tooth required approximately 10 minutes to complete etching and

treatment. The treatment of all the teeth in this group was completed in at least two sessions over two days.

The acid used in the acid-etch technique for the teeth assigned to ICON® treatment was 15% hydrochloric acid, which was supplied with the manufacturer's kit. The reason ICON® uses 15% hydrochloric acid because it is capable of eroding a greater thickness of surface layer as compared to 37% phosphoric acid. The etching acid was placed on the lesion directly with the syringe supplied in the kit. Once the material was placed on the lesion, it was spread across the lesion using a micro-brush. The lesions were etched twice for three minutes each. After each etching cycle, the etching material was rinsed under running tap water for 15 seconds and air-dried with moisture-free air for 30 seconds. After the second etch and before the treatment, the specimens were further dried with alcohol (methanol), to enable removal of water which was present in the enamel pores as a result of rinsing. The alcohol was allowed to stay on the surface for 30 seconds and the surface was again air-dried with moisture free air for 30 seconds.

The next step involved the application of the ICON® resin material on the surface of the lesion. The resin material was dispensed into a small dappen dish and applied to the lesion using a micro-brush. Once the material was applied, a micro-brush was used to spread the material on the lesion, and the material was allowed to stay on the lesion for three minutes. After three minutes, the excessive material (material left on the top of the lesion after the use of micro-brush to enhance the penetration of material) was removed with a cotton swab and the material was light-cured for 45 seconds. The ICON® material was re-applied, left for two minutes, excess material was removed. A second light-curing

was done again for 45 seconds. The curing light that was used for all ICON® treated specimens was the same as that used during the pilot studies.

All 26 teeth assigned to this group were treated in a similar fashion, under similar conditions at room temperature and humidity. QLF measurements were then recorded for the treatment step as was done for the other groups. As the control groups did not receive any treatment, the QLF measurements taken after the initial demineralization carried forward and used as control group measurements for after the treatment step.

Figure 19: ICON® material

Secondary Demineralization Cycle

The secondary demineralization cycle was performed for all three groups. Once the teeth were treated using the respective material in their groups (Sealant, ICON® and none for the control), a standardized custom-made adhesive sticker of slightly larger

diameter of 2.8 mm, obtained using a hole punch were applied around each treated area, which had a diameter of 2.5mm. The teeth were then painted with a nail varnish and allowed to dry. Once the varnish was dry, the stickers were peeled off using a tweezers, leaving a margin of 0.15mm around the treated area. A fresh acid-buffer demineralizing solution was prepared and the pH adjusted to 4.5. The teeth were then left in the demineralizing solution for the same amount of time (72 hours), as for the initial demineralization. All the teeth were suspended in the same solution with 8-10 teeth in each container.

The teeth were then taken out of the solution and dried thoroughly using moisture free air. Once dried, the varnish was scraped off the teeth using a surgical blade.

Figure 20*:* The figure below shows a tooth treated with ICON® on the white spot area after the varnish was scraped, for analysis with the QLF machine.

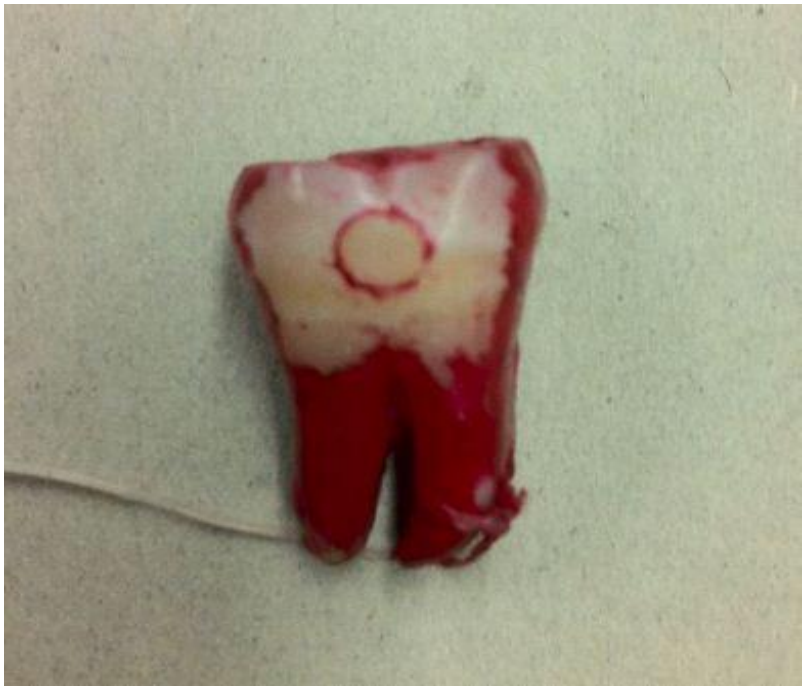
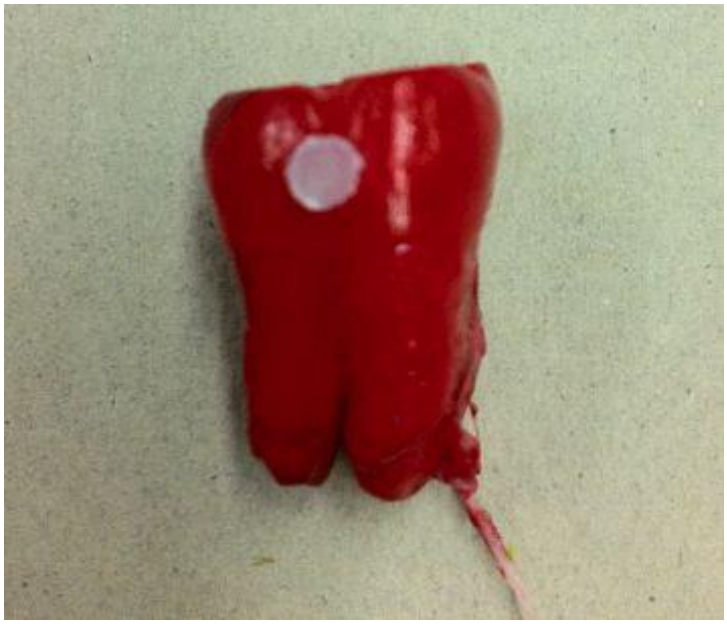


Figure 21*:* This tooth shows the lesion following treatment with a sealant material and shows secondary demineralization within the narrow ring left around the treated area.



Data collection

As mentioned earlier, the QLF analysis was done at every time point; at baseline, after initial demineralization, after treatment, and after secondary demineralization. The control group had only three time points, while the two treatment groups each had four time points to allow for the treatment step. After the secondary demineralization, two teeth in the sealant group were eliminated from the sample because they failed to show any signs of demineralization. Because of this, the overall sample size was reduced to 76, with 26 teeth in each of the control and ICON® groups, and 24 in the sealant group. The QLF data was used for the primary analysis of the data as opposed to the polarized light microscopy (PLM) which was used to assess the infiltration of materials into the lesion. The main reason behind doing this was that the QLF machine allows to follow and record

the lesion progression and regression over a period of time as compared to the PLM which is a destructive technique.

The QLF data for each step for every tooth in each group were downloaded from the QLF machine and transferred to a spreadsheet program to facilitate further analysis.

Data Analysis

Descriptive statistics were generated for each group at each time point, followed by one-way ANOVA (Analysis of Variance) with post-hoc Tukey-Kramer tests to assess the effects of treatment on the Delta F (ΔF) value at baseline, initial demineralization, and secondary demineralization. Shapiro-Wilk's tests were applied to assess assumption of normality. For the treatment point, the control group was not included. Because of the lack of normality of the QLF values, the non-parametric Wilcoxon rank-sum test was used to compare the differences between the sealant and ICON® groups after the treatment time point.

One-way ANOVA was also used to compare differences in ΔF values between two time points among the three experimental groups (initial demineralization to secondary demineralization: ΔF2-ΔF4 and treatment to secondary demineralization: ΔF3-ΔF4). A two-sample t-test was used to compare the differences from initial demineralization to treatment (ΔF2-ΔF3) between the treatment groups (sealant and ICON®).

For the baseline (ΔF1) comparison, the differences from treatment to secondary demineralization **(**ΔF3-ΔF4), and for the measures made after the treatment (ΔF3) (with all the three groups included), because of lack of normality, rank transformation was

performed. ANOVA based on rank-transformed data, with the post-hoc Bonferroni adjustment for multiple comparisons, was used to analyze the data.

Statistical analyses were performed using SAS for Windows (version 9.3, SAS Institute Inc., Cary, NC, USA). The statistical significance level was set at 0.05. Single measurements of QLF values were taken, and no repeats for reliability were done. The total sample size of the final analyses for the experiment was 76, as 2 teeth from the sealant group were lost after the secondary demineralization.

CHAPTER 4

RESULTS

Overview

The study was an *in vitro* study, done to compare the effect of two treatment protocols, the ICON® and sealant material, in prevention of secondary demineralization. The dependent variable for this experiment was the amount of demineralization, or ΔF (which is loss of mineral content within the tooth and a loss of fluorescence) at each time point, and the difference in the mean ΔF3- ΔF4. The independent variables were the types of treatment used, which were the resin-based sealant and the ICON® material. Chapter 4 presents the main results and statistical analyses for the study.

Descriptive statistics are presented for the time points of the study (ΔF1, ΔF2, ΔF3 and ΔF4) and for difference in time points for ΔF2-ΔF3, ΔF2-ΔF4 and ΔF3-ΔF4. Comparisons of ΔF across the three treatment groups of ICON®, sealant and control, was done at three time point (baseline, initial demineralization, and secondary demineralization) using one-way ANOVA. Wilcoxon rank-sum test was used to compare the ΔF values after the treatment step (as only two groups involved). Evaluations for differences in ΔF values were done between two time points among the experimental groups using the two sample t- test for ΔF-ΔF3, and one-way ANOVA for ΔF2-ΔF4, and ΔF3-ΔF4.

One hundred-ten teeth were demineralized, out of which seventy-eight teeth were chosen for use. During the experiment, two teeth were lost in the sealant group. Therefore, a total of seventy-six teeth were included in the main study, with group sizes of 26 for the control group, 26 for ICON® and 24 for sealant group.

Descriptive statistics

Table 1a displays the descriptive statistics for the ΔF values at each point by treatment groups. There were four time points where measurements of the ΔF were made: the sound tooth (ΔF1), after the first demineralization (ΔF2), after the treatment (ΔF3) and finally after the secondary demineralization (ΔF4). Descriptive measures of mean, median, standard deviation, minimum and maximum were performed for all three groups- the control, sealant and ICON® group. All the four time points for each group and comparison in ΔF value between two time points (ΔF2-ΔF3, ΔF2-ΔF4, and ΔF3-ΔF4) are represented in Tables 2-4. However, because the control group did not receive any treatment, data from only three time points is presented.

Descriptive Statistics of ΔF by Experimental Groups

Table 2: Control Group

Variable	Sample Size (N)	Mean	Standard Deviation	Minimum	Maximum	Median
Sound (ΔF1)	26	-7.78	2.65	-15.40	-5.75	-6.36
Initial Demineralization (ΔF2)	26	-29.03	4.75	-39.30	-18.50	-28.35
Treatment (ΔF3)	26	-29.03	-4.75	-39.30	-18.50	-28.35
Final (ΔF4) Demineralization	26	-37.38	4.03	-46.60	-27.40	-37.10
ΔF2-ΔF4	26	8.36	3.36	-2.40	12.50	8.95
ΔF3-ΔF4	26	8.36	3.36	-2.40	12.50	8.95

Note: Control=No treatment. The Values from initial demineralization were carried forward as treatment.

Table 3: Sealant Group

Variable	Sample Size (N)	Mean	Standard Deviation	Minimum	Maximum	Median
Sound (∆F1)	24	-7.81	3.79	-23.90	-5.60	-6.78
Initial Demineralization (∆F2)	24	-24.13	4.97	-33.70	-15.70	-23.25
Treatment (∆F3)	24	-11.44	3.78	-18.90	-6.37	-10.80
Final (∆F4) Demineralization	24	-21.70	3.86	-27.60	-12.50	-21.60
∆F2-∆F3	24	-12.69	5.63	-23.61	-4.00	-12.42
∆F2-∆F4	24	-2.43	4.58	-9.40	6.10	-2.70
∆F3-∆F4	24	10.26	4.32	3.00	16.80	9.70

Table 4: ICON® group

Variable	Sample Size (N)	Mean	Standard Deviation	Minimum	Maximum	Median
Sound (∆F1)	26	-7.65	1.89	-15.50	-5.77	-7.08
Initial Demineralization (∆F2)	26	-28.73	4.62	-38.70	-17.10	-29.05
Treatment (∆F3)	26	-12.71	5.98	-32.70	-6.46	-11.05
Final (∆F4) Demineralization	26	-23.04	2.74	-30.60	-17.10	-22.95
∆F2-∆F3	26	-16.02	7.36	-32.04	0.40	-15.57
∆F2-∆F4	26	-5.69	5.38	-17.50	6.00	-5.30
∆F3-∆F4	26	10.33	6.72	-10.80	19.90	11.89

Note: ∆F1=∆F at baseline; ∆F2=∆F at visit 2; ∆F3=∆F at visit 3; ∆F4=∆F at visit 4; Time points 1 (baseline), 2(initial demineralization), 3(treatment) and 4(secondary demineralization).

Comparison of ΔF values across the three experimental groups at each time point

A. At baseline: Since the assumption of normality was violated ($p<0.0001$) (Shapiro-Wilk test), a rank transformation was conducted. Subsequently, one-way ANOVA based on ranked data was used to analyze these results. The analysis revealed that there were no statistically significant difference in ΔF values among the three treatment groups at baseline ($F(2, 73) = 0.91$; $p=0.41$). Table 5 summarized the results of one-way ANOVA and Table 6 summarized the results of the post-hoc Bonferroni adjustment, for multiple comparisons.

Table 5: Results of one-way ANOVA for ΔF at baseline

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F Value	P Value
Treatment	2	891.54	445.77	0.91	0.40
Error	73	35677.96	488.74		
Total	75	36569.50			

Table 6: Mean ΔF values by experimental groups at baseline

Experimental Groups	N	Mean ΔF values	Group Comparisons
Control	26	-7.78	A
Sealant	24	-7.81	A
ICON®	26	-7.65	A

Note: Means with the same letter are not significantly different using post-hoc Bonferroni adjustment ($p>0.05$)

B. After initial demineralization: Since the assumption of normality was not violated (p=0.37, Shapiro-Wilk test), one-way ANOVA was conducted. The results revealed that there was a statistically significant effect of treatments on ΔF values after initial demineralization (F (2, 73) =8.14; p=0.0006). The post-hoc Tukey-Kramer test indicated that the mean ΔF value observed in the sealant group was significantly greater (lower in negative value) than the other two groups. However, there was no statistically significant difference between the mean ΔF values of control and the ICON® groups. Table 7 summarizes the results of one-way ANOVA and table 8 summarizes the results of the post-hoc Tukey-Kramer test.

Table 7: Result of one-way ANOVA for ΔF after initial demineralization.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F Value	P Value
Treatment	2	371.32	185.66	8.14	0.0006
Error	73	1665.95	22.82		
Total	75	2037.27			

Table 8: Mean ΔF values by experimental groups after initial demineralization (post-hoc Tukey-Kramer test)

Experimental Group's	N	Mean ΔF Values	Group Comparison
Sealant	24	-24.13	A
ICON®	26	-28.73	B
Control	26	-29.03	B

Note: Means with the same letter are not significantly different using post-hoc Tukey Kramer test (p>0.05)

C. After treatment: Analysis after the treatment was done including the control and excluding the control group.

Including the control group

When the control group was included assumption of normality was violated (p=0.0001) based on the Shapiro-Wilk test. Rank transformation was conducted, and subsequently one-way ANOVA based on ranked data was used to analyze the data. The analyses revealed a statistically significant difference in the ΔF value among the three groups was observed after treatment ($F(2, 73) = 61.49$; $p<0.0001$). The post-hoc bonferroni adjustment indicated that the mean ΔF values of sealant and ICON® statistically significantly lower (in negative values) than the control group. However, there was no statistically significant difference between the ICON® and sealant groups. The results of one-way ANOVA are summarized in Table 9 and Table 10 summarizes the group comparisons.

Table 9: Results of one-way ANOVA for mean ΔF after treatment.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F Value	P Value
Treatment	2	22946.46	11473.23	61.49	<0.0001
Error	73	13621.54	186.60		
Total	75	36568.00			

Table 10: Mean ΔF values by experimental groups after treatment.

Experimental Group	N	Mean ΔF Values	Group Comparison
Sealant	24	-11.44	A
ICON®	26	-12.71	A
Control	26	-29.03	B

Note: Means with the same letter are not significantly different using post-hoc Bonferroni adjustment (p>0.05)

Excluding the control group

When the control group was excluded from the analysis, the Shapiro-Wilk test was used to test normality of data and results indicated that the assumption of normality was violated for both the groups (sealant and ICON®), with p-values=0.0101 and p=0.0009, respectively. Therefore, the non-parametric Wilcoxon rank-sum test was used to compare differences between the two groups. No statistically significant difference in the mean ΔF was found between the ICON® and the sealant groups after the treatment (p=0.70). Table 11 summarizes the results of the Wilcoxon rank-sum test.

Table 11: Results from Wilcoxon rank-sum test after treatment.

Experimental Group	N	Mean /Median	P value
Sealant	24	-11.44/-10.80	0.70
ICON	26	-12.71/-11.05	

D. After secondary demineralization: Since the assumption of normality was not violated (p=0.74, Shapiro-Wilk test), one-way ANOVA without rank-transformation, was conducted. The results revealed that there was a significant effect of treatment on ΔF values after secondary demineralization (F (2, 73) =150.80; p<0.0001). The post-hoc Tukey-Kramer test indicated that the mean ΔF value observed in the sealant and ICON® groups were significantly greater (lower in negative value) than for the control group. However, there was no statistically significant difference between the sealant and ICON® groups after secondary demineralization. Table 12 summarizes the results of the one-way ANOVA and table 13 summarizes the results of the post-hoc Tukey-Kramer test.

Table 12: Results of one-way ANOVA: ΔF after secondary demineralization.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F Value	P Value
Treatment	2	3865.20	1932.60	150.80	<0.0001
Error	73	935.54	12.82		
Total	75	4800.74			

Table 13: Mean ΔF values by experimental groups after secondary demineralization.

Experimental Group	N	Mean ΔF Values	Group Comparison
Sealant	24	-21.70	A
ICON®	26	-23.04	A
Control	26	-37.38	B

Note: Means with the same letter are not significantly different using post-hoc Tukey Kramer test (p>0.05)

Evaluation of differences in ΔF values between pairs of points among the experimental groups

Differences in the ΔF values between pairs of time points were calculated, and the effects of the treatment on differences in ΔF values between two time points were evaluated.

1. Difference between initial demineralization and treatment (ΔF2-ΔF3)

Since the control group did not receive any treatment, it was excluded from this analysis. The Shapiro-Wilk test was used to test normality, and the results indicated that the data were normally distributed for both the sealant and ICON® groups (p= 0.52 for sealant and p=0.78 for ICON®).

A two-sample t-test was conducted. The results (Table 14) indicated that there was no statistically significant difference between the sealant and ICON® treatment groups for the mean ΔF values between the two time points.

Table 14: Mean ΔF values by experimental groups between time points ΔF2-ΔF3

Experimental Group	N	Mean difference in ΔF Values	P value
Sealant	24	-12.69	0.08
ICON®	26	-16.02	

Note: Results from the two sample t-test

2. Differences between initial demineralization and secondary demineralization (ΔF2-ΔF4)

The assumption of normality was not violated, which was tested using the Shapiro-Wilk test (p=0.5819), a one-way ANOVA was conducted. The results revealed that there was a statistically significant effect of treatments on ΔF values between the two time points (F (2, 73) =68.53; p<0.0001). The post-hoc Tukey-Kramer test indicated that the mean difference in ΔF value between the two time points observed in the control group was significantly greater than the sealant and ICON® groups, and the sealant group was significantly greater than the ICON® group. Table 15 shows the results of one-way ANOVA and Table 16 shows the results of the post-hoc Tukey-Kramer test.

Table 15: Results of one-way ANOVA: difference between ΔF2-ΔF4.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F Value	P Value
Treatment	2	2797.45	1398.72	68.53	<0.0001
Error	73	1489.88	20.41		
Total	75	4287.33			

Table 16: Mean difference in ΔF values between ΔF2-ΔF4 by experimental groups.

Experimental Group	N	Mean difference ΔF Values between F2-F4	Group Comparison
Control	26	8.36	A
Sealant	24	-2.43	B
ICON®	26	-5.69	C

Note: Means with the same letter are not significantly different using post-hoc Tukey Kramer test (p>0.05)

3. Difference between treatment and secondary demineralization (ΔF3-ΔF4)

The assumption of normality was violated, which was tested using the Shapiro-Wilk test (p=0.0007). One-way ANOVA based on the ranked data was used to analyze the data. The analysis revealed that there was no significant difference in F3-F4 among the three experimental groups (F (2, 73) =2.41; p=0.0967).

Table 17 summarizes the results of the one-way ANOVA and the table 18 shows post-hoc Bonferroni adjustment results.

Table 17: Results of one-way ANOVA for difference between ΔF3-ΔF4.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F Value	P Value
Treatment	2	2267.39	1133.69	2.41	0.0967
Error	73	34305.11	469.93		
Total	75	36572.50			

Table 18: Mean difference in ΔF values between ΔF3-ΔF4 by experimental groups.

Experimental Group	N	Mean difference ΔF values between two time points	Group Comparisons
ICON®	26	10.33	A
Sealant	24	10.26	A
Control	26	8.36	A

Note: Means with the same letter are not significantly different using the post-hoc Bonferroni test (P>.05)

Polarized Light Microscopy Results

Overview

Polarized light microscopy (PLM) was used as a secondary method of analysis for the present study. The working principle of the polarized light microscope is described earlier in the review of literature section.

The PLM was used to study the sections that were made at different time points of the experiment: at baseline, after initial demineralization, after treatment and after final demineralization. The PLM method helped to assess different steps of treatment with the ICON® and sealant material. Sections of sound tooth were made for comparison with different steps. Initial demineralization section showed the cross-section of the initial lesion and the treatment step assessed the amount of infiltration of the ICON® and sealant material into the lesions. The examples of pictures of sections from teeth after each time point are presented to better understand the effect of treatment and secondary demineralization on the teeth.

The final experiment had a control group, sealant group and ICON® group. The examples of pictures of sections show the pattern of demineralization after each time point for the three groups. The baseline and initial demineralization were common steps for all the three groups. The sealant and ICON® groups received treatment, whereas the control group did not receive any treatment, so no treatment images are presented for the control group. All the groups were exposed to the secondary demineralization.

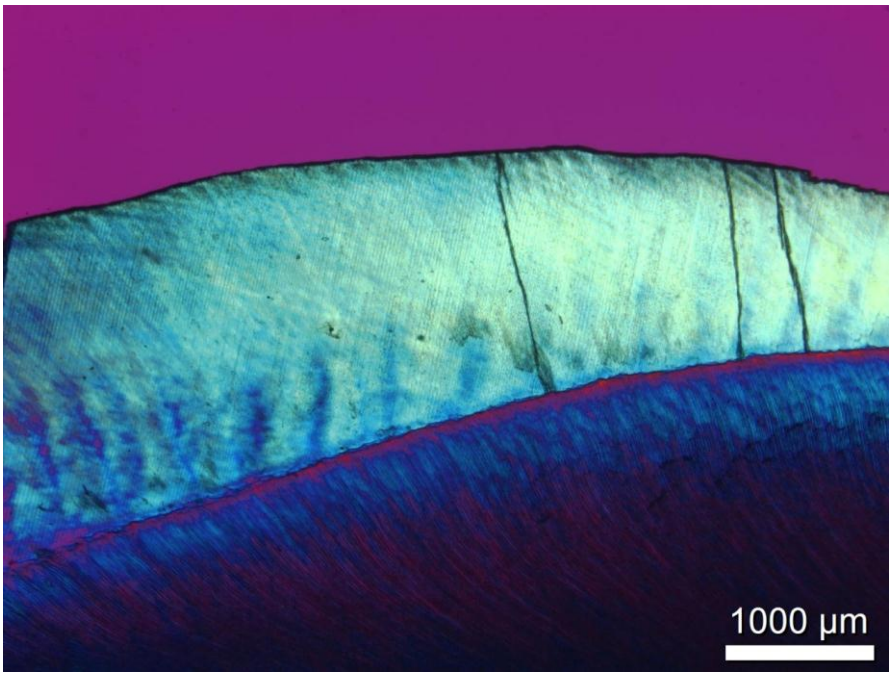
As described in Chapter 3, the sections depicted under the polarized light microscope were made with a high speed microtome, and were about 150 microns in

thickness. A brief discussion of the appearance of the pictures of the sections is presented in the pages that follow.

Baseline

Baseline measurements for the QLF were made for sound teeth from all the three groups, before they were exposed to the initial demineralization step. An example of picture of a section from a sound tooth is depicted in the Figure 22 below. The section shows that the tooth did not have any lesion.

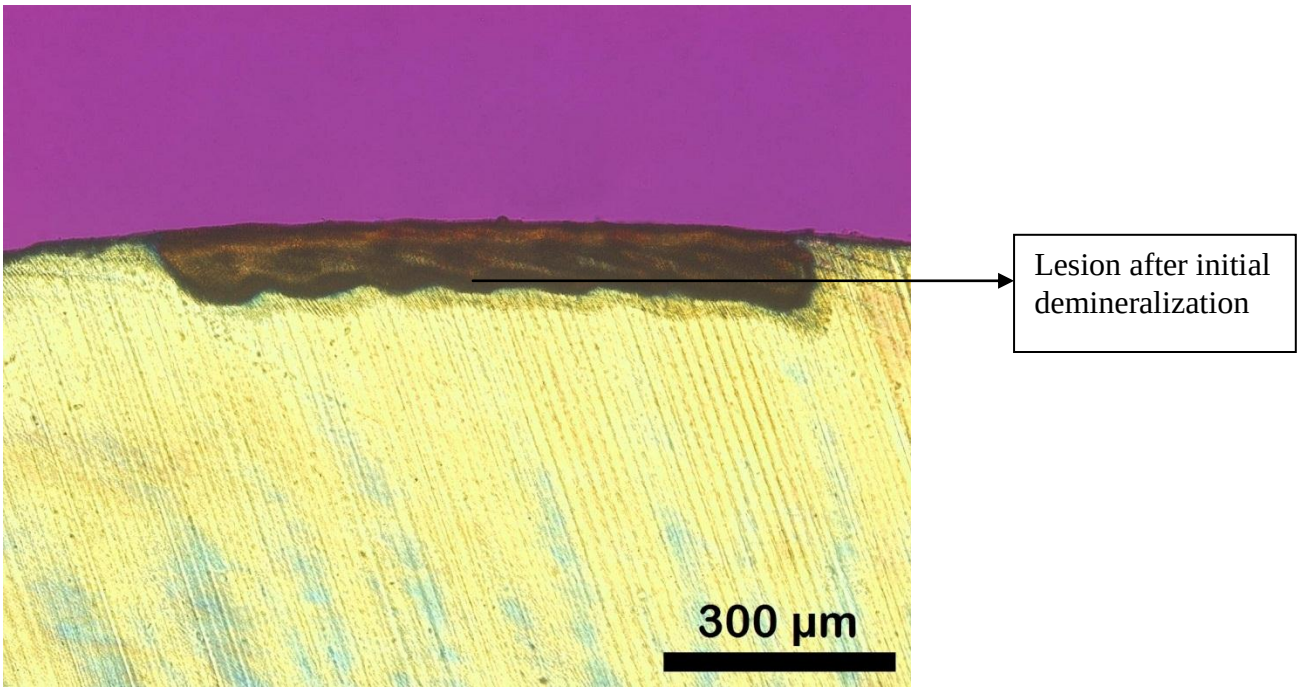
Figure 22: Baseline: At baseline, the tooth structure is intact with no lesion on the surface. Figure above shows the section from a sound tooth.



Initial demineralization

All three groups underwent the initial demineralization step. Using a microtome, sections were made out of the teeth after the initial demineralization step. An example of picture of lesion shown below is after the step of initial demineralization, which is similar for all the three experimental groups. Figure 23 shows a wide carious lesion on the buccal surface of the tooth.

Figure 23: Initial demineralization: The figure shows an example of section of the tooth after the initial demineralization. The lesion after initial demineralization is common to all the three groups



Treatment

The teeth in the sealant and the ICON® groups received treatment with a resin based sealant material and ICON® resin material, respectively.

ICON® treatment: The ICON® material works on the principle of infiltration, i.e., the material infiltrates into the porosity and restores the tooth structure. The teeth that received the treatment with the ICON® material showed a significant amount of infiltration into the porosity of the lesion. The lesions that were created here were shallow and the infiltration of ICON® penetrated almost to the base of the lesions. Figures 24 and 25 show examples of sections made out of the teeth treated with the ICON® material.

Sealant treatment: Sealants work on the principle of blocking the tooth surface mechanically. Therefore, for the success of the sealant, mechanical retention is very important and obtained by the resin tag formation. However, sealant materials were not designed to be used on smooth surfaces and the sealant does not readily infiltrate into the lesion. The example of section in the Figures 26 was treated with the sealant material do not show infiltration of the material. The sealant material stays on the surface of the lesion and blocks the porosity of the lesion superficially. The formation of resin tags into the tooth helps hold the material in place, as well as make the tooth less soluble.

Figure 24: The figure below shows an example of section of the tooth after it was treated with ICON® material.

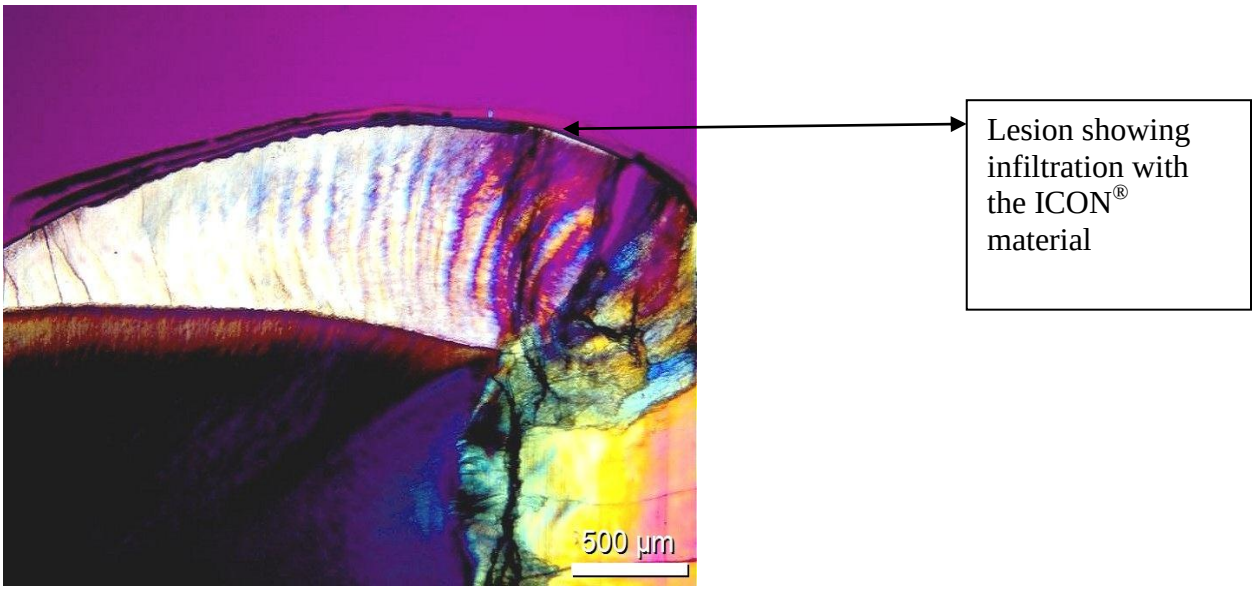


Figure 25: The figure below shows an example of section of the tooth after it was treated with ICON® material.

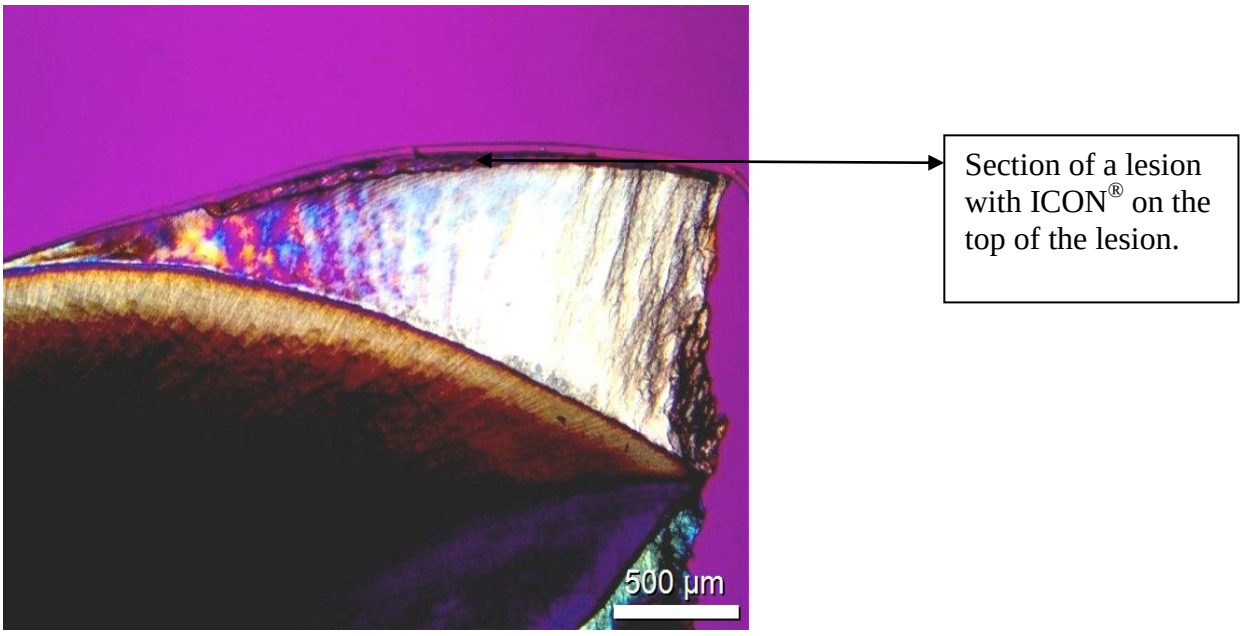
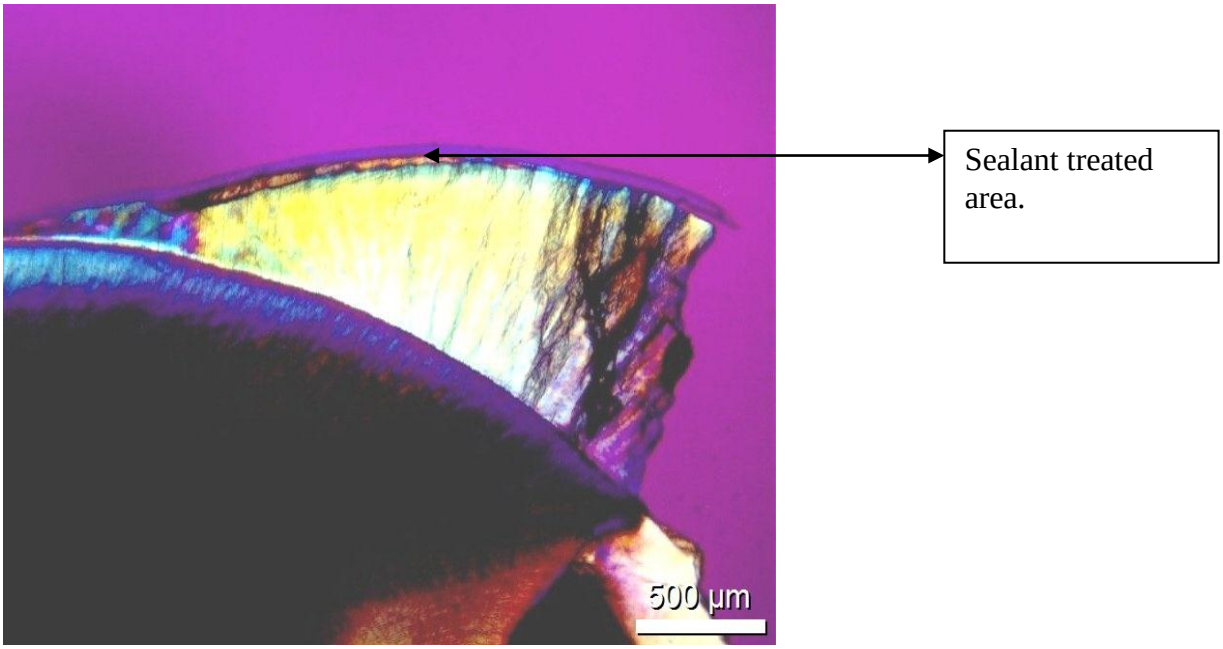


Figure 26: Below: Example of section from a tooth that was treated with a sealant material.



Secondary Demineralization

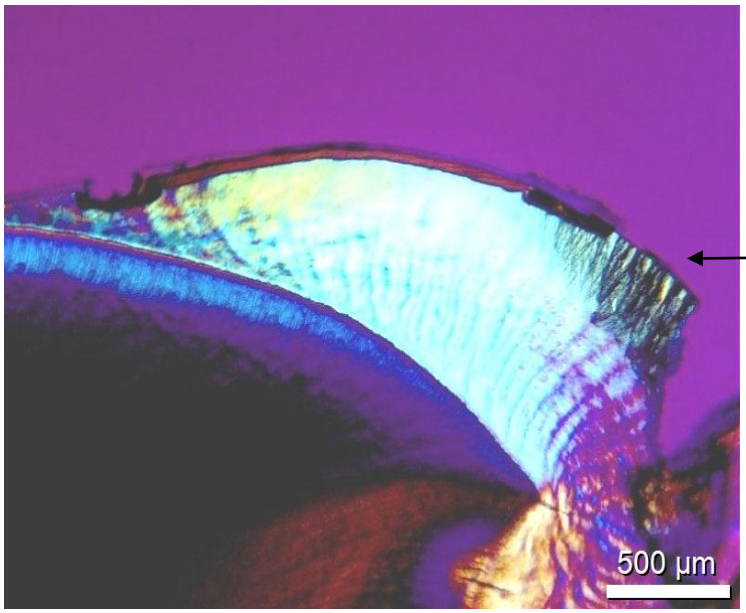
The teeth from all the three groups were subject to secondary demineralization after the treatment to assess the ability of the treatment material to prevent further demineralization of the tooth. For this step, a narrow strip of less than half a millimeter was left exposed around the treated area and subjected to a demineralization cycle.

Figure 27 shows an example of a section from a tooth treated with ICON®, after secondary demineralization. As stated earlier, the ICON® material infiltrates into the lesion and prevents the progression of the lesion. When the teeth treated with the ICON® material were exposed to secondary demineralization, the area around the treated lesion was eroded, which suggests that the ICON® was not effective in prevention of further

demineralization, however in the area that was treated, the restorative material did not allow the acids to seep into the restores area. One of the reasons might be that the material does not form any resin tags like the sealant does.

The sections treated with the sealant material did not show any kind of activity that suggested infiltration. After the secondary demineralization, examination images of the PLM sections showed protection (the area exposed to the secondary demineralization was intact) when compared to the sections from the teeth treated with ICON®. This can be attributed to the fact that the sealant material formed a resin coating which is held to the tooth surface by resin tags. Figures 28 and 29 show examples of the sections of the teeth treated with the sealant material after secondary demineralization.

Figure 27: The above section is from a tooth treated with ICON® material after the secondary demineralization. Note the crater on the gingival side of the lesion, which was the area around the lesion treated with ICON®, exposed to secondary demineralization.



Figures 28 Example of section cut from teeth treated with sealants after the secondary demineralization. The dark areas on the either side of the lesions are the effect of secondary demineralization.

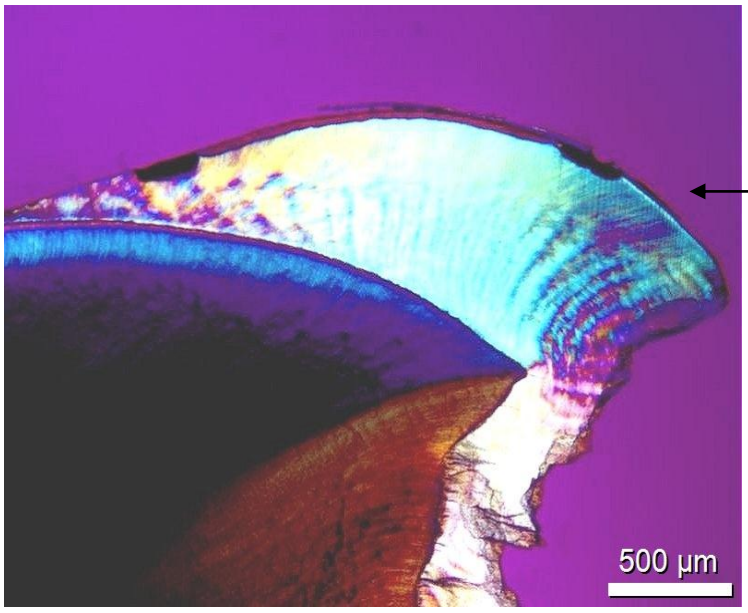
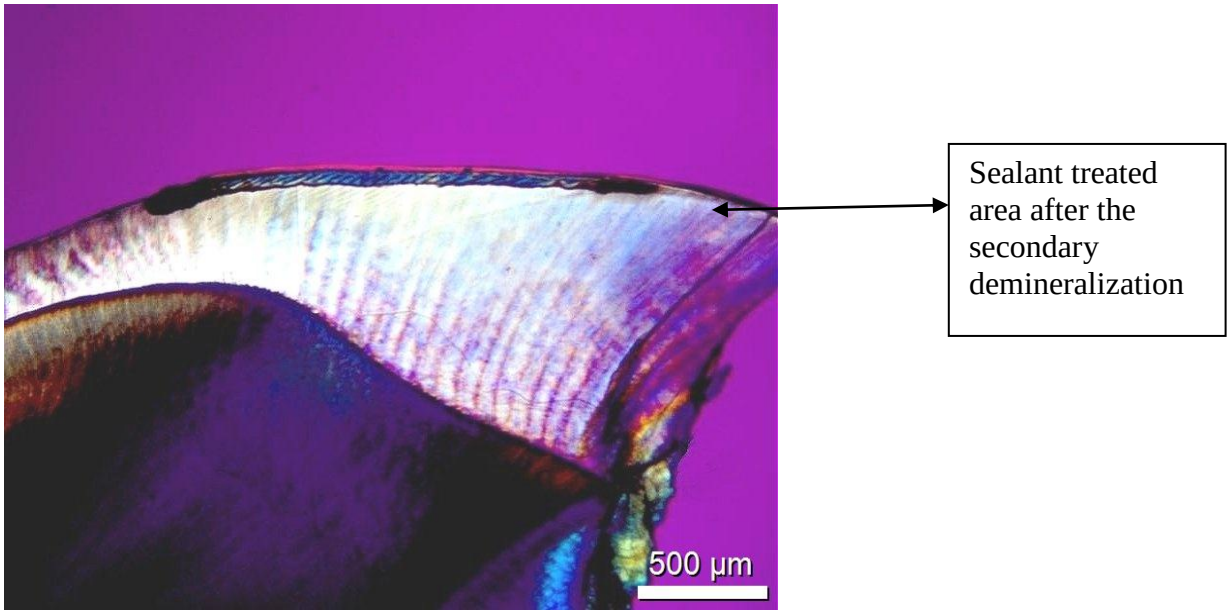


Figure 29: Examples of section cut from teeth treated with sealants after the secondary demineralization.



Summary

The polarized light microscopy was used as a secondary means of analysis to help interpret the infiltration level of the treatments given to the in vitro white spot lesion. Since no quantitative data were collected in the PLM analysis, it was not used in the statistical analysis.

The interpretation of the PLM sections was that ICON® significantly infiltrated into the lesion, although not to base of the lesion; the sealant material did not infiltrate into the lesions and stayed on the top of the lesion with the formation of resin tags.

The PLM results after the secondary demineralization concluded that the sealant material was equally protective when compared to the ICON® material in prevention of secondary demineralization, around the lesion.

CHAPTER 5

DISCUSSION

ICON® (infiltration concept) is a material used to treat white spot lesions on smooth surfaces of the teeth, and it can be used in the inter-proximal areas of the teeth as well. The white spot lesions on the smooth surfaces of the teeth can be reversed by various remineralization techniques such as fluorides (varnish), MI paste (mixture of casein phospho-peptides and amorphous calcium phosphate), but studies have demonstrated the effectiveness of the infiltration technique, along with fluoride, compared to fluorides only. The use of ICON® in the inter-proximal areas is indicated as opposed to restoration with a composite or an amalgam; however, the detection of such lesions present in the inter-proximal areas is difficult and careful radiographic evaluation is necessary.

ICON® is a relatively new material that was launched in September, 2010, with a very limited number of studies published before or since its launch. This is one of the first in-vitro studies done on the smooth surface that evaluated the ICON® material in comparison to a sealant material.

Discussion of results

The present study compared ICON® and a sealant material in prevention of demineralization from an acid attack after treatment (secondary demineralization) on a smooth surface. The assessment for this study was done using two methods. These were the quantitative light-induced fluorescence (QLF) and polarized light microscopy (PLM), with the QLF data used for primary statistical analyses. The reason was that the QLF technique can be used to follow the changes in caries lesions over a period of time. Also,

studies were done to assess the reliability of the QLF technique in diagnosis of caries lesions, and results suggested that it is highly reliable for detection of early caries lesions. The polarized light microscopy (PLM) was used to assess infiltration of the material into the teeth. There was no quantitative analysis done with the polarized light microscopy because the amount of infiltration into a lesion cannot be measured using this technique and only a qualitative measure of infiltration is possible with PLM. PLM is a destructive technique which would not allow for repeat measurements. A microscope with much higher magnification or a measurement of pore volume has to be done to assess the amount of infiltration of the material. The overall results of the QLF analysis were that there was no statistically significant difference between ICON® and sealant material after the treatment (material placement); however, both the treatments were significantly different and better than the control group. In addition, there was no statistically significant difference between the sealant and the ICON® material in prevention of secondary demineralization (around the treatment area) when compared to control, where no treatment was given.

The polarized light microscopy technique results were used to help understand the penetration of the ICON® material into the porosity of artificial white spot lesions. Polarized light microscopy was done with sections from all the three groups, and at every step: at baseline, after initial demineralization, after treatment (to see the amount of infiltration of the material into the lesion), and after secondary demineralization.

In this study the quantitative light-induced fluorescence (QLF) technique enabled the evaluation of two restorative materials in the prevention of secondary demineralization of treated white spot lesions in comparison to a control group. One

group was treated with a resin based material called ICON® (Infiltration concept) and compared to a group treated with a resin-based sealant material (Delton®). The control group did not receive any treatment. The QLF technique helped us to evaluate the effect of treatment, and the difference between a surface coating of sealant and infiltration of resin into the lesion in the prevention of secondary demineralization. Delta F (ΔF) is the measure of loss of fluorescence or loss of mineral content, and it is calculated based on sound tooth around the demineralized or treated area. The baseline (sound tooth) measurements are taken for the sound tooth structure. When the demineralization is highest, the ΔF is a highly negative value.

Delta F (loss of fluorescence) was considered as a measure of loss of mineral content from the tooth structure at different time points (at baseline and after initial demineralization, treatment and secondary demineralization), and the differences between the delta F values between the treatment and secondary demineralization (ΔF3-ΔF4) and were the main dependent variables in the study. At baseline, the fluorescence was measured for sound teeth and fluorescence was measured again at each step after that (initial demineralization, treatment, secondary demineralization); the ΔF for each step was measured in comparison to the sound tooth measure for each sample in each group.

Analysis was done among the three groups to detect the effect of treatment on the ΔF value after each time point. At baseline, the measurement of ΔF was similar for all three groups. Although the mean ΔF values varied among the groups, there was no statistically significant difference among the groups at baseline (Table 6). After the initial demineralization there was a statistically significant difference in the mean ΔF value for the sealant group compared to the control and ICON® groups (Table 9), since the sealant

group mean ΔF was less, as the teeth in sealant group demineralized to a lesser extent. The reason behind this could be that the amount of fluoride exposure prior to extraction of the teeth in that group was greater than for those in in the other groups; however, there is no scientific evidence for this. For the treatment step (ΔF3), the analysis was done with and without the control included. Without the control group, the analysis revealed that both the ICON® and sealant materials were able to alter the white spot lesions, with a significant reduction in ΔF values, and that no statistically significant difference in the mean ΔF were observed (Table 10). After the control group was included, the analysis revealed that there was a statistically significantly difference between the control and the treatment groups (sealant and ICON®), the treatments being better than the control group (Table 11). After the secondary demineralization step (ΔF4), in which both the treatment area and the area around it were exposed to secondary demineralization, the ICON® and sealant groups were protected when compared to the control group (Table 13). The mean ΔF value for both the treatment groups was lower than for control group, because the control group it did not receive any treatment.

Evaluations of the differences in mean delta F values between pairs of time points among the three experimental groups were done. The change in mean ΔF between initial demineralization and treatment (ΔF2-ΔF3) was calculated to see the effectiveness of treatment, and changes from treatment to secondary demineralization also were evaluated (ΔF3-ΔF4).

The difference in ΔF3-ΔF4 was mainly used to see the effectiveness of the sealant materials in the prevention of secondary demineralization, because the comparison was done based on the peripheral region adjacent to the treatment area, which was exposed to

the secondary demineralization. The analysis for this difference was done taking into consideration the area around the treatment that was exposed to the secondary demineralization along with the treatment area.

For the ΔF2-ΔF3 change, only the treatment groups were involved in the analysis, and the results showed that both the treatments (ICON® and sealant) were able to reduce the ΔF values significantly (Table 14). The ICON® was able to infiltrate and the sealant material stayed on the surface. The results for ΔF3-ΔF4, which assessed the change from the treatment step to the secondary demineralization step, showed that there was no statistically significant ($p=0.09$) difference among the three experimental groups (Table 17). Therefore both the sealant and ICON® materials were ineffective in prevention of secondary demineralization around the treated area, when compared to the control group. A group comparison revealed no statistically significant difference in the differential means among the three groups (Table 18). However, mean ΔF for the difference was least for the control group (without any treatment). To analyze the difference in mean ΔF values for the ΔF3-ΔF4 step, the treatment ΔF values for control group were carried forward from the initial demineralization step, since they did not receive any treatment. In a recent clinical study which compared ICON®, sealant and control group, there was no statistically significant difference between the treatment groups; however, they were both better than the control group. This suggests that the ICON® material has a tendency to perform well clinically, may not be better than sealants. Clearly, more such studies are required to assess the relative effectiveness of ICON® compared to other treatment modalities.

The PLM results suggested that the ICON® material penetrated substantially into the white spot lesions, but the sealant material formed resin tags that penetrated into enamel to a limited depth to hold the sealant in place, but there was no infiltration. According to the QLF analyses, both the treatments did not help in the protection of the tooth structure after the secondary demineralization, which was evaluated by exposing the periphery of the treated lesions to secondary demineralization. Compared to ICON®, the sealant material, although not made for the smooth surfaces, proved to be protective for the tooth after the secondary demineralization. This can be attributed to the formation of resin tags by the sealant material around the periphery of the treated area on the sound enamel.

Discussion of previous studies in relation to the present study

The present study concluded that the treatments (sealant and ICON®) were effective in altering or decreasing the mean delta F values compared to the control group where no treatment was given. However, after the secondary demineralization, the area surrounding the treatment area was not protected by both of the treatments, which was comparable to the control group, the mean delta F value being least for the control group. The polarized light microscopy results showed that the ICON® material penetrated into the white spot lesions substantially after the treatment compared to the sealant material which remained on the surface by formation of resin tags. The greater penetration for ICON® can be attributed to the etching with 15% HCl in the ICON® groups as opposed to phosphoric acid in the sealant group.

Several previous studies will be discussed in comparison to the results of the present study. Studies have been done in past with various adhesives and resin-based

materials to test the property of infiltration into the early caries lesions. Most of the studies concluded that a better resin material was needed to be developed for this purpose.

The study by Robinson et al[42] concluded that the resorcinol formaldehyde penetrated into the porosity and penetration increased after the second and subsequent applications. The compound used in the experiment was effective in the treatment of the white spot lesions. The etching material that was used in this experiment was 1N HCl, which was very similar to the ICON® etchant. The amount of penetration was measured by assessing the pore volume. Similarly, during the pilot studies conducted before our final study, the effectiveness of using 15% HCl was evaluated. It was found that, for the resin material to penetrate into the porosity, more than one etch was required. The polarized light microscopy results of the final study showed measurable penetration of the ICON® into the porosity.

Rodda[43] published a similar type of study that assessed the penetration depth of different resin materials available using polarized light microscopy techniques. HCl was used as etching agent for removal of the surface layer. However, the etching time in their study[43] was 60 seconds and the depth of the penetration in different resins varied from no penetration to a substantial penetration. Gray et al[20] assessed the extent to which carious lesions can be infiltrated with polymerizable resins. The resins that were used were Scotch Bond™ and Seal and Protect® (Dentsply). The results showed that the duration of etch (0, 5 and 10 seconds), and number of applications (1, 2 and 3 applications) affected the percentage penetration of the resin material; the maximum penetration was for a 10 second etch and three applications. They concluded that an optimal infiltration could be

achieved with a 5 seconds etching where the lesions are dehydrated with ethanol and two layers of resins are applied. During the pilot studies described in this thesis study, efforts were made to etch the teeth once with 15% HCl and apply the resin material, which showed no penetration. Penetration only increased after the number the etchings with the HCl was increased.

Similarly, a study done by Paris et al[29] described the application techniques for the ICON® material in detail. They concluded that the ICON® infiltration material required two applications to penetrate to the depth of the lesion. However, after following this protocol, significant penetration was achieved, but none of the lesions in the present thesis study exhibited penetration of ICON® material to the full depth of the lesion. Paris et al[29] concluded that the current available bonding resins have a potential to be used for infiltration technique. Paris et al [29] was one of the many studies based on which the ICON® material that was developed. The QLF results in the present thesis study showed the effectiveness of ICON® material in the treatment of white spot lesions on the smooth surfaces. However, after the secondary acid attack for the difference from treatment to secondary demineralization ($\Delta F3 - \Delta F4$) where only the area around the treatment area was taken into consideration, results showed that the treatments were not effective in prevention of demineralization around the lesion.

The QLF results of the present study suggested that, after secondary acid attack, both the ICON® and the sealant material were not effective in prevention of secondary demineralization on the smooth surface of teeth, when compared to a control group. A study done by Goepferd et al[50] was done in a similar way to our study. The lesions were created using 15% acidified gel at a pH of 4. After the lesions were created, etching was

done using 37% phosphoric acid. The treatment was done with an unfilled, light-cured resin material. The lesions were further exposed to acid attack, as we did in our study. A comparison was made with a control group where no treatment was given. The results showed that the resin-treated area was protective when compared to the control lesions. Also, the area around the treated area had shallower lesion depth compared to the untreated area. However, in the present thesis study, there was no statistically significant difference among the treatment and the control groups.

A study done by Garcia-Godoy et al[51] assessed the effectiveness of an adhesive material in the prevention of secondary demineralization in comparison with a control, where no treatment was given[51]. The adhesive used in the experiment was Prisma Universal Bond 2 adhesive ™. The results of the study showed that the lesions treated with the adhesive progressed less than did the controls, demonstrating effectiveness in prevention of secondary demineralization. However, in the present thesis study, the ICON® material and the sealant material were not significantly different or protective (since the amount of demineralization after the secondary acid attack was similar in both the treatment groups when compared with the control group where no treatment was given) when compared to the control group after the secondary demineralization, but the mean delta F for the control group was least among the three groups

Although many studies compared the effectiveness of resin material or an adhesive with a control group (no treatment) in prevention of secondary demineralization, the present study compared the ICON® resin material with a resin-based sealant material. In this study the results showed no statistically significant differences among the two materials and the control group in prevention of secondary demineralization.

Several studies were done in the past that assessed various adhesive and resin materials' infiltration of white spot lesions and prevention of further progression of these lesions. All the materials that were tested failed to either obtain any infiltration or achieved limited infiltration. Thus, the ICON® material was developed based on the previous studies' results to achieve infiltration to the base of the white spot lesion.

Laboratory studies were done to assess various physical properties and assess the infiltration of the material into white spot lesions during the development of the ICON® material.

In the present study, penetration abilities of the materials were assessed using the polarized light microscopy. The results concluded that the ICON® material showed a substantial amount of penetration (etching was done with 15% HCl) as compared to the sealant material (etching was done with 37% phosphoric acid) where no penetration was seen. One of the other laboratory studies was done to assess the effectiveness of 15% hydrochloric acid as an acid etch in comparison to the 37% phosphoric acid. This study by Lueckel et al[36] looked at the ability of etching acids to permit the resin infiltration into the porosity of the white spot lesions. The results concluded that the surface layer reduction with 15% HCl was significantly greater compared to the 37% phosphoric acid. In the present study that compared the ICON® and the sealant material, the ICON® group was treated with 15% HCl and the sealant group with 37% phosphoric acid.

Although the studies discussed above were done to evaluate the efficacy of ICON® in an in-vitro/in-situ setting, no studies have been done which compared the ICON® infiltration material with a sealant material. However, some clinical studies, described below, were conducted to assess the effectiveness of the ICON® material.

In the present study, ICON® and sealant material were used for treatment of smooth surface white spot lesions. Evaluation of the effects of treatment with these two materials was done using the QLF technique. Both the materials were effective in masking the white spot lesions. A clinical study done by Shin-Kim et al, at the Pusan National University, evaluated the ability of resin infiltration in masking labial enamel[52]. Subjects with white spot lesions that were either developmental enamel defects or post-orthodontic decalcification were included. The results showed that 25% of the teeth with developmental defects were completely masked, 35% partially masked and 40% were unchanged. Sixty-one percent of teeth with post-orthodontic decalcification lesions were completely masked, 33% partially masked and 6% remained unchanged. The color changes in both groups were statistically significant after one week of treatment when compared to baseline, which suggests that ICON® resin infiltration treatment is an effective way to mask white spot lesions[52]. The present study did not assess color change, but did demonstrate infiltration by ICON® material, suggesting that infiltration concept is a plausible means of masking such lesions.

A radiographic study done by Paris et al[40] assessed the lesion progression of teeth treated with ICON® material compared to placebo. The study was a randomized, split-mouth, placebo-controlled trial. Twenty-nine pairs of proximal caries lesions from 22 young adults were included in the study, with inclusion based on having lesions in the inner half of enamel or outer third of the dentin. Radiographs were made at the baseline for evaluation, and, out of 61 screened individuals, 22 met these inclusion criteria. ICON® infiltration was performed on the test group and placebo was used with the control group. After 18 months, a follow-up radiographic assessment was done for both

groups. The results were that 7% of the teeth treated with ICON® material showed lesion progression compared to 37% of teeth in the placebo group[40]. ICON® material's use in a clinical setting in this study showed promising results. Although there was no statistically significant difference among the ICON®, sealant and the control group after the secondary demineralization, studies in the future with larger sample size are required.

A recent clinical study was published in January 2012 by Martignon et al[72], after the present study's laboratory work was completed. It assessed the therapeutic effects of infiltration vs. sealant for arresting the caries progression on the proximal surfaces of teeth[72]. The split-mouth, placebo-controlled, randomized clinical trial was conducted with subjects aged 16 to 35 years. A total of 680 participants were approached for the three-year clinical study, out of which 90 agreed to participate. For each individual, the three lesions were randomly allocated to either infiltration with ICON® pre-product, a sealant (PrimeBond®) material or placebo (no treatment, but a micro-brush was passed between the teeth) . Follow-up at the end of each year was done for three years. Thirty-seven subjects were available for analysis at the end of three years. There was no statistically significant difference between ICON® and sealant in prevention of caries progression, although both were significantly better than the placebo[72]. The present study was similar to the one by Martignon et al[72], but was in-vitro. The present study results concluded that there was no statistically significant difference among all the three groups of control, sealant and ICON® after secondary demineralization, since both the treatment groups were not effective in protection around the area that was treated, when compared to the control group where no treatment was given.

All the above in-vitro studies using the ICON® material were either done to help develop the product or to test the physical properties of the material. The majority of the clinical trials reported were done in comparison with the controls without any treatment, except the one by Martignon et al[72]. In some of the clinical studies that used an adhesive or a sealant material for the infiltration of white spot lesions, these materials did not show significant results when considering complete infiltration or effective in prevention from secondary demineralization. The ICON® material was developed to have physical properties to allow for infiltration to the base of the lesion and prevention of lesion progression after secondary demineralization.

As discussed earlier, the ICON® material works on the principle of infiltration, where a lesion treated with the product will have the resin material infiltrated into the porosity. Pioneering research on prevention of progression of white spot lesions evolved since the study by Robinson et al published in 1976[42]. A number of studies were done to develop, and assess the efficacy and effectiveness of infiltration material in prevention of further progression of white spot lesions and the materials have evolved with better properties and infiltrating capacity. Many recent in-vitro study results have shown promising results in prevention of lesion progression. However, little clinical research has been done to assess the effectiveness of the infiltration materials on the white spot lesions. Among the few clinical studies that were done, most were either inconclusive or concluded that more clinical trials were required to reach a conclusion.

Advantages and Disadvantages: ICON® and Sealant

ICON® and sealants are both technique-sensitive materials. The two products have completely different working and setting times, and each have their own advantages and disadvantages. The working and setting times of the sealant material are less than for the ICON® material. The etchant used for the sealant material is 37% phosphoric acid (37% H_3PO_4) gel and the etching time is 30 seconds. The curing of the sealant requires about 30-45 seconds. The sealant requires maintenance of a completely dry field and any moisture on the tooth surface can cause the sealant to fail. The drying is accomplished using dry, moisture-free air.

While sealants are technique sensitive, the ICON® material is more so, as there were many problems encountered with the ICON® material. As discussed earlier, the ICON® works on the principle of infiltration, which also requires a very dry field. Apart from keeping the environment moisture-free, additional steps must be taken to dry the lesion. This is accomplished by treating the lesion area with alcohol, which evaporates the water within the porosities which can inhibit the process of infiltration. Another disadvantage of ICON® compared to the sealant material is the etching process. For ICON®, the etching is accomplished with 15% hydrochloric acid, which is more corrosive compared to the 37% phosphoric acid. The hydrochloric acid has to be kept away from the oral soft tissue, which requires placement of a rubber dam if done intra-orally.

In addition, during the etching procedures, problems arose concerning the number of etchings done on the white spot lesions. The ICON® manufacturer's manual suggested etching the white spot lesions once before the next step was to be performed, but it also

said that, after etching the lesion with 15% hydrochloric acid, the lesion surface was supposed to appear chalky white in color and texture. However, after etching once, neither of these characteristics was seen. A scientific paper on how to treat lesions with ICON® was released stating that the desired results may require etching twice[38]. We followed this revised protocol and etched the lesion twice for 45 seconds each, after which the lesion appeared white and chalky. The main concern with the etching procedure was that, with one etch, the 15% hydrochloric acid eroded a significant depth of enamel and the second etch only further reduces the thickness of enamel. The manufacturer's literature says that the etching steps open the porosity of the enamel to enable the infiltration of the ICON® material into the porosity. To check this, we treated some teeth with the ICON® material and made sections to assess them using polarized light microscopy, which showed that the material did not infiltrate to the base of the lesion, even after two etches.

Other challenges were encountered with the ICON® material during its use. These included packaging of the ICON® material, which includes a syringe from which the material is dispensed. As the plunger of the syringe is depressed, the amount of material that is dispensed is much more than is required and the excess material has to be wiped away with cotton swab. Therefore, there should be a better dispensing tool for the ICON® resin material so that the desired amount of material is dispensed onto the treatment surface and less is wasted. Finally, during the curing of the material the manufacturer suggested to cure the material for 45 seconds after treating with ICON®, but while performing the treatment in the study, we found that two cycles of curing of three minutes each were required for the material to cure.

These challenges of working with the ICON® material raise several issues as to whether it would be appropriate to use in a public health setting. First, the ICON® material is a very technique-sensitive material to be used in a public health setting. The materials that are typically used in such settings are easier to use; in contrast, the procedure involved with the ICON® material must be followed carefully and every step from etching to infiltration and curing has to be done carefully and in a timely manner. The other challenges for ICON® use in both public health setting and a private practice are the high cost of the material and the fact that the treatment and that the costs incurred by the treatment are not reimbursable by insurance companies. Given the need to minimize costs in public and private settings, it is not clear that use of ICON® is cost-effective in routine practice for the prevention of caries. Research is needed specifically investigate the cost-effectiveness of ICON® compared to other treatments. It should be noted that there are challenges with the sealant placement that aren't a concern with the use of the infiltration technique. Most importantly once the sealant material is placed on the tooth periodic checks have to be done for retention, and replaced if the sealants are lost.

Limitations of laboratory-based studies

Laboratory studies such as the present study have some inherent limitations. Studies done in the laboratory are conducted in artificial and very controlled settings, so study results cannot be generalized (external validity). Laboratory-based studies are artificial by nature, and subject to human error and bias, so results may be difficult to replicate.

Limitations specific to the present study

As this study was *in-vitro*, the treatment was conducted on extracted teeth and the process for and time required for lesion creation, the application of the treatment material, the way the material acts on the lesions and the process of secondary demineralization may be different than what occurs *in-vivo*. Also, the lesions themselves might be different in each tooth, i.e., the amount of demineralization in each tooth could vary depending on the amount of fluoride they were exposed to prior to extraction. Some of the limitations specific to the study are that the pH cycling that was conducted cannot completely simulate the oral environment. The lesions were created in approximately 72 hours of exposure to demineralization acids, whereas lesions in the oral cavity could develop over a period of months or years. In addition, lesions in mouth develop when both demineralization and remineralization occurring simultaneously. Similarly, the cycle of secondary demineralization is time-bound in an *in-vitro* setting, but could occur over a longer time span in vivo. Finally, the treatment materials that were used in the laboratory study could act differently when used in vivo.

There were limitations in the study related to the methodologies used. The final data collection was done using the QLF technique. While using the QLF technique, it assesses the lesion area, but not the depth or size of the lesion. Another limitation with using the QLF technique is that it cannot differentiate between an active and an inactive lesion.

The PLM technique was used in this study to assess the depth of infiltration of the treatment materials (sealant and ICON®). No data for analysis was collected using the PLM technique since it is a destructive technique.

The final experiment included a sealant group in addition to the ICON® and control group. Due to the limited time, material and other resources, no pilot study was conducted with all three groups, and the final sample size for the experiment was decided based mainly on the previous studies.

Implications

The study found that both the restorative materials i.e., the sealant and the ICON® were able to reduce the lesion area as measured by delta F after the treatment, but not after the secondary demineralization. The sealant material's primary purpose is its use on the occlusal and pit and fissure surfaces, whereas the primary purpose of the ICON® material is to be used on active smooth surface lesions that are encountered after orthodontic treatment, or initial active carious lesions on any other surface of the tooth. So, in this study, neither of the materials was used in its ideal application. Thus, further testing and additional studies are required to fully assess the relative value of each material in varied applications.

However, the present findings were that the sealant material and the ICON® performed equally well after the treatment step, but the procedure involved in the application of the ICON® was cumbersome as compared to the sealant. The acid etching and the number of etches with the hydrochloric acid used for ICON® causes more erosion of enamel as compared to the etching by phosphoric acid. Future studies are required to assess the amount of loss of enamel after the etching with 15% HCl and if it is a concern. The ICON® material is used as an infiltrant and the sealant works on the principle of mechanically blocking the pits and fissures. As anticipated, there was some infiltration

achieved with the ICON® material and the sealant material stayed on the surface of the lesion and prevented the further demineralization by formation of resin tags (where the material penetrates into the enamel superficially) on the surface. The use of sealant material in a clinical setting is comparable to its use in the laboratory, where the etching is done with phosphoric acid with a curing time of 30 seconds. The only precaution that has to be taken is to maintain a dry field. The sealant and ICON® material, when used in a clinical setting, are both very technique-sensitive materials. The acids used for etching for the two materials are HCl and phosphoric acid, which are corrosive, and can cause tissue damage if not handled carefully. The use of a rubber dam is a must when using the ICON® in a clinical setting.

Lastly, given that there was no advantage in using ICON® vs. sealant; ICON® is cost issues must also be considered. ICON® is much more expensive than the sealant material, with the cost of treating a single lesion approximately $30-$40. This is based on the ICON® kit cost of about $120, for which the manufacturer claims that 4-5 lesions can be treated. During the pilot studies being conducted for the present thesis study we found that, if used properly 8-10 lesions could be treated with one ICON® kit. However, it is not clear whether this is possible in a clinical situation, and the additional time involved in using ICON® must also be considered.

The analyses of the data showed that there was no statistically significant difference among the three groups of sealant, control and ICON®, which means neither of the treatments is effective when compared to the control group. Looking at the cost, the cumbersome nature of the procedure and being a technique-sensitive material, the ICON® material does not appear to be appropriate for use in a public health setting. The sealant

material, although effective in prevention of decay on the occlusal and other pit and fissure surfaces, is not effective on the smooth surface. However, since the ICON® material was developed and first launched into the European market, the use of the material by dental professionals there may be more favorable due to the cost which may be lower, and also that treatment cost there may be covered by national health insurance programs. Again, more formal assessments of the relative cost-effectiveness of ICON® are needed.

In brief, more clinical studies are required in the future to promote the use of ICON® material in a clinical setting.

Future Directions

During the study, we encountered many challenges with the ICON® material. The polarized light microscopy analysis showed that the ICON® resin material penetrated to only a certain depth, i.e., in none of the lesions did the material penetrate to the full depth of the lesion. However, there are no studies that have investigated whether the material penetration to the depth of the lesion is necessary for optimal caries prevention. To better analyze this, we are planning to do a study on deeper lesions to see if the infiltrating capacity of the ICON® will be increased.

Studies can also be done to assess the retention of the sealant material in comparison to the ICON® material, to see which one is more effective. Longitudinal studies to follow the lesions after they have been treated with the ICON® material over a certain period of time can be done to see the effectiveness of treatment in further progression of the lesion.

Another avenue for future research would be to conduct a study on the occlusal surfaces of the teeth, if challenges with assessment of the lesions can be overcome. The study was originally planned to be done on the occlusal surfaces, but to better understand the material, and to overcome the many hurdles, the study was done on the smooth surfaces. However, future study on the occlusal surfaces should be done to evaluate the ICON® material's use on the occlusal surfaces.

With the sample size for the study, no statistically significant differences were observed among the groups after secondary demineralization. However, there was a difference that approached statistical significance ($p=0.09$), suggesting that a larger sample may have yielded significant differences. Thus, more pilot studies using a sealant group should be done to calculate of power and sample size, or future studies should use large sample sizes.

Finally, more clinical trials with larger sample size more comparisons (sealant, resins, ICON®) would be the best way to assess the efficacy of ICON® material.

CHAPTER 6

CONCLUSIONS

The present study was an in-vitro laboratory study that assessed the effectiveness of ICON® and sealant materials in comparison to the control group, where no treatment was given. Teeth were divided into three groups (ICON®, sealant and control) and subjected to demineralization, treatment (no treatment for control group) and then re-exposed to secondary demineralization. Data collection was done using the QLF (quantitative light fluorescence) technique. Delta F was the measurement unit for the loss of fluorescence, and data collection was done for each time point (baseline ΔF1, initial demineralization ΔF2, treatment ΔF3, and secondary demineralization ΔF4) and evaluation of difference in mean ΔF values was done for ΔF3- ΔF4 to see the effect of treatment on the secondary demineralization, around the treatment area.

In the present study, the primary analysis was done using the quantitative light fluorescence technique. At the end of the secondary demineralization the results showed no statistically significant difference among the treatment groups of ICON® and sealant, compared to the control group. Thus, it can be said that that, within the limits of this study (*invitro* study), the ICON® and sealant material were not protective in prevention of further demineralization of the tooth.

Polarized light microscopy (PLM) was used to evaluate the amount of infiltration of the material into the lesions. The PLM results showed that the ICON® material penetrated into the lesion porosity substantially, but the sealant material did not. The PLM results were only of descriptive results since no measurements of the infiltration could be taken.

The ICON® and sealant material did not perform any better in prevention of secondary demineralization in comparison to control where no treatment was given. Although the sealant material is not designed to be used on the smooth surfaces, it was equally effective compared to ICON® material. However, since the sealant material is not designed to be used on the smooth surfaces and retention over a period of time might be a matter of concern. So, long term longitudinal studies are required to assess the effectiveness of sealants on the smooth surfaces.

Thus, in considering the cost of ICON® material, and the cumbersome procedures for its use, it may be of questionable value in treatment of white spot lesions as a means of preventing further demineralization or decay.

APPENDIX A: TABLE OF SYSTEMATIC REVIEW OF PRR STUDIES

Table A1

Author, Year	Study Period	Number of Subjects (age range)	Restorations	Attrition	Findings
Raadal, 1978	30 months	281 (5-7 years)	647 sealants, 249 PRR (enamel)	30%	25% sealant/17% PRR failures. Loss of sealant main cause. No caries under sealant.
Azhdari et al, 1979	18 months	Not given	130 PRR, 116 amalgam	N/A	No difference in 1 yr, 14% PRR lost sealants.
Simonsen, 1980	3 years	123 (6-8 years)	88 sealants, 73 PRR (Enamel), 71 PRR (Dentin)	14%	No caries. 97-100% sealant retention, but deteriorations required additions.
Welbury et al,1990	5 years	126 (6-18 years)	174 PRR, 174 amalgam	37.4%	Failure: 10% Amalgam, 7.3% PRR and 50% sealant loss. Projected Survival similar
Granath et al, 1992	2 years	111 (5-14 years)	87 PRR, 48 Class I composite	28%	3.4% PRR failures (caries 1.1%).
Roth and Conry, 1992	27 months (7-71)	64(child)	100 PRR(Retrospective)	N/A	26% loss of sealant, 4% caries with sealant loss.
Cloyd et al, 1992	3 years	38 (8-35 years)	74 PRR, 52 amalgam	7-10%	No amalgam failures, 8% caries in PRR, all due to sealant loss.

Stadtler, 1993	5 years	N/A	292 PRR, 242 sealant	N/A	94.3% survival. Total (5.7%) and partial (22.8%) sealant loss.
Gray and Paterson, 1994	1 year	N/A (11 years)	128 PRR (GI)	23%	7.1% caries. 62% GI PRR required additional sealant.
Houpt et al, 1994	9 years	110 (6-14 years)	322 PRR	76.2%	24% caries, 45% sealant loss. No caries with intact sealant.
King et al, 1996	16 months	351	532PRR(retrospective)	N/A	97.7% caries free, only 28.4% complete sealant
Kilpatrick et al, 1996	17 months	67 (children)	80PRR, 80 PP(GI)	17.5%	No failures, Sealant loss: 21.5% PRR (Composite) 40.9% PRR (GIC)
Walker et al, 1996	Up to 6.5 years	N/A(6-18 years)	5,185PRR(retro-spective)	N/A	16.8% replacements: 13.1% sealant loss (including 6.9% caries) and 3.7% proximal caries.
Gray and Paterson, 1998	2 years	N/A (6-16 years)	115 PRR (Enamel) 163 PRR (Dentin)	42%	90.5% adequate. 9.1% failures, 41.5% required additional sealant.
Mertz-Fairhurst et-al, 1998	10 years	123(8-52 year)	156 PRR (Cariostatic), 156 Amalgam	5-8%	Arrest of caries (radiographic) in PRR placed over caries, 75-84% showed some loss of sealant.
Gray, 1999	2 years	164 (avg 24 years)	164 PRR (GI) (49% second molars)	8.1%	All PRR present. Sealant loss 31-33%. More sealant loss from GI causing crevices.

APPENDIX B: FINAL DATA-SET

Table B1

Tooth No	Group No	ΔF1	ΔF2	ΔF3	ΔF4	ΔF2-ΔF3	ΔF2-ΔF4	ΔF3-ΔF4
1	1	-6.25	-22.8	-22.8	-35.3	0	12.5	12.5
2	1	-6.1	-23.5	-23.5	-34.8	0	11.3	11.3
3	1	-5.75	-28.5	-28.5	-40.5	0	12	12
4	1	-6.19	-18.5	-18.5	-27.4	0	8.9	8.9
5	1	-6.26	-25.9	-25.9	-36.2	0	10.3	10.3
6	1	-6.04	-30.3	-30.3	-33.5	0	3.2	3.2
7	1	-8.14	-28.2	-28.2	-33.8	0	5.6	5.6
8	1	-8.23	-27.7	-27.7	-38.3	0	10.6	10.6
9	1	-6.29	-32	-32	-39.8	0	7.8	7.8
10	1	-6.07	-33.7	-33.7	-42.7	0	9	9
11	1	-6.17	-25	-25	-31.9	0	6.9	6.9
12	1	-9.71	-35.1	-35.1	-42.5	0	7.4	7.4
13	1	-6.12	-28.1	-28.1	-40.5	0	12.4	12.4
14	1	-6.04	-25.7	-25.7	-35	0	9.3	9.3
15	1	-5.95	-29.9	-29.9	-37.2	0	7.3	7.3
16	1	-15.4	-35.6	-35.6	-41.3	0	5.7	5.7
17	1	-10.6	-28.6	-28.6	-40	0	11.4	11.4
18	1	-5.77	-26.7	-26.7	-32.9	0	6.2	6.2
19	1	-8.37	-28.2	-28.2	-38.3	0	10.1	10.1
20	1	-6.42	-30.1	-30.1	-38	0	7.9	7.9
21	1	-6.97	-38.9	-38.9	-36.5	0	-2.4	-2.4
22	1	-14.4	-25.1	-25.1	-37	0	11.9	11.9
23	1	-6.45	-30.1	-30.1	-34.4	0	4.3	4.3
24	1	-11.9	-39.3	-39.3	-46.6	0	7.3	7.3
25	1	-9.23	-29.7	-29.7	-40.6	0	10.9	10.9
26	1	-7.34	-27.5	-27.5	-37	0	9.5	9.5
1	2	-23.9	-24	-7.8	-18.4	-16.2	-5.6	10.6
2	2	-6.94	-22.5	-6.46	-22.4	-16.04	-0.1	15.94
3	2	-13.7	-22.9	-10.4	-18.7	-12.5	-4.2	8.3
4	2	-6.1	-16.9	-9.08	-12.5	-7.82	-4.4	3.42
5	2	-6.39	-22[1].2	-10.1	-18.9	-12.1	-3.3	8.8
6	2	-5.6	-26	-11.6	-20.5	-14.4	-5.5	8.9
7	2	-5.98	-21.7	-11.2	-27	-10.5	5.3	15.8
8	2	-6.72	-18.7	-6.37	-20.5	-12.33	1.8	14.13
9	2	-8.31	-20.1	-10.9	-26.2	-9.2	6.1	15.3

[1] Table B1 continued

10	2	-7.07	-18.6	-11.6	-17.5	-7	-1.1	5.9
11	2	-6.3	-31.6	-9.66	-24.4	-21.94	-7.2	14.74
12	2	-6.29	-18.7	-10.8	-20.3	-7.9	1.6	9.5
13	2	-7.28	-21.2	-16.5	-24.2	-4.7	3	7.7
14	2	-7.42	-33.7	-18.5	-27.3	-15.2	-6.4	8.8
15	2	-6.83	-28.9	-18.9	-22.3	-10	-6.6	3.4
16	2	-7.31	-28.9	-6.9	-21.7	-22	-7.2	14.8
17	2	-6.44	-24.5	-18	-25.3	-6.5	0.8	7.3
18	2	-6.22	-23.6	-10.8	-27.6	-12.8	4	16.8
19	2	-5.85	-21.5	-17.5	-20.5	-4	-1	3
20	2	-7.59	-28.2	-12.6	-26.1	-15.6	-2.1	13.5
21	2	-6.32	-28.6	-9.55	-19.8	-19.05	-8.8	10.25
22	2	-9.21	-29.1	-11.6	-21.5	-17.5	-7.6	9.9
23	2	-6.59	-15.7	-10.1	-15.3	-5.6	-0.4	5.2
24	2	-6.99	-31.3	-7.69	-21.9	-23.61	-9.4	14.21
1	3	-6.82	-26.4	-7.49	-26.3	-18.91	-0.1	18.81
2	3	-6.94	-38.5	-6.46	-22.4	-32.04	-16.1	15.94
3	3	-6.04	-38.7	-7.46	-21.2	-31.24	-17.5	13.74
4	3	-9.03	-25.7	-10.7	-30.6	-15	4.9	19.9
5	3	-7.41	-30.1	-8.44	-25.5	-21.66	-4.6	17.06
6	3	-15.5	-30.5	-12	-23	-18.5	-7.5	11
7	3	-8.2	-29.9	-11.2	-26.1	-18.7	-3.8	14.9
8	3	-8.73	-25.1	-17.1	-19	-8	-6.1	1.9
9	3	-6.27	-30.4	-18.2	-22	-12.2	-8.4	3.8
10	3	-8.25	-26.5	-21.6	-23	-4.9	-3.5	1.4
11	3	-8.58	-24.3	-8.66	-21.5	-15.64	-2.8	12.84
12	3	-6.63	-30.6	-17.9	-24.8	-12.7	-5.8	6.9
13	3	-5.77	-17.1	-7.41	-23.1	-9.69	6	15.69
14	3	-6.21	-22.2	-9.06	-21.7	-13.14	-0.5	12.64
15	3	-8.76	-26.9	-14.2	-24.2	-12.7	-2.7	10
16	3	-6.54	-25.4	-8.01	-17.1	-17.39	-8.3	9.09
17	3	-6.25	-29.2	-9.87	-25.5	-19.33	-3.7	15.63
18	3	-6.08	-32.4	-10.9	-24.2	-21.5	-8.2	13.3
19	3	-8.51	-26.8	-20.4	-22.9	-6.4	-3.9	2.5
20	3	-8.22	-31.3	-7.83	-19.5	-23.47	-11.8	11.67
21	3	-6.8	-32.3	-32.7	-21.9	0.4	-10.4	-10.8
22	3	-7.05	-33.7	-15.4	-25.4	-18.3	-8.3	10
23	3	-7.11	-25.2	-11.7	-23.8	-13.5	-1.4	12.1
24	3	-8.22	-27.5	-12	-22.7	-15.5	-4.8	10.7
25	3	-8.01	-28.9	-16	-19.7	-12.9	-9.2	3.7
26	3	-6.99	-31.3	-7.69	-21.9	-23.61	-9.4	14.21

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