LONG-TERM CARIES INHIBITORY EFFECTS OF FLUORIDE RELEASING TOOTH-COLORED RESTORATIVE MATERIALS

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Summary

Objectives: The objectives of this research were to compare the demineralization inhibition properties of the continuum of fluoride releasing tooth colored restorative materials. The effects of aging on the caries inhibition properties of the materials were also assessed.

Methods: Materials evaluated included a giomer (Reactmer, Shofu [RM]); a conventional glass ionomer (Fuji II, GC [FJ]); a resin modified glass ionomer (Fuji II LC, GC [FL]) and a compomer (Dyract AP, Dentsply [DY]). A non-fluoride releasing composite (Spectrum TPH, Dentsply [SP]) was used for comparison. Class V preparations on buccal and palatal/lingual were made at the CEJ of 75 freshly extracted molar teeth. The teeth were randomly divided into 5 groups of 15 and restored with the various materials. The occlusal half of each restoration was in enamel, while the gingival half was in dentin. The restored teeth were sectioned into two halves, half stored for 2 weeks, and the other half for 6 months in distilled water at 37°C. All restorations were subjected to artificial caries challenge (18 hours demineralization [pH 5.0] followed by 6 hours of remineralization [pH 7.0]) for 3 days. Sections of 130±20 μm were examined with PLM, and outer lesion depth [OLD] and wall area [WA] lesion/inhibition measurements made using image analysis software. All data were subjected to statistical analyses.

Results: At 2 weeks, OLD ranged from 54.55 to 65.86 and 124.68 to 145.97 μm in enamel and dentin respectively. WA (positive values (+) indicates wall inhibition, (-) negative values indicates wall lesion) ranged from -2356.13 to 1398.20 and -3011.73 to 5095.80 μm^2 in enamel and dentin respectively. At 6 months, OLD

ranged from 43.40 to 59.53 and 112.99 to 166.27 μm in enamel and dentin respectively. WA ranged from -1604.53 to 1975.23 and -3444.27 to 2653.87μm^2 in enamel and dentin respectively. Results of ANOVA/Scheffe's post-hoc test ($p<0.05$) were as follows: At 2 weeks, enamel OLD – no significant difference between materials; Dentin OLD – SP & DY > FJ, FL & RM; Enamel WA inhibition – FJ, FL & RM > DY & SP; and Dentin WA inhibition – FJ > FL > RM > DY > SP. At 6 months, enamel OLD – FJ, RM, DY, SP > FL; Dentin OLD – SP > FJ, FL, RM, DY; Enamel WA inhibition – FJ > FL, RM > DY > SP; and Dentin WA inhibition – FJ > FL, RM > DY > SP.

Significance: The present study showed that dentin is more susceptible to demineralization than the enamel at regions away from restorations. The demineralization inhibition effect of giomers, conventional and resin-modified glass ionomer cements appear to be more evident at the margins of restorations. The demineralization inhibition effects of materials were time and tissue dependent. At both time intervals, FJ & RM had similar enamel and dentin OLD. At both time intervals, enamel and dentin WA inhibition by glass ionomers and giomer was significantly greater than the compomer and composite.

List of Publications

1. E. De Hoyos, A.U. J. Yap, S. Hsu (2002) In vitro caries inhibition by fluoride releasing tooth-colored restoratives. 1st NHG Scientific Congress "YEARS TO LIFE- LIFE TO YEARS" in Singapore August 17 &18, 2002
2. E. De Hoyos, A.U. J. Yap, S. Hsu (2002) In vitro caries inhibition by fluoride releasing tooth-colored restoratives. (Abstract) 17th International Association for Dental Research (South-East Asian Division) Annual Meeting / 13th South-East Asia Association for Dental Education Annual Meeting 18 – 20 September 2002. (IP-47) page 44.
3. EG De Hoyos, AUJ Yap, SCY Hsu (2004) Demineralization Inhibition of Direct Tooth-colored Restorative Materials *Operative Dentistry* **29(5)** 578-585

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1 INTRODUCTION

Recurrent Caries or secondary caries has been one of the major reasons for failure of a dental restoration (Kidd, Toffenetti & Mjör, 1992; Mjör, 1985). It is by definition found at the tooth-restoration interface and is, in general, the result of microleakage (Arends, Dijkman & Dijkman, 1995). Microleakage is defined as the clinically undetectable passage of bacteria and fluids between cavity walls and restorative materials (Mjör & Toffenetti, 2000). The loss of marginal integrity between the aforementioned provides potential pathways for reinfection, as cariogenic bacteria can easily penetrate into the underlying dentin through these defects (Brännström & Nordenvall, 1978). These micro-organisms are responsible for the demineralization of adjacent dentin and/or enamel via a chemical process presumed to be similar to those in primary caries (Arends, Dijkman & Dijkman, 1995). As the marginal seal of tooth-colored restoratives to tooth tissues is still not perfect (Sjodin, Ursitalo & Van Dijken, 1996; Yap, Lim & Neo, 1995), antibacterial properties are desirable.

During the last decade, more emphasis has been placed on the desirable properties of having fluoride in a soluble form, as it can dissolve in saliva and/or plaque fluid and slowly supply low concentrations of ambient fluoride, which promotes the demineralization and remineralization kinetics at the tooth surface during the carious process (Clarkson, 1991). Furthermore, the low incidence of caries around silicate restorations containing fluoride (Halse & Hals, 1976) has led to the incorporation of fluoride into various dental restorative materials including sealants, composite resins, amalgam, cements and even core build-up materials (Ewoldsen & Herwig, 1998; Hickel & others, 1998; Mount, 1994). The mechanisms and cariostatic effects of both systemic and topical fluoride have been well documented (ten Cate & van Loveren,

1999). Fluoride release has been postulated to have anticariogenic potential by protecting both surrounding tooth structure and adjacent teeth against caries and demineralization (Forss & Seppa, 1990; Friedl & others, 1997). Hence, a slow release of fluoride from a restoration is desirable because of the potential of secondary caries inhibition (Arends, Ruben & Dijkman, 1990; Diaz-Arnold & others, 1995; Forsten, 1990; 1994). However, a therapeutic dose of fluoride release necessary for "curing" carious lesions and for anticariogenic effects has not been documented and may vary depending on different factors (Mjör & Toffenetti, 2000). The content of fluoride in the restorative materials should, however, be as high as possible without adverse effects on physico-mechanical properties and the release should be as great as possible without causing undue degradation of the filling (Yap & others, 2002). The properties of GIC's to take up and release fluoride have been widely substantiated (Creanor & others, 1994; Nagamine & others, 1997; Tam, Chan & Yim, 1997; Wandera, 1998). Fluoride ions penetrating dentin produced mineralization of the dentin and reduced demineralization (Damen, Buijs & ten Cate, 1998). Therefore, dentin penetrated by fluoride ions offers resistance against secondary caries attack (Itota & others, 2001).

Glass ionomer cements were introduced to the dental profession in the early 1970's (Wilson & Kent, 1972). Their favorable adhesive and fluoride-releasing properties have led to their widespread use as luting, lining and restorative materials (Sidhu & Watson, 1995). Disadvantages of these cements, however, include sensitivity to moisture, low initial mechanical properties and inferior translucency compared to composite resins. Hybrid materials combining the technologies of glass ionomers and resin composite were subsequently developed to help overcome the problems of conventional glass ionomer cements (GIC) and at the same time maintain their clinical

advantages. Examples of these hybrid materials include resin-modified glass ionomer cements and compomers (polyacid-modified resin composites). Recently a new category of hybrid aesthetic restorative material was presented to the dental profession. Known as giomers, they employ the use of pre-reacted glass ionomer (PRG) technology to form a stable phase of glass ionomer in the restorative. Unlike compomers, the fluoro-alumino silicate glass is reacted with polyacrylic acid prior to inclusion into the urethane resin. The manufacturer's claims include fluoride release, fluoride recharge, biocompatibility, smooth surface finish, excellent aesthetics and clinical stability. Like compomers, giomers are light polymerized and require the use of bonding systems for adhesion to tooth structure. Although the enamel and/or dentin caries-inhibiting effects of these fluoride-releasing materials had been widely reported, no literature is available regarding the caries-inhibiting effect of giomers.

Objectives of this study are:

1. To evaluate and compare the caries inhibition of the continuum of tooth-colored restorative materials.
2. To determine the effects of aging on the caries inhibition properties of these materials.

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2 LITERATURE REVIEW

2.1 The Structure of Enamel and Dentin

2.1.1 Normal Structure

Enamel is a semi-translucent grey or bluish-white secretory product of cells derived from the stratified epithelium of the oral cavity and is the most densely calcified tissue in the human body. Except at the unworn biting edges of the incisors, its color is modified by that of the underlying dentin, producing the characteristic yellowish-white appearance of the crown. In its adult state, enamel has a specific gravity of approximately 3.0, denoting a tissue very high in mineral and low in nitrogen content (Stack & Fernhead, 1965). It is birefringent; its average refractive index is high (1.62) and the microscopic appearance of the tissue is dependent upon the refractive index and degree of penetration of the mounting medium.

The inorganic content of enamel consists of a crystalline calcium phosphate known as hydroxyapatite, which is also found in bone, calcified cartilage, dentin, and cementum. Enamel has a rigid highly organized structure consisting of innumerable microscopic crystals of the mineral hydroxyapatite arranged in larger structural units, known as prisms or rods. In the permanent teeth, the rods are approximately 4-7 μm in width (Mortimer, 1970). The enamel rods, when viewed in cross section with an electron microscope, appear as a group of keyhole-shaped structures, approximately 6-8 μm in diameter with the enlarged portion of the keyhole called the head and the narrow portion called the tail (Boyde, 1997).

However, since the keyhole analogy does not adequately account for some of the variations in the structural arrangement of enamel components or coordinate with the pattern of secretion by Tomes' process, this terminology has been largely dropped.

Inside the head of the rod, the long axis of the crystals, called the c-axis, is parallel to the enamel rod. Submicroscopic amounts of organic matrix are present between crystals along the c-axis (Boyde, 1997). At the periphery of the rod, the crystals assume an angle to the more central crystals (Meckel, Griebstein & Neal, 1965). The crystals are hexagonal in shape, with slightly flattened ends; this theoretic description is based on X-ray diffraction studies. The smallest space unit of the hydroxyapatite crystal is called the unit cell, containing 10 calcium ions, 6 phosphate ions, and 2 hydroxyl ions. Each of the millions of crystals in each rod has three axes, a- and b-axis representing the longest and the shortest cross-sections of the basal face respectively, and c-axis that parallels the long axis (Boyde, 1997).

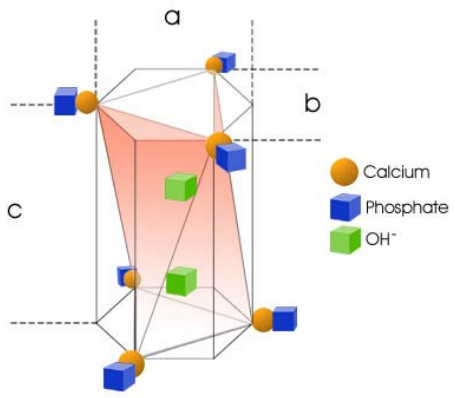

Figure 2-1. Theoretical 3D illustration of a hydroxyapatite crystal.

Three calcium ions form an equilateral triangle lying parallel to the a-b plane centered on this column. Immediately peripheral to each calcium atom is a phosphate grouping. Successive calcium triangles are rotated 180° with respect to each other, in accord with the screw axis symmetry. The c-axis is comprised by a crystallographic symmetry element known as a screw axis, where hydroxyl ions are arranged at distances of one-fourth and three-fourths the height of the axis (Figure 2-1) (Boyde, 1997). The apatite

structure permits considerable variation in its structure because other atoms can replace each one of these atoms; calcium ions can be replaced by strontium ions, hydroxyl ions can be substituted by fluoride ions, and a phosphate group can be replaced by a carbonate ion (Elliott, 1969). Ionic exchange is continual throughout life when a great number of random hydroxyl groupings are replaced with fluoride, the crystal is termed as fluoroapatite.

The inter-rod region is an area surrounding each rod in which the crystals are oriented in a different direction from those making up the rod. Condensations of the organic matter are found at the rod junctions. Submicroscopic spaces occur in the inter-rod area through which fluids can diffuse (Frank, 1966).

Dentin in the other hand is yellowish in color. This is due to the ease of the light passing readily through thin, highly mineralized enamel and reflecting the underlying dentin. It is the hard tissue portion of the pulp-dentin complex and forms the bulk of the tooth. Its inorganic component consists mainly of hydroxyapatite, and the organic phase is type I collagen with fractional inclusions of glycosaminoglycans, proteoglycans, phosphoproteins, glycoproteins, and other plasma proteins. About 56% of the mineral phase is within the collagen. The inorganic phase makes dentin slightly harder than bone and softer than enamel. Its elastic quality provides flexibility to prevent fracture of the overlying brittle enamel.

Dentin is characterized by the presence of a multitude of closely packed dentinal tubules that transverse its entire thickness and contain the cytoplasmic extensions of the odontoblasts. Dentinal tubules are small, canal-like spaces within the dentin filled with tissue fluid and occupied by odontoblast processes. They follow an S-shaped path from the outer surface of the dentin to the perimeter of the pulp in a coronal dentin.

This S-shaped curvature is less pronounced in root dentin and is least pronounced in the cervical third of the root beneath incisal edges and cusps, where they may run an almost straight course. These primary curvatures move towards the center of the pulp. Dentinal tubules make the dentin permeable, providing a pathway for the invasion of caries.

In the human teeth, three types of dentin can be recognized. Primary dentin forms most of the tooth and outlines the pulp chamber of the fully formed tooth. Its outer layer (mantle dentin) is formed by newly differentiated odontoblasts and has loosely packed coarse collagen fibrils. The secondary dentin represents the continuing, but much slower deposition of dentin by the odontoblasts after root formation has been completed. Tertiary dentin is also known as reactive, reparative or irregular secondary dentin, it is produced in reaction to noxious stimuli, such as caries or restorative dental procedures.

2.1.2 Macroscopic Changes of Enamel and Dentin

At the time of eruption, many of the apatite crystals are not fully mineralized (Crabb, 1976). Once the tooth is exposed to saliva, considerable uptake of ions occurs in the crystals making up the outer 10 to 100 μm layer of the enamel rods. This physiologic mineralization process (post-eruptive maturation) permits the mineral-deficient crystals to add calcium, phosphorus, fluoride, and other ions from the saliva, resulting in an enamel surface layer that is more mature and more resistant to dental caries.

The physico-chemical integrity of the dental enamel in the oral environment is entirely dependent on the composition and chemical behavior of the surrounding fluids. The two main factors governing the stability of the enamel apatites in saliva are the pH and the concentrations of calcium, phosphate and fluoride in solution.

Hydroxyapatite is very permissive in incorporating foreign ions in the crystalline lattice. These may be either positively charged sodium, potassium, zinc or strontium ions or negatively charged fluoride or carbonate ions. The concentration of these impurities in the tissue is influenced by their presence during its formation. These mineral modifications may have either a positive or a negative effect on the solubility; carbonate incorporation makes the apatite more soluble, while fluoride incorporation makes it less soluble.

The solubility of the apatite mineral depends highly on the pH of the environment. In an acidic environment (low pH), the concentration of ions in the liquid phase surrounding the crystallites necessary to maintain saturation is higher than at high pH. The local pH is therefore the driving force for dissolution and precipitation of hydroxyapatite. Apart from the physico-chemical considerations other regulatory mechanisms exist in saliva. The saliva bathing the teeth is normally supersaturated with respect to the calcium and phosphate of enamel (Suddick, Hyde & Reller, 1980).

The concentration of calcium and phosphate ions in the saliva bathing the tooth at the plaque-tooth interface is extremely important, since these are the same elements of the hydroxyapatite crystal.

However, after eating foods or drinks containing fermentable carbohydrates, acids are formed in plaque leading to a fall in pH called Stephan curve (Stephan, 1940).

If allowed, a microbial biofilm will be formed in the plaque-tooth interface, especially in surfaces with irregularities such as occlusal fissures, or in the gingival and proximal niches, that will result in bacterial deposits. All bacterial deposits irrespective of their age of maturation are metabolically active. These metabolic activities will result in pH fluctuations that if extended for overtime, such fluctuations will result in mineral loss (Fejerskov, 1997).

When the pH is lowered, the level of supersaturation drops, the concentration of ions needed for saturation rises, at pH around 5.6, the tissues starts to dissolve to maintain saturation (McCann, 1968; Tenvuo & Lagerlof F, 1994). As a result, the phosphate and hydroxyl ions released will take up protons (H^+) thus slowing down or reversing the fall in pH.

Consumption of foods or drinks containing fermentable carbohydrates also increases salivary flow; the increased buffering power of saliva, and the washing out of remaining sugars and acids from plaque, also contribute to the pH-rising phase of the Stephan curve.

During the recovery phase the plaque gradually becomes supersaturated with hydroxyapatite, and mineral may reprecipitate (ten Cate, Jongebloed & Arends, 1981). Ideally, this occurs at the sites 'damaged' during the demineralization. If the frequency of carbohydrate consumption is too high, the redeposition of mineral is far from completed and there is cumulative loss of enamel substance. Then a carious lesion will be formed, which is often the 'forerunner' of the caries cavity. A carious lesion is characterized by subsurface loss of mineral at the intact surface layer.

Typically, in vitro demineralization of the crystals occurs in two stages: (1) dissolution of the cores of the individual apatite crystals, and (2) subsequent dissolution of the remaining "shell" of crystal. The destruction of the crystal begins with the formation of etch pits, small indentations in the centre of the terminal ends of the apatite crystals, which progressively deepens as the dissolution continues down the centre of the crystal. The preferential dissolution of the crystal core is demonstrated by in vitro experiments in which the cores are completely dissolved in a few minutes by dilute lactic acid, whereas dissolution of the remaining shell requires several hours (Moreno & Zharadnik, 1974).

The earliest macroscopic evidence of enamel caries is known as the white spot lesion. It is best seen on dried, extracted teeth where the lesion appears as a small, opaque, white area. The color of the lesion distinguishes it from the adjacent sound enamel. Sometimes this lesion may appear brown in color due to exogenous material absorbed in its porosities.

Root Caries on the other hand, are soft irregularly shaped lesions either totally confined to the root surface or involving the undermining of enamel at the CEJ, but clinically indicating that the lesion initiated on the root surface (Katz, 1984)

Dentin or root caries occurs only after the surfaces are exposed in the oral environment (Wefel, 1994). The *Lactobacillus, Mutans Streptococci,* and some subspecies of *Actinomyces* are regarded to be important in the pathogenesis of root caries (Van Houte & others, 1990; Zambon & Kasprzak, 1995). Also involved in root caries formation are proteolytic organisms that can hydrolyze collagen matrix and a number of additional species which affect the formation of a complex microbial ecology necessary for the development of root surface caries (Zambon & Kasprzak, 1995). This creates the so-called microbial biofilm. The presence of a microbial biofilm does not necessarily result in caries lesion, but it is a necessary factor (Nyvad & Others, 1997)

Mineral dissolution is induced by various organic acids produced from fermentation of carbohydrates in the plaque, thus adhering to the teeth, and going further with subsequent proteolytic breakdown of the collagen matrix (Clarkson & others, 1986; Wefel, 1994).

The carious process at the root can be described as a dynamic process, alternating episodes of demineralization and remineralization on a daily basis (Becker, 1966;

Biesbrock & others, 1998; Koulourides, 1982). In fact, root caries is a result of the disturbance of the balance between demineralization and remineralization when the frequency and/or relative amount of organic acid produced by the plaque bacteria is large (Featherstone, 1994) and the net loss in mineral determines whether a decay is progressing or not (Wefel, 1994).

Critical pH for root is known to be as high as about 6.5 (Wefel, 1994). Root surfaces appear to be more soluble than enamel, with only half the mineral content of enamel and substantially smaller crystal size (Wefel, 1994), which would explain the initial caries development in root surfaces which is about 2.5 times faster than in enamel (Ogaard & others, 1988a).

After demineralization, denaturation and enzymatic degradation of the organic matrix, the final step in the destructive phase of root caries process occurs with the breakdown of the major portion of the collagen matrix (Clarkson & others, 1986; Frank, 1990; Wefel, 1994).

Most of the root caries initiate at or near the Cemento-Enamel Junction (CEJ), where plaque retention is more likely to happen (Axelsson, 2000). It is usually seen as a shallow, softened area, often discolored, and characterized by destruction of cementum with penetration to the underlying dentin. Furthermore, advanced lesions may cause pulpal involvement (Axelsson, 2000; Zambon & Kasprzak, 1995).

2.1.3 Macrostructural Changes of Enamel and Dentin

The outer layer of the enamel has a higher organic content than deeper layers. The mineral component of the outer surface of enamel is rarely exposed in the mouth since a layer of organic material always covers it. A thin surface cuticle lying immediately

upon the enamel surface has been described (Meckel, 1965). When this organic layer thickens to become 1 μm in thickness, it is usually referred to as pellicle (Silverstone, 1978). Beneath the pellicle, a dendritic network of organic material extends into the superficial enamel structure. In addition to these organic membranes, exogenous organic material derived from salivary mucopolysaccharides penetrates up to 10μm into the defects in the surface enamel (Silverstone & Johnson, 1971; Silverstone, 1977). The presence of surface and subsurface organic integuments may play a significant role in the initiation and progress of the carious lesion by controlling the diffusion of ions into, and out of the enamel.

Organic matrix allows the transport of mineral salts, thereby acting as the diffusion medium for acid entry during enamel demineralization (Travis & Glimcher, 1964). It was shown in earlier studies that demineralization occurred before histological change could be demonstrated in the organic matrix (Darling, 1956). The time at which organic change in the matrix became histologically identifiable was only a short time before cavitation of the lesion occurred. Electron microscopic studies on the organic component of carious enamel have revealed less dense and frequently missing fibrillar network of organic matrix from the prisms and interprismatic areas (Johnson, 1962; Johnson, 1967b). Apparent increase in organic material in carious areas has been documented in several studies (Bhussry, 1958; Hardwick & Manley, 1952; Stack, 1954). The additional organic material is amorphous in appearance and may be of bacterial, or mixed salivary and bacterial origin. The outer layer of carious enamel has a higher organic content than deeper layers (Johnson, 1967b; Meckel, 1965). Another change in early enamel caries is the accentuation of the incremental striae of Retzius (Mortimer & Tranter, 1971). Gaps occurred between the prisms, which were thought to be the result of demineralization.

Ultrastructurally, the observed features in carious enamel include: (a) scattered destruction of individual apatite crystals both within the enamel prisms and at their borders. The progressive dissolution of the crystals results in broadening of the intercrystalline spaces (Johnson, 1967a). Larger crystals at the periphery of the enamel prisms were observed. This has been interpreted as evidence for some recrystallization taking place during carious process; (b) High resolution electron microscopy clearly shows that carious dissolution starts in the centre of one end of the crystal and develops anisotropically along the c-axis (Johnson, 1962; Johnson, 1967a); (c) Differences in size distribution and density of crystals in the different zones of the lesion.

The chemical changes associated with the caries include: (a) lower mineral density; (b) lower Ca/P ratio; (c) decrease in magnesium concentration; (d) decrease in carbonate concentration; (e) increase in HPO_4^{2-} content, and (f) increase in relative fluoride concentration (LeGeros, 1991).

2.2 Relation between Polarized Light Microscopy and Carious Tooth Structure

The polarized light microscope (PLM) has been used to evaluate mineralized samples for over 150 years (Schmidt & Keil, 1971). PLM has proved to be a valuable technique in the evaluation of carious lesions (Silverstone, 1968; Wefel & Harless, 1987). Basically, PLM is a combination of a conventional light microscope with the addition of a polarizer between the light source and condenser lens; a rotating stage which facilitates the position and orientation of the specimen; an analyzer located opposite the specimen relative to the polarizer; and a ¼ wavelength or quartz tint that changes background from black to magenta, to determine the boundaries of the lesions and

distinguish the edge of the section from the usual black background (Olympus, 1997) (Figure 2-2).

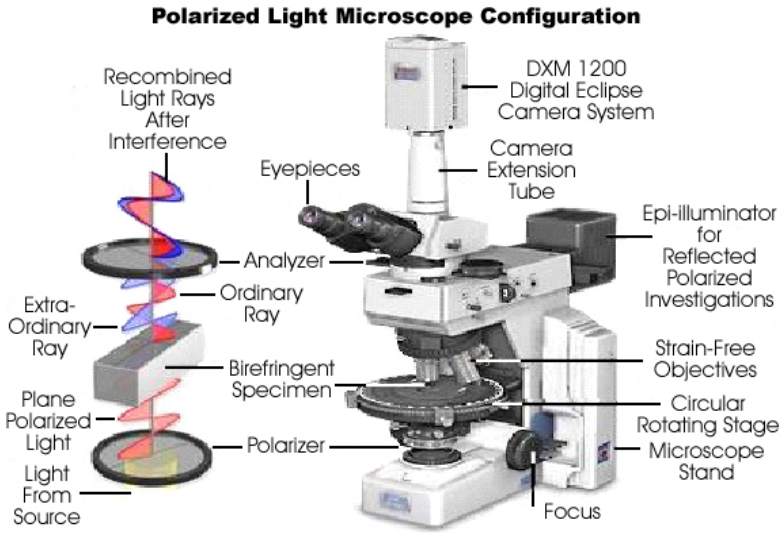

Figure 2-2. PLM configuration.

The combination of dense oriented, crystalline mineral, interspersed by tiny water-filled pores makes enamel suitable for study with the PLM. The optical characteristics of hydroxyapatite cause light to travel at two different velocities and directions, which is known as birefringence, and is indirectly measured by PLM. During the caries process, the inter-crystalline spaces become considerably larger when mineral is dissolved and the tightly-packed arrangement of the HAP crystals is disrupted (Silverstone, 1973), resulting in birefringence alterations.

When an anisotropic, uniaxial crystal is oriented at 45^{o} to the plane of the polarized light, the crystal splits the light into two beams, the ordinary ray with refractive index (n_o) and an extraordinary ray (n_e). The birefringence or difference between n_e and n_o has both quantity and sign. If n_e is greater than n_o, the sign is positive, and if n_e is less than n_o, the sign is negative. In sound enamel, which is predominantly hydroxyapatite crystals, the sign of birefringence is negative with respect to prism length. The small

volume of organic material exhibits a tiny amount of positive birefringence, but has been shown to be insignificant and can be disregarded (Theuns, Arends & Groeneveld, 1980).

During carious dissolution, there is an increase in the total volume of microspaces in enamel. These spaces give rise to the form birefringence. Form birefringence is produced when the spaces in the tissue contain a medium having a refractive index (RI) different from that of the enamel crystals (RI=1.62). In other words, intrinsic birefringence is produced by the crystals in tooth hard tissues, while form birefringence is produced by the spaces between crystals containing a medium having a different refractive index. Thus, enamel will show a negative intrinsic birefringence due to its orientated crystal component and positive form birefringence due to the intercrystal spaces. The observed birefringence is the total of these two. When enamel is examined in longitudinal ground section with the PLM, the image formed depends on both the refractive index of the mounting medium and its degree of penetration into the tissue. The greater the difference between the refractive index of the mounting medium and the enamel, the more positive form birefringence will be produced. Likewise, as the internal pore volume increases, the amount of form birefringence will also increase (Silverstone, 1968; Silverstone & others, 1981).

The observed colors of a thin section of enamel viewed with polarized light are produced by inserting a 1/4 lambda color tint into the light path. The specimen will change color as the stage is rotated every 90^{o}. The two quadrants in which sound enamel is blue-green in color are said to be negative while the opposite quadrants are positive (Silverstone, 1968; Silverstone & others, 1981). The negative birefringent sound enamel becomes positively birefringent due to the increased form birefringence after demineralization.

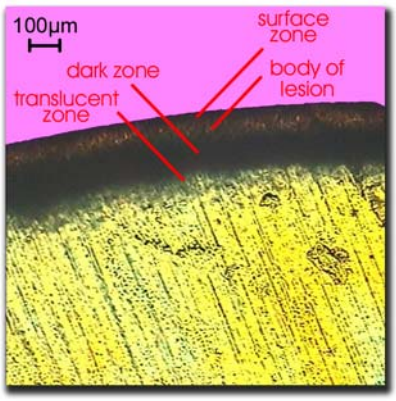

Figure 2-3. Histological zones in enamel lesion.

The enamel lesion has been divided into four distinguished zones based upon its histological appearance when longitudinal ground sections are examined with the PLM. Two zones – the translucent zone and the body of the lesion, represent areas of demineralization; while the dark zone and the surface zone represent areas of remineralization within the lesion of the enamel (Silverstone, 1973, 1983).

2.2.1 The Translucent Zone

The translucent zone of enamel caries is not seen in all lesions, but when present it lies at the advancing front of the lesion and is the first recognizable alteration from normal. This zone is only seen when a longitudinal ground section is examined in quinoline, which has the same refractive index as that of enamel, since it is more porous than sound enamel.

2.2.2 The Dark Zone

The dark zone lies superficial to the translucent zone and is the second zone of alteration from sound enamel. In fact, it is more porous than the translucent zone, having a pore volume of 2-4%. In this zone the pores vary in sizes, large and small.

Quinoline being a large molecule cannot penetrate the small pores that remained filled with air, giving a dark appearance.

2.2.3 Body of the Lesion

The body of the lesion comprises the largest proportion of carious enamel in the small lesion. It lies superficial to the dark zone and deep to the relatively unaffected surface layer of the lesion. The body of the lesion is positively birefringent and has a minimum pore volume of 5% at its periphery, increasing to 25% or more in the central portion. The water molecules enter the pores in the tissue, and since the refractive index of water is different to that of enamel, the area appears dark.

2.2.4 The Surface Zone

The small lesion remains covered by a surface layer, which appears relatively unaffected by the acid attack. The surface zone appears to be relatively unaffected when compared with adjacent healthy enamel. However, it is negatively birefringent, has a pore volume of approximately 1 and 5% and is between 10 and 50 times more porous than sound enamel.

2.3 Recurrent Caries (Secondary Caries)

The ability of a restorative material to resist a secondary caries attack and microleakage at its margins will, to a great extent, determine whether a restoration will succeed or fail. Causes of restoration failure can be classified in two ways: a) new disease, which includes the development of secondary caries, primary caries, pulpal

problems, periodontal disease; b) abrasion and technical failures, which includes fractures, marginal breakdowns, defective contours, overhanging margins, failures in cavity preparation, and poor anatomical appearance (Kidd, Toffenetti & Mjör, 1992).

Caries is a multifactorial disease resulting from the interplay of three principal factors for over time: the host (primarily the saliva factors and teeth resistance), cariogenic (acidogenic and aciduric) bacteria within dental plaque, and the substrate (fermentable dietary carbohydrates) (Van Houte, 1994). For caries to occur, conditions within each of these factors must be favorable (Newbrun & Ernest, 1989). Principally, modification in any component of this triad can alter the development of caries (Kleinberg, 1979; Van Houte, 1994).

Secondary caries is the same as primary caries; the difference is established because secondary caries is located at the margin of a restoration (Mjör & Toffenetti, 2000). As it is well known, the term primary caries is used to describe the carious process in the tooth before or without any restoration placement.

The Federation Dentaire Internationale in 1962 defined secondary caries as a "positively diagnosed carious lesion which occurs at the margins of an existing restoration". The lesion usually consists of two carious regions: an outer lesion formed in the enamel or cementum of the tooth surface, similar in histology to a primary lesion, that can be used by trapped plaque in the restoration's margin; and a wall lesion, which is narrower defect in the enamel or dentin along the cavity wall restoration interface (Hals & Kvinnsland, 1974; Kidd, Toffenetti & Mjör, 1992).

Secondary caries is by far the most frequent reason for replacement of restorations (Kidd, Toffenetti & Mjör, 1992; Mjör, 1985). It is by definition found at the tooth-restoration interface and is in general, the result of microleakage (Arends, Dijkman &

Dijkman, 1995). However, conflicting data regarding microleakage has been widely reported. Mjör & Toffenetti (2000) define the term "*Leakage*" as the act of letting fluid in or out accidentally and "*Micro*" refers to something small or minute. Therefore, *microleakage* means minute amounts of fluid passing in or out.

Moreover, Dérand, Birkhead & Edwardsson (1991) suggest that if there is no microleakage, there will be no wall lesion. Özer (1997) explains that the size of the gap between the tooth and the restoration has no influence on the initiation of caries, unless the gap exceeds 250μm, and then only if the gaps are not accessible to physical forces, including oral hygiene measures to clean the defects. The author considered that plaque accumulation on the surface at the site of development of secondary caries was the decisive factor and that such accumulation is most often associated with gingival overhangs on Class II amalgam restorations.

Secondary caries has been shown to diminish at a rate similar to that of primary caries, mainly as a result of topical fluoride available in the oral environment (Eriksen & others, 1996). However, the concentration of fluoride required to prevent caries has not been determined and may vary depending on different factors (Mjör & Toffenetti, 2000; Yap & others, 2002).

The outer lesion of secondary caries on the root surface is considered to develop in the same way as primary caries, but differentiation between marginal staining and caries is difficult (Tyas & Wassenaar, 1991). Root surface caries starts off as a subsurface lesion and when demineralization progresses, the surface become dark yellow or brown and soft, depending on extrinsic factors.

2.3.1 Recurrent Caries Adjacent to Glass Ionomer based Restorations

A number of similar studies have used *in vitro* methods to produce artificial caries lesions in an attempt to define the role of demineralization and remineralization effects of tooth structures adjacent to restorative materials. They incorporated the use of acidified gels (Attar & Onen, 2002; Dionysopoulos & others, 1998; Dunne & others, 1996; Hicks & Flaitz, 2000; Millar, Abiden & Nicholson, 1998; Tam, Chan & Yim, 1997), buffered solutions (Donly & Grandgenett, 1998; Heilman & others, 1997), and incubation with natural plaque (Gilmour, Edmunds & Newcombe, 1997; Hsu & others, 1998; Itota & others, 2001; Nagamine & others, 1997; Torii & others, 2001). These *in vitro* studies have shown the ability to mimic the demineralization and remineralization process of the tooth structure around restorations and determine if the restorative material will decrease demineralization in tooth structure (Donly, 1994; Erickson & Glasspoole, 1995; Featherstone, 1996; Wefel, Heilman & Jordan, 1995). Most demineralization studies conducted *in vitro* / *in vivo* have predominantly agreed that glass ionomers adjacent to restoration margins, offers protection against demineralization substances produced when an acid attack challenge occurs.

Previously, Attar & Onen (2002) showed that conventional glass ionomer provide significantly higher protection against caries attack and non-fluoride releasing composite resin restoration provided the least. Dionysopoulos & others (1998) performed a similar study including silver-reinforced glass ionomers and fluoride containing and non-fluoride amalgams. Similar conclusions that glass ionomer materials provided higher protection against caries attack, and high copper amalgam restoration provided the least were reported. Non-fluoridated composite resin also provided the least protection of the tooth colored restorative materials.

Similar to this study, Dunne & others (1996) observed a typical zone of inhibition at the cavity wall and concluded that both RM-GICs and GICs inhibited caries *in vitro* without significant differences.

Hicks & Flaitz (2000) compared the lesion initiation and progression effects of one RM-GIC and one resin composite. They shared that RM-GICs had lesser values of surface lesions and less frequency of cavosurface wall lesions than resin composites. Concurrently, they described wall lesions adjacent to resin composite restorations as more defined wedge-shaped structures within the cavosurface enamel and with RMGIC as an ill-defined, wedge-shaped portion of the body of the lesion projecting toward the cavity wall.

The authors firstly concluded that RM-GIC restorations reduce susceptibility of unrestored adjacent enamel surfaces and cavosurfaces to a constant cariogenic challenge. And secondly, that the caries resistance imparted to the surface enamel and cavosurface is most likely due to the fluoride release from the RM-GIC material.

Tam, Chan & Yim (1997) studied the fluoride release/uptake of the materials to resist artificial caries challenges. In addition, they studied the effect of using intermediary dentin bonding agent components on the development of surface and wall carious lesions adjacent to RM-GICs. Their consistent results showed higher fluoride release/uptake of the glass ionomer cements without primer/adhesive materials, as well as less surface depth in the body of the enamel/dentin caries. Evidently, they observed the presence of narrow zones of non-carious dentin between the restoration and the body of dentin decay extended directly to the restoration interface. Concurrently, they also observed that the resin composite was the only one to show the development of wall lesions along the dentin/restoration interface below the body of dentin decay. The mean depths of the dentin lesions for all groups were higher than the maximum

recorded depths for enamel lesions. Their conclusion was that both conventional and resin- modified glass ionomer restorations imparted resistance to dentin against the development of recurrent wall lesions *in vitro*. This effect was also attributed to fluoride release and uptake.

Gilmour, Edmunds & Newcombe (1997) assessed the effectiveness of a conventional GIC compared with a fluoride releasing composite restoration. Their results showed a 20% reduction in enamel and a 24% reduction in dentin outer lesion depths, when compared with those adjacent to composite restorations.

Nagamine & others (1997) evaluated the caries inhibitory effect of three RM-GICs, one GIC and a composite resin. They found that depth of the outer lesion and the thickness of the acid resistant layer showed no significant differences between the GICs and the RM-GICs and confirmed significant differences of GICs and RM-GICs materials in comparison with the resin composite. Whereas, the composite resin restoration did not result in demineralization inhibition of the enamel and dentin lesions. In fact, the lesion extends along the cavity wall but no deeper that the part of the lesion away from the restoration. However, they suggest that the fluoride concentration taken in the dentin may be related to the migration of fluoride ions rather than the amount of fluoride released from GICs, since they were able to detect fluoride ions released from the GICs in the cavity wall. Torii & others (2001) estimated the effects of materials on the inhibition of artificial secondary caries around restorations and concluded that RM-GICs presented a particularly strong effect, compared with compomers and fluoride releasing resin composites.

It has been proposed by several authors that demineralization not only depends on the material used, fluoride ions penetrating the hard dental tissues also plays a major role upon demineralization and remineralization challenge (Itota & others, 2001; Nagamine

& others, 1997; Skartveit & others, 1990; Tam, Chan & Yim, 1997; Torii & others, 2001; Wandera, 1998). Several *in vitro* studies have shown the uptake of fluoride ions from GICs into adjacent cavity walls (Nagamine & others, 1997; Skartveit & others, 1990) as well as the release of other ions which may complement the effects of the fluoride such as calcium, sodium, aluminum and strontium.

2.3.2 Recurrent Caries Adjacent to Resin based Restorations

On the other hand, most of demineralization studies conducted *in vitro* have predominantly agreed that compomer and resin composite materials adjacent to restoration margins had shown development of wall lesions adjacent to restorations instead of inhibition. However, controversial results regarding caries inhibition between *in vivo* and *in vitro* studies including resin-based restoratives have been reported.

2.3.2.1 Recurrent Caries Adjacent to Compomer Restorations

Millar, Abiden & Nicholson (1998) compared the *in vitro* caries inhibition of two compomers with one conventional glass ionomer and observed no significant differences in enamel surface lesion depths between GICs and compomers. The compomers showed wall lesions while the GIC showed wall inhibition areas. The authors concluded that compomer restorations offer an alternative to existing restorative materials but lack the benefits of caries inhibition similar to that for conventional GICs.

Donly & Grandgenett (1998) evaluated the dentin demineralization inhibition of two compomers in comparison with a RM-GIC and composite resin and showed that the RM-GIC and compomers had significantly less demineralization adjacent to restoration margins than the composite resin. They reported that seventy percent of glass ionomer cement restorations demonstrated inhibition zones adjacent to dentin,

while no dentin inhibition zones were demonstrated with the compomer restorations. Itota & others (2001) evaluated the effect of adhesives on the inhibition of secondary caries around compomer restorations *in vitro*. In their discussion they suggest that the type of adhesive used with compomers might play a major role in fluoride release. The authors finally concluded that applying an adhesive without Bis-GMA resin to compomer restoration will not have a suppressive effect on the fluoride release and therefore might be beneficial for inhibiting secondary caries *in vitro*.

Considering clinical implications, Meyer, Cattani-Lorente & Dupuis (1998) suggested in their study that compomers could not simply be used as substitutes for composite resins in clinical applications, since the overall *in vitro* behavior of the compomers tested were considered somewhat inferior to that of the composite resin. In a 5-year clinical study using USPHS criteria, Van Dijken (1999) reported no significant differences between compomers and resin-modified glass ionomers with reference to recurrent caries incidence.

Folwaczny & others (2001) also evaluated the 5-year clinical performance also using USPHS criteria of resin-modified glass ionomer and compomer restorations in non-carious cervical lesions of adults. The authors reported that a high and almost overall failure rate was seen for both restorative materials. However, although not significant, a considerable number of Dyract-restorations were dislodged whereas none of the Fuji II LC restorations were lost within the study period.

2.3.2.2 Recurrent Caries Adjacent to Composite Restorations

Arends, Ruben & Dijkman (1990) reported that the presence of the fluoride releasing composite resins reduced the lesion depth measured after an acid attack by about 35%. However, there is little evidence to suggest that a composite resin inclusive of fluoride provides caries inhibition, since several limitations were shown in the study. In order

to establish an accurate comparison, the authors fail to include a well-known caries inhibition material as a control group such as glass ionomers. In my personal point of view, when the effect of a restorative material shows shallower lesion than other material, it is inappropriate to infer that a better achievement has been obtained, since it still shows lesion rather than inhibition. In the other hand, when the results dramatically change from lesion to inhibition, one can speculate that the material may satisfactorily improve its efficacy in a clinical situation. Tam, Chan & Yim (1997) reported that for the resin composite, the body of dentin decay extended directly to the restoration interface, thus showing wall lesions instead of inhibition.

2.4 Cariostatic Mechanism of Fluoride

Extensive evidence shows that fluoride has a major effect at low concentrations on the demineralization and remineralization of dental tissues and, at relatively high concentrations, on acid production of cariogenic bacteria. However, it has also been shown that inappropriate fluoride concentrations and/or exposure periods could be physiologically harmful, as systemic administration of fluoride runs the risk of causing fluorosis.

During the last decades, more emphasis has been placed on the desirable properties of having fluoride in a soluble form, as it can dissolve in saliva and/or plaque fluid and slowly supply low concentrations of ambient fluoride which promotes the demineralization and remineralization kinetics at the tooth surface during the caries process (Clarkson, 1991). Hence, a slow release of fluoride from a restoration is desirable because of the potential to secondary caries inhibition (Arends, Ruben & Dijkman, 1990; Diaz-Arnold & others, 1995; Forsten, 1990; 1994). Fluoride release has been postulated to have anticariogenic potential by protecting both surrounding

tooth structure and adjacent teeth against caries and demineralization (Forss & Seppa, 1990; Friedl & others, 1997). It also enhances remineralization of early demineralized lesions of enamel, and increase enamel resistance to subsequent acid attacks.

The mechanism of fluoride starts when fluoride is released through an ion exchange mechanism or through diffusion of fluoride through the dental material.

In enamel, fluoride ions binds calcium (Ca_2) and phosphate (PO_4) dissolving, as a result of the acid penetration into the tissue and the resulting acid dissolution of the apatite, during a period of acid challenge (Larsen, 1974). This reprecipitation prevents the mineral constituents of the enamel to be leached away into the plaque and saliva (ten Cate & van Loveren, 1999). As a matter of fact, Koulourides (Koulourides, 1982) showed that enamel placed in a solution with the addition of fluoride increases the rate of mineral deposition.

In dentin, the smaller crystallites dissolve faster when placed in an undersaturated solution. The collagen fraction is the matrix onto which the apatite crystallites were precipitated during dentinogenesis (ten Cate & van Loveren, 1999). During demineralization, the apatite fraction is the first to be dissolved, only exposing the collagen after its dissolution; the collagen serves as a diffusion barrier slowing down demineralization (Kleter & others, 1994; Klont & ten Cate, 1990; Klont, Damen & ten Cate, 1991).

2.4.1 Fluoride as an Inhibitor of Demineralization

It has been widely accepted that fluoride, when taken up in the apatite lattice in the form of fluorhydroxyapatite, reduces the solubility of the crystal and improves its crystallinity (DePaola, 1991). An increase of the fluoride concentration in the outer enamel was supposed to impart a lifetime of caries resistance which is the aim of many

studies (ten Cate & van Loveren, 1999). The application of fluoride for cariostatic purposes has to a large extent been based on the above theory (ten Cate & van Loveren, 1999).

On the other hand, a small amount of aqueous fluoride in saliva and dental plaque was demonstrated to reduce the rate of mineral loss dramatically (ten Cate & van Loveren, 1999). The amount of mineral loss during demineralization was found to be a function of both pH and fluoride concentration (ten Cate & Duijsters, 1983a; b). Since the dissolved fluoride in the oral environment could be rinsed away, this mechanism implies the necessity of a continued supply of fluoride, so that caries prevention can be maintained at any time with reasonable results (ten Cate & van Loveren, 1999; Wefel, 1990).

The above-mentioned roles of incorporated and aqueous fluorides in inhibiting demineralization could be illustrated by the following reaction (ten Cate & van Loveren, 1999):

$Ca_{10}(PO_4)_6(OH)F \leftrightarrow 10Ca^{2+} + 6PO_4^{3-} + OH^- + F^-$

It is apparent that if the solid material has a low solubility due to incorporated fluoride, less calcium, phosphate, hydroxyl and fluoride are required to prevent the dissolution. It is, however, equally clear that high concentration of any of the ions, including fluoride, in the aqueous phase inhibits dissolution as well (Margolis & Moreno, 1992; Wefel, 1994).

In considering the reaction above, it can be concluded that the incorporated and aqueous fluoride work in concert in preventing demineralization (Margolis & Moreno, 1992; Wefel, 1994). In addition, during the demineralization and remineralization episodes, the incorporated fluoride could be released into plaque and saliva, while

aqueous fluoride could be incorporated into crystalline lattice and replace carbonate, resulting in a mineral with lower solubility (ten Cate & van Loveren, 1999).

In the past decades a lot of attention has been given to the relative importance of firmly versus loosely bound fluoride in caries prevention. Firmly bound fluoride refers to fluoride incorporated in the crystalline lattice of hydroxyapatite, whereas loosely bound or labile fluoride pertains to fluoride adsorbed to apatite and to fluoride leaching from relatively soluble fluoride-containing deposits. The latter includes calcium fluoride, whereas firmly bound fluoride concerns fluorhydroxyapatite. In the early days of development of caries preventive products and treatment strategies, the adage was that the formation of calcium fluoride should be prevented because its formation would draw away calcium from enamel and, because calcium fluoride was thought to dissolve quickly, this calcium would leach away from the oral cavity and thus be lost from the dental tissue. Moreover, the prevailing thinking at the time was that fluoride should be deposited in a stable form in the dental tissues, which would, as a result of this, be safeguarded against caries for life.

Findings on the effects of low concentrations of fluoride on demineralization and remineralization, however, initiated discussions on the effects of fluoride in solution or released from calcium fluoride (like) deposits versus fluorapatite in inhibiting enamel demineralization.

A very elegant experiment in this category was the in situ study described by Ogaard & others (1988a). They placed shark enamel (consisting of fluorapatite) on Hawley retainers in subjects participating in their intraoral research program and studied the enamel demineralization, post-in vivo, by microradiography. The enamel specimens were covered with orthodontic bands to create a space for plaque formation. Besides

the experimental group with shark enamel, a group with human enamel specimens was investigated. After 4 weeks in situ caries lesions were formed not only in human enamel but also, although less severely, in shark enamel. This observation indicated that structurally bound fluoride was not very effective in inhibiting enamel demineralization. Additional information was obtained from a third group of subjects who rinsed their mouth daily with a 0.2% sodium fluoride rinse. In their case caries was inhibited to a significantly greater extent in the shark enamel group. In this direct comparison ambient fluoride showed a greater caries preventive effect than firmly bound fluoride. Consequently, fluoridation of enamel, with the aim of producing high levels of incorporated fluoride, is not a sufficient method of inhibiting tooth decay.

Calcium fluoride, as a fluoride reservoir on the tooth surface, only forms during treatments with high-concentration fluoride solution. Fluoride topical applications, in particular when acidified, result in the formation of globular deposits of calcium fluoride (like) materials. These globules do not dissolve as quickly as expected on the basis of their solubility (Rølla & Ogaard, 1986). This is attributed to the presence of a phosphate- and protein- rich surface covering these globules (Rykke & others, 1989; Saxegaard, Lagerlof & Rølla, 1988). The dissolution of the fluoride from the globules is pH dependant, presumably because the phosphate ions on the surface are released when protonated at low pH. By this mechanism fluoride is dissolved from the globules at the time fluoride is most needed (i.e., at a low pH). Various studies have shown that calcium fluoride is found in demineralized, porous tissue, more so than on sound enamel. Also the amount of fluoride that can be mobilized is decreased during an acid challenge, whereas at the same time the fluoride firmly bound in the lattice is increased. The acid cycle thus contributes to the conversion of loosely to firmly bound

fluoride, and consequently the reduction of the source of fluoride that can be mobilized.

2.4.2 Effect of Fluoride in Remineralization

The effect of fluoride on remineralization has received considerable attention during the past decades (ten Cate & van Loveren, 1999). A small amount of fluoride in the oral fluid (saliva, plaque) has been found to strongly promote remineralization of dentine and enamel, resulting in a shift from a net negative balance leading to caries to a positive balance where the tissue can be further mineralized, remineralized, or hypermineralized (Featherstone, 1994). The hyper-mineralization of dentine, evidenced by multiple radiodense bands within the lesion after the use of topical fluoride agents, was found *in vitro* and *in situ*, implying the mineral content and acid resistant exceeding that of sound dentine (Inaba & others, 1996; ten Cate & van Duinen, 1995). A dose response between the fluoride concentration and remineralization has been shown (ten Cate, Buijs & Damen, 1995). *In situ* studies also have demonstrated that fluoride treatments could shift the balance in a demineralizing environment to a condition of remineralization, not only for enamel but also for dentine (Kashani & others, 1998; Stephen, Damato & Strang, 1992; Sullivan & others, 1997; Wefel & Jensen, 1992).

Not only the aqueous fluoride, but also the incorporated fluoride account for the enhanced remineralization. The incorporation of fluoride into crystal with a resultant fluorohydroxyapatite of lower solubility than hydroxyapatite leads to a larger degree of supersaturation at a given calcium and phosphate level in saliva or plaque fluid. This thermodynamic driving force for precipitation determines the rate at which minerals precipitate (Wefel, 1994).

The natural repair of early lesions is also described as lesion remineralization and for many decades has become one of the cornerstones of the fluoride treatment strategy. It was first described by Head in 1909 when the author observed that the teeth become harder when placed in saliva and attributed this to saliva properties or carbon dioxide.

The chemistry, including the mechanism of enamel remineralization, was studied in detail by many investigators. Silverstone (Silverstone, 1983) did histomorphometric analyses of lesions after remineralization and concluded that the crystallites in the inner zone of the lesion (at the advancing lesion front) and in the surface layer had dimensions greater than the crystallites of sound enamel. This was the result of remineralization and indicated that these zones in the lesion apparently were favorable for remineralization and indicated that these zones in the lesion apparently were favorable for remineralization. Ten Cate & others (1982; 1983a; b; 1994; 1995) investigated mineral deposition from saliva-like remineralizing solutions on etched enamel and enamel lesions. They concluded that mineral is deposited as calcium hydroxyapatite, that the deposition is crystalline, and that remineralization was the result of regrowth of crystallites affected by the caries process. In addition, it was reported that the relative orientation of the crystallites in remineralized tissue was not as perfect as in sound enamel. Whereas in sound enamel crystallites are arranged in a parallel orientation, in remineralized lesions many crystallites were also seen in a random orientation. One of the consequences of this is that the mineral density after remineralization could never regain the value.

The mechanism of fluoride-enhancement of remineralization is as follows: fluoride can be incorporated in the crystal lattice of calcium hydroxyapatite. The resultant fluorohydroxyapatite has a lower solubility than hydroxyapatite. The result of this is that, at given calcium and phosphate level in saliva or plaque fluid, the degree of

supersaturation to fluorohydroxyapatite is larger than hydroxyapatite (ten Cate & van Loveren, 1999). This thermodynamic driving force for precipitation determines the rate at which minerals precipitate. The provision is obviously that the nucleus for precipitation, such as crystals already present or organic material that allows epitaxic growth, is present and the mineral ions needed for precipitation are available. In the case of an enamel lesion with narrow transport channels, diffusion of mineral ions is the rate-limiting step of enamel remineralization. In particular, with fluoride present, mineralization in the surface layer draws away mineral ions from the lesion pores and this affects the diffusion gradient. This is another explanation of why complete remineralization is difficult to achieve (ten Cate & Duijsters, 1982; ten Cate & van Loveren, 1999).

Many of the mechanistic studies have been made in simplified in vitro models, in which some of the complicating factors from saliva are excluded. One of these, salivary proteins forming salivary pellicle, need to be considered in this respect because for some of these proteins, crystallization-inhibiting properties have been reported. If these proteins bind to the crystallites in the outer regions of the lesion, this prevents the described surface blocking effect as a result of remineralization (ten Cate, 1994).

A similar mechanism of remineralization in enamel can occur in dentin. Supporting evidence of the mineralization properties of the dentinal tissue is the formation of secondary and tertiary dentin. However, these are processes of mineralization that are driven by the odontoblast cells that lay down the organic matrix, which is then filled in with apatite crystallites. In sclerotic dentin, a purely physicochemical crystallization takes place. In the advancing front of dentin caries the deposition of calcium phosphate crystals in the tubules has been reported. This contributes to the underlying tissue

becoming less accessible for invading bacteria, acids, and other metabolites. Thus, although the initial dentinal tissue is porous due to the tubular structure, this character is changed at a time when porosity is detrimental rather than advantageous (ten Cate, Damen & Buijs, 1998; ten Cate & van Loveren, 1999). Research on dentin has proved that it is not able to withstand the oral challenge. For that reason the tooth crown is covered with enamel. Nyvad, (Nyvad, 1993) concluded that the fluoride in conjunction with improved oral hygiene was able to convert active lesions to arrested lesions, and advises that an initial remineralization of the root surfaces affected offers a better starting point for a restorative treatment if such is requested for aesthetic reasons.

2.4.3 Effect of Fluoride on Tooth Morphology and the solubility of Tooth Structure

Fluoride uptake in tooth, in the loosely-bound form (calcium fluoride) and the firmly-bound form (apatitic fluoride), has been regarded as a marker of tooth resistance to caries (Caslavska, Moreno & Brudevold, 1975; DePaola, 1991).

The fluoride uptake into the apatite lattice as apatitic fluorides, resulting in a less soluble mineral than the original enamel apatite through the compositional and crystallographic alternations, has drawn considerable attention of dental researchers for many years (Caslavska, Moreno & Brudevold, 1975; DePaola, 1991; ten Cate, 1997).

The finding that caries-like lesions were developed when enamel was exposed to a liquid unsaturated with respect to hydroxyapatite and supersaturated with respect to fluorapatite (Larsen & Fejerskov, 1977) has indicated that the formation of fluorapatite could increase the caries resistance substantially.

The enamel resistance to lesion formation was found to be positively related with the firmly-bound fluoride content, while the loosely-bound fluoride was essentially absent (Takagi, Liao & Chow, 2000). In the experiment, the loosely-bound fluoride was

removed from the teeth after topical fluoride treatment. The mineral loss after a 5-day pH cycling process was reduced by 55% due to the formation of firmly-bound fluoride. When dicalcium phosphate dihydrate (DCPD) was adopted to increase the firmly-bound fluoride formation, the mineral loss was reduced by 77% (Takagi, Liao & Chow, 2000). It was demonstrated that a more acid-resistant surface of tooth hard tissues could result from the incorporation of firmly-bound fluoride (Driessens & others, 1980). In view of the long-lasting presence of firmly-bound fluoride in the tooth structures, firmly-bound fluoride was supposed to impart a long-term cariostatic effect (Takagi, Liao & Chow, 2000).

In addition to reducing solubility of tooth, during acid attacks the dissolved apatitic fluoride can also cause de novo prevention of demineralization, as evidenced by *in vitro* (Hoppenbrouwers & others, 1988; LeGeros & others, 1983) and *in vivo* (Ogaard & others, 1988a) studies. Apatitic fluoride, which could be released during the initial dissolution of mineral, may also serve as a reservoir of fluoride for the inhibition of acid production (Birkeland & Charlton, 1976; Harper & Loesche, 1986; Pearce, Hancock & Gallagher, 1984), especially under low pH conditions (White & Nancollas, 1990).

The cariostatic effect of loosely-bound fluoride was acknowledged later and has been well established in these several decades (Arends & others, 1983; Borsboom, vd Mei & Arends, 1985; Margolis, Moreno & Murphy, 1986).

When tooth hard tissues were exposed to high concentration fluoride application, CaF_2-like globules were formed on the surface and in intercrystal regions (Arends, Reintsema & Dijkman, 1988; Tsuda & others, 1993). In the oral environment, loosely-bound fluoride is easily dissolved and fluoride is released into the plaque and saliva, resulting in an slightly elevated and beneficial fluoride levels which may account for a

shift of the mineral uptake and loss pattern to favor overall remineralization (ten Cate, 1997; Wefel, 1990). In addition, calcium fluoride deposits may enhance root surface resistance by acting as a diffusion barrier (Caslavska, Moreno & Brudevold, 1975; DePaola, 1991; ten Cate, 1997). During demineralization, the released loosely-bound fluoride could also be incorporated into tooth crystal, to form apatitic fluoride (Wefel, 1990).

Although the loosely-bound fluoride could be washed away, resulting in the exponential decrease of fluoride levels in saliva and plaque after a topical fluoride application (ten Cate, 1997), the finding that loosely-bound fluoride tends to be released at the time it is most needed, namely during a cariogenic challenge, has stirred greater interest in the role of loosely-bound fluoride (ten Cate & van Loveren, 1999). In addition, CaF_2 was found to be less soluble and stay within the tooth surface for a long time in *in vivo* conditions (Caslavska & others, 1991; Lagerlof & others, 1988; ten Cate, 1997). The presence of CaF_2-like deposits in tooth may therefore act as a reservoir for fluoride to be mobilized into the underlying tooth surface (Wefel, 1990).

The effects on the solubility of enamel are well documented and have been, for many years, the cornerstone for the prevention of dental caries. Nevertheless, the previously mentioned experiment involving fluorapatite-containing shark enamel (Ogaard & others, 1988b) raised doubts regarding the importance of the fluoride-induced decreased solubility in terms of the overall effect of fluoride in caries prevention. This prompted experiments in which not fluoride in the apatite crystals, but fluoride in the ambient solution during a period of acid challenge, was investigated. Larsen (Larsen, 1974) showed that the presence of fluoride, more specifically a condition of

supersaturation with respect to fluorapatite, is crucial for the formation of a surface layer during enamel demineralization.

The mechanism of fluoride is twofold. Firstly, it binds calcium and phosphate dissolving, as a result of the acid penetration into the tissue and the resulting acid dissolution of the apatite. This reprecipitation prevents the mineral constituents of the enamel to be leached away into the plaque and saliva. Also, by this reprecipitation the pores of the tissue are narrowed, which, in turn, affects the diffusion of acid into the tissue and the efflux of dissolving ions. Ten Cate and Duijsters (1983a), performed a series of studies to investigate the fluoride in solution (between 0 and 10 ppm) in the pH range relevant for the occurrence of caries (pH 4 to 5). They showed that the amount of mineral loss during demineralization is a function of both pH and fluoride concentration. In particular, at the lower pH values studied the concentration of fluoride is an important determinant for the rate of mineral dissolution. The authors commented that the largest inhibition occurs at the fluoride concentration where the solution is supersaturated with respect to calcium fluoride and hypothesize that the different morphology of a calcium fluoride deposit in the lesion pores may result in a more effective inhibitor than the fluorapatite growing onto existing hydroxyapatite crystallites.

With the specimens from the study on pH and fluoride effects on demineralization subsequent analyses were performed to assess the mineral density profiles in the lesion. Microradiographic assessment of the lesions showed that the surface layer was a function of the fluoride concentration in the solution and that also the lesion depth was affected by fluoride, as was the overall mineral loss (ten Cate & Duijsters, 1983a).

There are many similarities in the structure of enamel and dentin from which it could be speculated that the effects of fluoride on demineralization of the two tissues should be similar; however, there are also many dissimilarities. These include the presence of a large proportion (about 35%) of organic matrix, which is composed mainly of collagen, and some structural aspects of apatite crystallites. Dentin contains about 5 vol% by weight of carbonate, which is double the amount for enamel and gives rise to a higher solubility (Featherstone, 1994).

The solubility is also influenced by the size of the crystallites, which are considerable smaller in the case of dentin than enamel. Smaller crystallites dissolve faster when placed in an undersaturated solution. The collagen fraction is the matrix onto which the apatite crystallites were precipitated during dentinogenesis. During demineralization, the apatite fraction is the first to be dissolved, only exposing the collagen after its dissolution. The collagen, while still present in the dentin, serves as a diffusion barrier slowing down demineralization, but it is also subject to denaturation, enzymatic degradation, and solubilization (Kleter & others, 1994; Klont & ten Cate, 1990; Klont, Damen & ten Cate, 1991). Once the matrix is removed it no longer can nucleate new apatitic crystals. Regarding the demineralization of dentin it should be remembered that not only is the solubility larger but also the amount of apatite in the sound tissue is small in comparison to enamel. At double the demineralization rate and half of the mineral content of sound dentin, this leads more rapidly to a condition of irreparable damage (i.e., no remineralization) of this substrate. Various experiments have shown that dentin demineralization is also partly inhibited by fluoride (ten Cate, Damen & Buijs, 1998) when it is supplied during short-term treatments or when continually present. Featherstone (Featherstone, 1994; 1999) reported a dose response between the fluoride concentration in the dentifrice and demineralization.

2.5 Fluoride Containing Tooth-Colored Restorative Materials

2.5.1 Conventional Glass Ionomers

The glass-ionomer cement has been used in dentistry for more than 3 decades and is now well established as a material with an important role in clinical dentistry. Wilson and Kent first reported its unique characteristics in 1972. The original glass-ionomer cement was far from an ideal material with poor setting characteristics, the clinical consequences of which extended beyond limited working time and delayed hardening, and rendered the cement, before it fully hardens, vulnerable to the effects of both moisture and desiccation. Therefore, glass ionomer cements has undergone continuous development, improvement and diversification. Glass ionomers are widely used in dentistry because of a variety of beneficial properties such as, chemical diffusion-based adhesion to enamel and dentin, fluoride release, biocompatibility with tooth structure, simple application, aesthetic appearance, acceptable abrasion resistance and capacity to be retained on unsupported enamel or non-undercut cavities (Mount, 1994). Conventional glass ionomer cement is based on an acid-base reaction derived from aqueous polymeric acids, such as poly acrylic acid homo polymer or acrylic/ itaconic/ maleic copolymers. The glass component is usually fluoro-alumino-silicate (Wilson & Kent, 1972) which is the "base" part of the reaction. The fluoride is released by the glass material for overtime (de Araujo & others, 1996). In the first 2 weeks there is a very high fluoride release, then it dissipates to a level of around 10% of the original level in 3-4 weeks, and remains at this level for 1 year or more (Berg, 1998).

Amounts of fluoride release in deionized water have been found to range between almost 100ppm to less than 10ppm at 28 days in some conventional GICs (Arends & Ruben, 1988; de Araujo & others, 1996). However, they can be "recharged" in the

presence of ambient fluoride, which can replenish the fluoride in the material (Forsten, 1991). GICs are the only dental materials with a true chemical bond to tooth structure (Hse & Wei, 1997). Bonding to hydroxyapatite occurs by the standard diffusion based adhesion system in as much, the polyalkenoic acid will soften the surface of the tooth structure and the chains will diffuse into the surface of the tooth, displacing calcium and phosphate ions. Both calcium ions and phosphate ions are displaced equally to maintain electrical neutrality. Then, displaced ions combine with the surface of the tissue and form an intermediate layer of new material (interdiffusion zone), which is firmly attached to tooth (Akinmade & Nicholson, 1995). It's also suggested to have some degree of bonding to collagen of the dentin, through either hydrogen bonding or metallic ion bridging between the carboxyl groups of the polyacid and the collagen molecules (Akinmade, 1994).

The introduction of high powder-to-liquid ratio glass ionomer materials, improved the compressive and flexural strengths, from 190 to 250 MPa and 30 to 45 MPa, respectively, it also provides a "condensable" feel, and this allows to be used in posterior teeth.

GICs also can be used as bases in conjunction with resin composites, and the "sandwich" or "laminate" technique (Li & Others, 1996; Davidson, Abdalla & De Gee, 1993). However, bond strength to the resin composite is limited by the low cohesive strength of the GICs (Li & Others, 1996), and this make the cements unsuitable for use in high-stress sites, such as Class I and Class II restorations.

Other indicated clinical applications are: Repair of defective restoration margins, restoration of root surfaces for overdentures, temporary restorations, lining/base under composite and amalgam, pit and fissure sealants, bonding of orthodontic brackets, bonding agent for composites, core build-up.

Some general contraindications are in stress-bearing areas such as large class II and IV cavities in permanent teeth, replacement of lost cusps and also in those large aesthetic areas such as class IV cavities.

Some examples of GICs are: Fuji II™ /-Caps™ (GC Corp), Glasionomer Fx™ (Shofu), Ketac – Molar Aplicap™ / – Molar Quick Aplicap™ /– Fil Plus Aplicap™ (3M ESPE).

2.5.2 Resin-modified Glass Ionomers

In recent years, developments in the field of glass ionomer cements have led to the introduction of hybrid versions of the material which can be light cured. They were introduced to help overcome the problems of moisture sensitivity and low early mechanical strengths associated with the conventional glass ionomer cements and at the same time maintain their clinical advantages. In these materials the fundamental acid-base curing reaction is supplemented by a second curing process, which is initiated by light. In their simplest form they are glass ionomer cements with the addition of small quantities of resin components (13 wt % resin to 87 wt % glass ionomer liquid) such as hydroxyethyl methacrylate (HEMA) or Bis-GMA. Some of the water component of the conventional glass ionomer cement is replaced by water / HEMA mixture (Wilson, 1990). In addition, there are traces of photoinitiators as well.

The setting reaction is said to be a dual mechanism. Hence, the acid-base reaction of true glass ionomer cements is supplemented by a polymerization reaction in these materials. The set material has two inter-penetrating matrices, *i.e.* the ionic matrix from the acid-base reaction and the polymerization from the free acid radical reaction (Wilson, 1990). The GIC component offers fluoride release, while the resin component offers strength and better esthetics than conventional GICs (Uno, Finger & Fritz, 1996; Berg, 1998). RM-GICs have a dual cure system, they are self-cured and also

photocured, because the glass can be silanized to allow an adherence of the glass within the resin matrix and also because of the addition of the photoinitiator.

The major disadvantages of RMGICs are the handling properties, because the material must be mixed, due to the reaction of GIC and the self-cured resin elements. Hence, these components must be separated. Therefore these materials are in both hand-mixed and capsulated versions.

RM-GICs possess higher measured bond strength than conventional GICs (Xie & Others, 2000). Their recent study showed that RMGICs were at least 200% higher in flexural strength and generally more than 60% higher in diametral tensile strength.

A true resin-modified glass ionomer cement material is therefore a two-part system which is characterized by an acid-base reaction critical to its cure, a diffusion-based adhesion between the tooth and the cement, and lastly, continuing fluoride release.

Some examples of RM-GICs are: Fuji II LC™, Fuji IX GP™ (GC Corp., Tokyo, Japan), Vitrebond™, Vitremer™ (3M), Photac-Fil Quick™/-Aplicap™ (3M ESPE).

2.5.3 Prereacted Glass Ionomer-Composites (Giomers)

Pre-reacted Glass Ionomer – Composites (PRG-C) are also known as Giomers.

These new generation of hybrid material are an anhydrous resin-based restorative that uses pre-reacted glass ionomer technology (Roberts & others, 1999). In the chemical composition, the material incorporates fillers that are produced from the complete or partial reaction of ion-leachable glasses with polyalkenoic acids. Basically, the fluoro-alumino silicate glass is reacted with polyacrylic acid prior to inclusion into the urethane resin (Yap & others, 2002). In the fully pre-reacted type (F-PRG), the remaining soft, siliceous hydrogel is freeze-dried, ball-mined, and silanized to form PRG fillers. Unreacted FASG particles, silica particles, and fumed silica are included

to optimize the physical properties of this material. Since PRG fillers are already pre-reacted, acidic monomers are not necessary for *in situ* acid-base reactions. A hydrophilic monomer, hydroxyethyl methacrylate (HEMA), is included with urethane dimethacrylate to produce a resin matrix that is conductive to water uptake and ion exchange. It is postulated that this PRG phase promotes sustained fluoride release via ligand exchanges within the ion-rich hydrogel, without disrupting the integrity of the filler-matrix interface that was speculated to occur in fluoride releasing resin-based materials such as compomers (Roberts & others, 1999; Tay & others, 2001a).

The manufacturer reports biocompatibility, fluoride release, fluoride recharge, clinical stability, smooth surface finish and excellent esthetics. Giomers are required to use a bonding system for adhesion to tooth structure. When using Reactmer™, the manufacturer bonding agent suggested is Reactmer Bond™, a glass ionomer based, tri-curable, all-in-one, filled adhesive that combines etching, priming and bonding. It consists of two components, which must be hand-mixed prior to application. It is left for 20 seconds on the tooth surface, air thinned and subsequently light-polymerized for 20 seconds prior to the placement of Reactmer. Manufacturer indications include restorations for root-caries, non-carious cervical lesions and class V cavities in permanent teeth and all classes of deciduous teeth. In addition to conventional FASG fillers, Reactmer Bond utilizes a novel filler material known as fully pre-reacted glass polyalkenoate fillers (F-PRG) to enhance the sustained fluoride releasing and recharging potential of the material. These fillers are formed by the complete reaction of FASG glass with polyalkenoic acids in the presence of water to form a wet siliceous hydrogel. Upon freeze-drying, the dissicated 'xerogel' is further milled and silane-treated to form F-PRG fillers of a specific size range. It was proposed that of the use of

F-PRG fillers promotes rapid fluoride release through a ligand exchange within the pre-reacted hydrogel (Tay & others, 2001b).

There's still little data regarding the physical properties of these materials in the literature. However, a recent study compared the shear bond strength of a PRG-C (Reactmer Bond/Reactmer Paste) and a Compomer (Clicker/F2000) (Miyazaki & others, 2001). They found mainly cohesive failure in the bonding agent with no significant difference between both materials. A recent TEM study (Tay & others, 2001b), evaluated the existence of the GI phase on different fluoride release materials and concluded that the variable extent of the GI phase is determined by differences in the resin composition of the restoratives. However, as the initial *in vitro* trials of new or experimental materials do not always reveal their full limitations or assets, clinical data is essential to prove the success of these materials.

Some examples of PRG-Cs are: Reactmer™ used in combination of Reactmer Bond™ (Shofu) and Beautifil™ used in combination with FL-Bond™ (Shofu).

2.5.4 Polyacid Modified Composites (Compomers)

Polyacid-Modified Composites (PAM-C) are also known as Compomers, these materials are essentially resin composites, the main difference is that the resin monomers are modified to contain acidic functional groups, capable of participating in an acid/base glass ionomer reaction after the polymerization of the resin molecule has taken place (Berg, 1998).

The photo-initiated polymerization is the only setting reaction in PAM-C, (Meyer & others, 1998) although a limited acid-base reaction takes place upon water absorption. This reaction results in a fluoride release, but not involved in the hardening process of the material (McCabe, 1998). Compomers does not have inherent adhesion to tooth

structure, it is essential to use a bonding agent, although it has been demonstrated that this significantly decreases the fluoride release (Castro, Gray, Buikema, 1994; McKnight-Hanes & Whitford, 1992). Meyer & Others (1998) have shown that the diffusibility differs significantly among RMGICs, PAM-C, and Resin Composites. The authors suggested that different formulations of bonding agents exhibit different permeabilities. A recent study suggests that the bonding agent acts like a barrier impeding the diffusion of compomers such as water and fluoride into and out of the PAM-C respectively (Vercruysse, De Maeyer & Verbeeck, 2001). Moreover, Itota & others (2001) suggested that the type of adhesive used with compomers might play a major role in fluoride release. The authors finally concluded that applying an adhesive without Bis-GMA resin to compomer restoration will not have a suppressive effect on the fluoride release and may help to overcome the problem of fluoride release. However, it should not be expected that compomers would provide properties similar as that of glass ionomers (Mount, 2002). Therefore it is suggested that the compomers be used carefully, with an understanding of their limitations, in as much as they show all the inherent problems associated with composite resins.

Some examples of PAM-Cs are: Dyract™/-AP™, Dyract Flow™, (Dentsply), Hytac (3M ESPE), Compoglass F™/-Flow™ (Ivoclar Vivadent), F2000™ (3M).

2.5.5 Fluoride Releasing Composites/Resins

Traditionally, composite resin materials are manufactured with four major components: organic polymer matrix, inorganic filler particles, coupling agent and the initiator-accelerator system. The organic polymer matrix in most composites is an aromatic or urethane diacrylate oligomer. The inorganic filler particles may consist of

several inorganic materials such as quartz, glass and/or colloidal silica. The coupling agent is a silane, which contains functional groups that can hydrolyze and react with the inorganic filler. Initiator-accelerator systems allow self-curing, light curing and dual curing.

The two most common oligomers used in composite resin materials are Bis-GMA (2,2-bis[4(2-hydroxy-3 methacryloyloxy-propyloxy)-phenyl] propane and urethane dimethacrylate (UDMA).

Dental composites can be classified by the particle size, shape, and distribution of filler. Early composite resins contained large spherical particles (20 to 30 μm), followed by large irregularly shaped particles, microfine particles (0.04 to 0.2 μm), fine particles (0.4 to 3 μm), and finally blends (microhybrids) containing mostly fine particles with some microfine particles. Based on the type of filler particles, composites are classified as microhybrid and microfilled products.

Microhybrid composites contains irregularly shaped glass or quartz of fairly uniform diameter plus microfine filler (5% to 15%) and may contain filler 60% by volume and 72% to 80% by weight. Microfilled CR may contain 40% to 70% filler by volume and approximately 77% to 84% by weight.

Traditional composites were initially non-fluoride releasing restorative materials. In recent years, composite resins have been formulated to release fluoride (Arends & Ruben, 1988; Strother & others, 1998; Swift, 1989; Young & others, 1996). Cariostatic effect in dental substrates by fluoride uptake of fluoride releasing composites have been shown in several previous studies (Arends, Ruben & Dijkman, 1990; Jensen, Wefel & Hammesfahr, 1991; Zimmerman, Rawls & Querens, 1984). However, fluoride is released in lesser amounts than glass ionomers (Fortin & Vargas, 2000) and the fluoridated composite resins did not consistently create inhibition zones in adjacent

dentin. The presence of a smear layer and the pH seemed to have an affect on the presence of an inhibition zone (Segura, Donly & Quackenbush, 2000).

A bonding agent to achieve bond strength is required. A mechanical interlocking is achieved by flowing the water-tolerant primer into the surface of the dentin where it penetrates the spaces in the networked structure of the collagen, created by acid etch (Berg, 1998).

Hybrids are suitable for anterior and posterior restorations due to the polishability of the microfiller size and durability of larger particle size. This property gives the most esthetically desirable material. Mechanical properties of hybrid composites such as compressive, flexural and tensile strengths are excellent.

The major disadvantages of resin composites other than the low fluoride release, includes shrinkage due to polymerization, which is higher in flowable composites due to the filler content, and perhaps can be lowered when using lower intensity lights. However, shrinkage of the material will compromise the marginal integrity forming gaps, which we can assume these gaps to have some leakage and the latter, resulting in secondary caries formation depending upon the population of origin (Tyas & others, 2000). Another disadvantage is that composite resins are less susceptible to fluoride recharge due to the complexity of the resin-based matrix. Some examples of fluoride releasing composites were: FluorEver™ (MacroChem Corp), Heliomolar Radiopaque™ (Vivadent Inc).

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3 METHODS AND MATERIALS

3.1 Materials Selection

The materials evaluated in this study represent the entire continuum of direct aesthetic restorative materials currently available to the dental practitioner and are summarized in Tables 3-1 and 3-2. They included a conventional glass ionomer cement (Fuji II Caps [FJ]), a resin-modified glass ionomer cement (Fuji II LC [FL]), a giomer (Reactmer [RM]), a compomer (Dyract AP [DY]), and a non-fluoride releasing composite resin (Spectrum TPH [SP]) control group.

3.2 Sample Preparation and Restorative Material Placement

Seventy-five freshly extracted human third molars were randomly divided into 5 groups of 30 teeth. The teeth were free from caries, structural defects and extraction flaws (assessed under a stereomicroscope [Olympus SZ40, Tokyo, Japan] at 10x magnification). Immediately following extraction, the teeth were placed in 10% formaline-saline solution for 10 minutes, cleaned and stored in distilled water at 4°C.

Buccal and palatal/lingual class V cavities (2mm deep, 4mm long [mesio-distal] and 3mm wide [occlusal-gingival]) were carefully prepared at the cemento enamel junction (half above and half below the CEJ) of each tooth by a single operator. The cavities were prepared by means of a high-speed hand-piece with a #330 carbide bur and #1311 diamond bur (Shofu, Kyoto, Japan) under water spray. Standardization of the cavities was ensured by measurement with a digital caliper (Fowler Ultra-cal Mark III, Sylvac, Sweden). Conditioners, coating and priming materials were thoroughly mixed prior to use as indicated in the manufacturer's instructions. One hundred and fifty cavities were restored with a one-increment procedure following manufacturer's

instructions (Table 3-3) with the aid of transparent cervical matrices (Hawe-Neos Dental 721; Gentilino, Switzerland). Materials were placed in subsequent order within a 5-day interval starting with FJ, FL, RM, DY and SP. Date of placement for each material was recorded and strictly followed up. Placement protocols for the different materials are summarized in Table 3-3. Cervical matrices were replaced after every 15 restorations. All restorations were grossly finished 10 minutes after placement using a high-speed hand-piece and eight flute tungsten carbide burs (Robot Carbide SH134; Shofu, Kyoto, Japan) under water spray. Finishing burs were replaced after every 15 restorations. FJ and FL restorations were covered with a layer of unfilled resin (FujiCoat) and light-cured for 15 seconds after gross finishing. The restored teeth were then stored in distilled water at 37°C for 1 week and finished/polished with 10 strokes of coarse, medium, fine and extra-fine Sof-lex discs (3M Dental Products, St. Paul, MN 55144, USA), at 10,000 rpm. Special care was taken to ensure that no overhangs were present at the margin of the restorations on both enamel and dentin substrates. After treatment with Sof-lex discs, the restored teeth were returned to distilled water at 37°C for an additional week.

The crowns of the restored teeth were separated from the roots and bisected mesio-distally using a diamond impregnated disc with an alloy grinder motor (DEMCO, Dental Maintenance Co., Inc. Bonsall, California, U.S.A). This resulted in one hundred and fifty samples corresponding to the five groups of materials (n=30).

The tooth fragments were coated with two layers of acid-resistant nail varnish (Max Factor, Procter and Gamble, Surrey, UK), except for a zone approximately 1mm wide around the restorations. The restorations in each group were then randomly divided into 2 groups of 75. The first group was subjected to artificial caries challenge (pH cycling), while the remaining half was stored in distilled water at 37°C for an

additional five months and two weeks more (total of six months after placement) before pH cycling. The pH cycling protocol will be described in detail in section 3.3.

3.3 Artificial Caries Challenge

Each group of specimen restorations (*n*=15), was exposed to a 3-day cyclic treatment regime which produced artificial recurrent caries around restorations. Specimens were immersed in demineralizing & remineralizing solutions. The demineralizing solution used contained acetic acid buffer with 2.2 mM calcium ($CaCl_2$), 2.2 mM phosphate ($NaH_2\ PO_4$), 0.05 M acetic acid. The remineralizing solution used contained 1.5 mM of calcium, 0.9 mM of phosphate, 0.15 M KCl (Damato, Strang & others, 1988). The pH of the demineralizing solution was adjusted to 5.0 using KOH (Konishi, Fried & others, 1999) and the pH of the remineralizing solution was carefully adjusted to 7.0 using KOH (Hsu, Jordan & others, 2000). Each group of specimens were immersed in demineralizing solution for 18 hours at 37°C, removed, washed with distilled water for 5 minutes and immersed in remineralizing solution within another vial for 6 hours at 37°C. All solutions were constantly stirred at 132 RPM with a Data Plate Hot Plate / Stirrer [Model 735, Barnstead|Thermolyne, Dubuque, Iowa, USA] (Hsu & others, 2000). The pH cycling process started with demineralizing solution. Solutions were renewed after each cycle.

3.4 Lesion Measurement and Data Collection

Longitudinal sections of 130±20μm in thickness were obtained with a Silverstone-Taylor hard-tissue microtome (Series 1000 Deluxe, Sci Fab, Colorado, USA) with a diamond-wafering blade of 7.6cm diameter and 0.15mm thickness (Series 15 LC Diamond, Buehler, Illinois, USA). The enamel/dentin margins were photographed in an imbibition media of distilled water with a digital color video camera (Sony

ExwaveHAD SSC-DC58AP, Sony Co.,Tokyo, Japan) attached to a polarized light microscope (Olympus BX51, Tokyo, Japan). Photomicrographs were traced with an image analysis software (Microimage v4.0; Olympus Optical Co. Europa GMBH, Hamburg, Germany) and outer lesion depths [OLD] / wall area [WA] lesion or inhibition were measured according to Hsu & others (1998). We determined the average depth of each outer lesion by measuring a lesion area of 200µm in length and dividing it by 200. The wall lesion area or inhibition area was defined by a 100µm area adjacent to the restoration and three peripheral lines: (1) the vertical line representing the cavity wall or the tooth restoration interface; (2) the inner border of the demineralization area curving up, axially, to meet the cavity wall; and (3) the imaginary line following the horizontal portion of the inner border of the demineralization area and extending straight to the cavity wall at 90° (Figure 3-1) (Hsu, Donly & others, 1998). The same protocol was followed for restorations that were aged for 6 months.

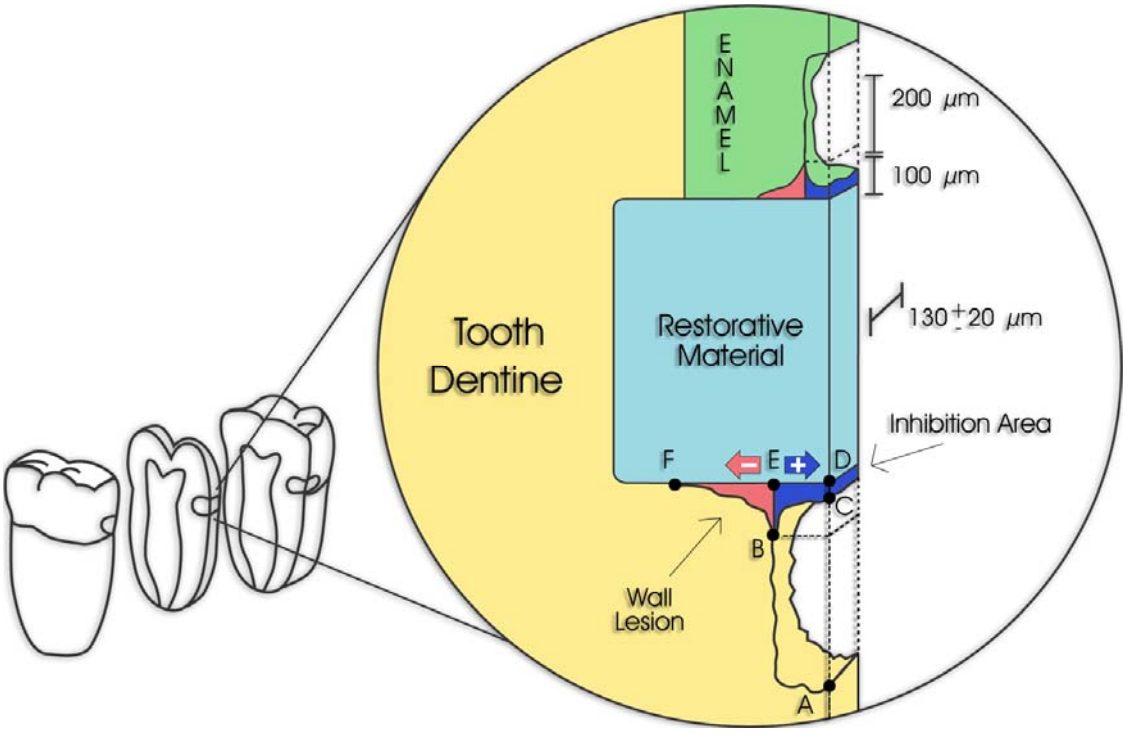

Figure 3-1. Lesion measurement and data collection.

OLD was determined by measuring a lesion area of 200µm in length and dividing it by 200. WA inhibition/lesion was computed based on a 100µm area adjacent to the restoration margins. Three major patterns of demineralization along cavity walls evaluated with polarized light microscopy in both enamel and dentin. Three types of lesions along the cavity wall can be categorized based on three different directions that the inner border of demineralization may take toward the cavity wall (DEF line). If the inner border of demineralization curved upward (ABC line), the effect was an "inhibition" represented by the BCDE area with a positive value (+). If the inner border of the demineralization extended straight to the cavity wall at 90° (ABE line), the lesion exhibited "no effect". If the inner border of demineralization curved downward (ABF line), the effect was a "lesion" represented by the BEF triangular area with a negative value (-).

3.5 Statistical Analysis

All data were subjected to statistical analysis at a significance level of 0.05. Paired Samples T-Test was used to compare OLD & WA values between tooth tissues and aging periods. One-way ANOVA and Scheffe's post-hoc tests were performed to compare mean OLD and WA values between materials. Non-parametric Kruskall-Wallis test and Mann Whitney U tests were used to compare wall lesion patterns between tissues and materials.

References

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Hsu CY, Jordan TH, Dederich DN & Wefel JS (2000) Effects of low-energy CO2 laser irradiation and the organic matrix on inhibition of enamel demineralization. *Journal of dental research* **79(9)** 1725-1730.

Konishi N, Fried D, Staninec M & Featherstone JD (1999) Artificial caries removal and inhibition of artificial secondary caries by pulsed CO2 laser irradiation. *American Journal of Dentistry* **12(5)** 213-216.

Table 3-1. Technical profiles and manufacturers of the materials evaluated.

Material	Manufacturer	Chemical Composition		Color	Lot no.
Fuji II Capsule	GC Corp. Tokyo, Japan	**Powder:**	**Liquid:**	Shade A2	0007186
		FASG PAA	PAA TA Water		
Fuji II LC Capsule	GC Corp. Tokyo, Japan	**Powder:**	**Liquid:**	Shade A2	9912202
		FASG Pigments	PAA Water HEMA CQ		
Reactmer Paste	Shofu Inc., Kyoto, Japan	**Resin:**	**Fillers:**	Shade A2	100102
		FASG UDMA HEMA Photo Initiator	F-PRG, Silica, Aerosil silica, Glass fillers		
Dyract AP	Dentsply-De Trey, Konstanz, Germany	**Resin:**	**Fillers:**	Shade A2	0003001521
		UDMA TCB	Strontium-fluoro-Silicate glass		
Spectrum TPH	Dentsply-De Trey, Konstanz, Germany	**Resin:**	**Fillers:**	Shade A2	0006000747
		BisGMA-adduct Bis-EMA TEGDMA	Bariumaluminum-Borosilicate, Silica		

CQ: Camphorquinone
Bis-EMA: Ethoxylated bisphenol-A-glycidil-methacrylate
BisGMA: Bisphenol-A-glycidyl-metacrylate
BisGMA -adduct: Adduct of 2,2-Bis[4-2-hydroxy-3-methacry loyloxpropoxy)-phenyl]propane with hexamethylene diisocyanate
FASG: Fluoroaluminosilicate-glass
HEMA: Hydroxyetylmetacrylate
TA : Tartaric Acid
PAA: Poly Acrylic Acid
TEGDMA: triethylene glycol dimethacrylate
TCB: Reaction product butane tetracarboxylic acid and HEMA
UDMA: Urethane dimethacrylate

Table 3-2. Technical profiles and manufacturers of the bonding/coating agents.

Material	Manufacturer	Chemical Composition	Lot no.
GC Cavity Conditioner	GC Corp. Tokyo, Japan	20% PAA 3% Aluminum Chloride Hexahydrate	0006301
GC Fuji Coat LC	GC Corp. Tokyo, Japan	Methyl metacrylate	9911021
Reactmer Bond A & **B**	Shofu Inc., Kyoto, Japan	F-PRG FASG NI Water Acetone 4-AET 4-AETA HEMA UDMA Photo Initiator	0901
Conditioner 36	Dentsply-De Trey, Konstanz, Germany	Phosphoric Acid 36%	9904000621
Prime & Bond NT	Dentsply-De Trey, Konstanz, Germany PENTA	PENTA UDMA Nano F Acetone *Resins	0001000767

4-AET: 4-Acryloxyethyltrimellitic acid
4-AETA: 4-Acryloxyethyltrimellitate anhydride
FASG: Fluoroaluminosilicate-glass
F-PRG: Full reaction type pre-reacted glass-ionomer filler
HEMA: Hydroxyetylmetacrylate
Nano F: Nano-filler initiators
NI: New Initiators
PENTA: phosphonated penta-acrylate ester
PAA: Poly Acrylic Acid
*Resins: Resin R5-62-1, T-resin, D-resin
UDMA: Urethane dimethacrylate

Table 3-3. Tooth restoration procedure.

Fuji II Material 1	Fuji II LC Material 2	Reactmer Material 3	Dyract AP Material 4	Spectrum TPH Material 5
GC Cavity Conditioner (10 seconds) ↓ Rinse (5 seconds) & Dry (3 seconds) ↓ Fuji II Caps ↓ ST (4 minutes) ↓ Gross finishing w/ Tungsten carbide 8 fluted bur ↓ GC Fuji Coat ↓ Light Cure 15 seconds ↓ Stored 1 week in distilled water** ↓ Finish/Polish (w/Sof-lex*) ↓ Stored 1 week in distilled water**	GC Cavity Conditioner (10 seconds) ↓ Rinse (5 seconds) & Dry (3 seconds) ↓ Fuji II LC Caps ↓ Light Cure 20 seconds ↓ Gross finishing w/ Tungsten carbide 8 fluted bur ↓ GC Fuji Coat ↓ Light Cure 15 seconds ↓ Stored 1 week in distilled water** ↓ Finish/Polish (w/Sof-lex*) ↓ Stored 1 week in distilled water**	Reactmer Bond (20 seconds) ↓ Dry 3 seconds ↓ Light Cure 20 seconds ↓ Reactmer Paste ↓ Light Cure 30 seconds ↓ Gross finishing w/ Tungsten carbide 8 fluted bur ↓ Stored 1 week in distilled water** ↓ Finish/Polish (w/Sof-lex*) ↓ Stored 1 week in distilled water**	Conditioner 36 (20 seconds) ↓ Rinse (20 seconds) & Dry (3 seconds) ↓ Prime & Bond NT (20 seconds) ↓ Dry 5 seconds ↓ Light Cure 10 seconds ↓ Dyract AP ↓ Light Cure 40 seconds ↓ Gross finishing w/ Tungsten carbide 8 fluted bur ↓ Stored 1 week in distilled water** ↓ Finish/Polish (w/Sof-lex*) ↓ Stored 1 week in distilled water**	Conditioner 36 (20 seconds) ↓ Rinse (20 seconds) & Dry (3 seconds) ↓ Prime & Bond NT (20 seconds) ↓ Dry 5 seconds ↓ Light Cure 10 seconds ↓ Spectrum TPH ↓ Light Cure 20 seconds ↓ Gross finishing w/ Tungsten carbide 8 fluted bur ↓ Stored 1 week in distilled water** ↓ Finish/Polish (w/Sof-lex*) ↓ Stored 1 week in distilled water**
*3M Dental Products, St. Paul, MN 55144, ** 10 ml with distilled water at 37°C, changed every 24 hours. Above instructions were obtained from manufacturer booklets. ST=Setting Time.				

4 RESULTS

The means and standard deviations of the outer lesion depths and wall lesion/inhibition areas (positive values indicates wall inhibition, negative values indicates wall lesion) in enamel and dentin are summarized in Tables 4-1 and 4-2. The statistical comparison of means of outer lesion depth and wall inhibition area between tissues and materials are summarized in Tables 4-3 and 4-4. Frequencies of wall lesion/inhibition patterns are reflected in Tables 4-5 and 4-6. The comparisons of wall area patterns are summarized in Tables 4-7 and 4-8. Time comparison of outer lesion depth in μm and wall inhibition in μm^2 between tissues and materials are summarized in Table 4-9. Time comparison of frequencies of wall area patterns are summarized in Table 4-10.

4.1 Histological Features of Demineralization Lesions

In the present study, three major types of demineralization patterns were observed. They have been previously described by Hsu & Others (1998) and are summarized in Figure 3-1. Figures 4-1a to h and 4-2a to h show typical photomicrographs of enamel and dentin lesions associated with the various materials.

Ideally for outer lesions, the acidic artificial caries solution will penetrate freely and evenly into tooth structure, dissolve the basic tooth structure crystal units of hydroxyapatite (HAP) and leave a demineralized area with a rectangular shape on the outer tooth surface away from the cavity wall. It is believed that the stronger the acid attack, the deeper the outer lesion and thus the greater the lesion depth. In the same way, the greater the concentration of cariostatic fluoride ions released from adjacent restorative materials, the shallower the outer lesion and the smaller the outer lesion depth. An interesting observation was that enamel outer lesions were non-erosive

compared with dentin outer lesions, which were frequently erosive in nature (Figures 4-1a with Figures 4-1b).

If no microleakage is presented adjacent to the cavity wall, the demineralized area along the cavity wall should follow the same pattern as the outer surface. If this happens to be the case, we assigned this type of lesion an outer lesion with wall inhibition area. The latter occurred in the majority of cases in enamel/dentin adjacent to glass ionomer restorations. Figure 4-1a demonstrates the typical histological photomicrograph of enamel outer lesion with inhibition area. The inner border of demineralization area extends upward, vertically, toward the junction of the restoration and enamel/dentin on the tooth surface, forming a rectangular outline of lesion area.

In the presence of microleakage, the microspace allows the acid attack to take place in the gap between the cavity wall and restoration. Therefore, the demineralization area along the cavity wall extends downward axially along the microspace to meet the cavity wall. This type of lesion was defined as a wall lesion. Figures 4-1g and h shows the typical histological photomicrograph of enamel/dentin wall lesion with outer lesion respectively. Dyract and Spectrum TPH restorations demonstrated this type of lesion in most of their specimens.

With the presence of fluoride ions in the tooth/restoration interface released by the restorative material, an inhibitory effect of the demineralization process can be noticed along the cavity wall. Figures 4-1a to d and 4-2a to d shows the typical photomicrographs of enamel/dentin outer lesion with wall inhibition area, where the inner border of the demineralization area extends horizontally from the distal and middle portions of the lesion and then curves up to meet the tooth restoration interface. The enamel wall inhibition area was noted to be smaller than those in dentin lesions (Figures 4-1a and c with Figures 4-1b and d; and Figures 4-2a and c with Figures 4-2b

and d). All of FJ and FL and 90% of RM restorations demonstrated this feature (Tables 4-5 and 4-6). In addition, the typical erosive demineralization photomicrographs in dentin are illustrated in Figures 4-1b, d, f and h and 4-2b, d, f and h.

Due to their different organic and inorganic constituents and structure, the enamel and dentin usually show different manifestations of demineralization under the same environmental conditions, such as concentration of fluoride in solution and pH. Figure 4-1f shows a typical result of the dentin outer demineralization area with wall lesion or inhibition. The depth of the demineralization area along the cavity wall is no more than that on the dentin outer surface. This type of lesion was defined as the “no effect” lesion. In this type of lesion, it is possible to presume that a balance between the cariostatic agent (fluoride) in the ambience and the tooth structure took place.

The existence of microleakage may result in the enhanced demineralization of the cavity wall. Figures 4-1e, g and h and 4-2e to h illustrate the typical photomicrographs of a enamel/dentin outer lesion with a wall lesion. The wall lesion area was defined in our study by three peripheral lines as previously mentioned in Chapter 3. Specimens restored with SP and FJ showed 100% enamel/dentin wall lesion and inhibition respectively at both 2 weeks and 6 months. Specimens restored with FL and RM showed a mixture of wall inhibitions and “no effects” at 2 weeks, while at 6 months FL and RM showed a 100% enamel/dentin wall inhibition. Specimens restored with DY showed a mixture of enamel and dentin wall lesion/inhibition and “no effect” at 2 weeks and 6 months.

4.2 Outer Lesion

4.2.1 Material Effect

For the various materials, OLD ranged from 54.55 to 65.86 μm and 124.68 to 145.97 μm in enamel and dentin respectively at 2 weeks, while at 6 months OLD ranged from 54.06 to 59.53 μm and 112.99 to 166.27 μm in enamel and dentin respectively. Comparison of means between tissues revealed that the dentin had significantly greater outer lesion depth for all materials compared to enamel ($p<0.05$) (Tables 4-3 and 4-4). At 2 weeks no significant difference in enamel OLD was observed. At 6 weeks significant difference in enamel OLD was observed as follows: FJ, RM, SP > FL. Significant difference in dentin OLD was observed at 2 weeks and 6 months. At both time intervals SP > FJ, FL and RM.

4.2.2 Aging Effect

The six-month aging process produced a variable effect on demineralization of all groups (Table 4-9). On the enamel outer surfaces, FL had shallower lesions after aging. Aging had no significant effect on the enamel surfaces for FJ, RM, DY and SP. On the dentin outer surfaces, the FL, RM and DY had shallower lesions and the SP group had deeper lesions after aging. The FJ group exhibited no significant changes in OLD after aging.

4.3 Wall Area

Demineralization (lesion), inhibition or no effect can take place at the wall area. Tooth sample sections in the SP group never had wall inhibition areas; while the FJ, FL and RM never revealed wall lesions. The DY group showed a mixture of all three types of

lesions using our experimental conditions. In this study, WA increase in the wall lesion area and the wall inhibition area were observed with aging.

Regarding the WA material effect, positive values (+) indicates inhibition, while (-) negative values indicates lesion. In enamel WA ranged from –2356.13 to 1398.20 μm^2 and –3011.73 to 5095.80 μm^2 in enamel and dentin respectively at 2 weeks, while at 6 months WA ranged from –1604.53 to 1915.23 μm^2 and –3444.27 to 2653.87 μm^2 in enamel and dentin respectively. Comparison of means between tissues revealed that for all materials the dentin had significantly greater WA inhibition than enamel ($p<0.05$). However for SP, greater WA inhibition occurred in enamel than dentin (Tables 4-3 and 4-4). At 2 weeks significant difference in enamel WA inhibition was observed as follows: FJ, FL, RM > DY, SP, while in dentin WA inhibition was observed as follows: FJ > FL > RM > DY > SP. At 6 months significant difference in WA inhibition was equally observed in both tissues as follows: FJ > FL, RM > DY > SP.

4.3.1 Wall Inhibition Areas

The results of the present study revealed that in enamel, FJ, DY and SP had greater inhibition effect after aging, while FL and RM had no significant differences. In dentin, FJ and FL had smaller inhibition areas after aging, while RM, DY and SP had no significant differences (Table 4-9).

4.3.2 Wall Lesion Area

As previously mentioned in Section 4.3, FJ, FL and RM never showed a wall lesion. For DY and SP no significant differences in dentin WA lesion were observed between time intervals. Significant difference in enamel WA lesion was however observed in DY, which shown less lesion patterns after aging (Table 4-10).

Table 4-1. Means of outer lesion depths (OLD) and wall lesion/inhibition area (WA) of the various materials at 2 weeks.

Material	OLD - µm(SD)		WA - μm^2(SD)	
	Enamel	Dentin	Enamel	Dentin
Fuji II	54.99(6.83)	128.81(15.36)	1398.20(505.20)	5095.80(1574.64)
Fuji II LC	54.55(10.66)	124.68(10.20)	1283.73(636.43)	2903.33(598.19)
Reactmer	56.04(12.96)	129.02(15.50)	689.53(460.24)	1691.53(1128.13)
Dyract	58.30(12.53)	134.35(18.32)	-1399.33(1508.89)	-327.20(549.50)
Spectrum TPH	65.86(16.87)	145.97(14.67)	-2356.13(968.46)	-3011.73(566.84)

Standard Deviation in parentheses. For WA, positive values (+) indicates wall inhibition, negative values (-) indicates wall lesion.

Table 4-2. Means of outer lesion depths (OLD) and wall lesion/inhibition areas (WA) of the various materials at 6 months.

Material	OLD - µm(SD)		WA - μm^2(SD)	
	Enamel	Dentin	Enamel	Dentin
Fuji II	58.16(10.05)	128.29(14.76)	1915.23(415.96)	2653.87(353.62)
Fuji II LC	43.40(8.90)	112.99(10.02)	984.36(288.86)	1941.60(256.08)
Reactmer	59.53(14.18)	118.33(8.64)	875.07(145.72)	1787.07(478.12)
Dyract AP	54.06(12.19)	120.26(10.52)	-558.68(536.53)	-357.47(896.09)
Spectrum TPH	58.45(11.49)	166.27(20.46)	-1604.53(338.43)	-3444.27(837.47)

Standard Deviation in parentheses. For WA, positive values (+) indicates wall inhibition, negative values (-) indicates wall lesion.

Table 4-3. Comparison of means (OLD & WA) between tissues and materials at 2 weeks.

Variable		OLD - µm	WA Inhibition - μm^2
Materials*	FJ, FL, RM, DY	Dentin > Enamel	Dentin > Enamel
	SP	Dentin > Enamel	Enamel > Dentin
Tissues**	Enamel	NS	FJ, FL, RM > DY, SP
	Dentin	SP > FJ, FL, RM	FJ > FL > RM > DY > SP

Results of Paired Samples T-Test*, and one-way ANOVA/post-hoc Scheffe's tests ** ($p<0.05$). > Indicates significantly greater OLD lesion depths and significantly greater WA inhibition. NS indicates no statistical significance between materials. FJ= Fuji II; FL= Fuji II LC; RM= Reactmer; DY= Dyract AP; SP=Spectrum TPH.

Table 4-4. Comparison of means (OLD & WA) between tissues and materials at 6 months.

Variable		OLD - µm	WA Inhibition - µm^2
Materials*	FJ, FL, RM, DY	Dentin > Enamel	Dentin > Enamel
	SP	Dentin > Enamel	Enamel > Dentin
Tissues**	Enamel	FJ, RM, SP > FL	FJ > FL, RM > DY > SP
	Dentin	SP > FJ, FL, RM, DY	FJ > FL, RM > DY > SP

Results of Paired Samples T-Test*, and one-way ANOVA/post-hoc Scheffe's tests ** ($p<0.05$). > Indicates significantly greater OLD lesion depths and significantly greater WA inhibition. NS indicates no statistical significance between materials. FJ= Fuji II; FL= Fuji II LC; RM= Reactmer; DY= Dyract AP; SP=Spectrum TPH.

Table 4-5. Frequency of wall lesion/inhibition patterns at 2 weeks.

Tissue	Material	Frequencies			Total
		Inhibition	No Effect	Lesion	
Enamel	Fuji II	15	0	0	15
	Fuji II LC	13	2	0	15
	Reactmer	12	3	0	15
	Dyract	0	5	10	15
	Spectrum TPH	0	0	15	15
	Total	40	10	25	75
Dentin	Fuji II	15	0	0	15
	Fuji II LC	15	0	0	15
	Reactmer	12	3	0	15
	Dyract	1	9	5	15
	Spectrum TPH	0	0	15	15
	Total	43	12	20	75

Results of descriptive statistics – crosstabs.

Table 4-6. Frequency of wall lesion/inhibition patterns at 6 months.

Tissue	Material	Frequencies			Total
		Inhibition	No Effect	Lesion	
Enamel	Fuji II	15	0	0	15
	Fuji II LC	15	0	0	15
	Reactmer	15	0	0	15
	Dyract AP	3	0	12	15
	Spectrum TPH	0	0	15	15
	Total	48	0	27	75
Dentin	Fuji II	15	0	0	15
	Fuji II LC	15	0	0	15
	Reactmer	15	0	0	15
	Dyract AP	5	2	8	15
	Spectrum TPH	0	0	15	15
	Total	50	2	23	75

Results of descriptive statistics - crosstabs.

Table 4-7. Comparison of wall area patterns at 2 weeks.

Variable	WA Patterns	
Materials	All	Dentin > Enamel
Tissues	Enamel	SP, DY > FJ, FL, RM
	Dentin	SP > DY > FJ, FL, RM

Results of Kruskall Wallis and Mann Whitney U tests ($p<0.05$). > Indicates significantly higher frequency of wall lesions. FJ= Fuji II; FL= Fuji II LC; RM= Reactmer; DY= Dyract AP; SP=Spectrum TPH.

Table 4-8. Comparison of wall area patterns at 6 months.

Variable	WA Patterns	
Materials	All	Dentin > Enamel
Tissues	Enamel	SP, DY > FJ, FL, RM
	Dentin	SP > DY > FJ, FL, RM

Results of Kruskall Wallis and Mann Whitney U tests ($p<0.05$). > Indicates significantly higher frequency of wall lesions. FJ= Fuji II; FL= Fuji II LC; RM= Reactmer; DY= Dyract AP; SP=Spectrum TPH.

Table 4-9. Time comparisons of OLD and WA inhibition between tissues and materials.

Material	OLD - µm		WA Inhibition - μm^2	
	Enamel	Dentin	Enamel	Dentin
Fuji II	NS	NS	6M > 2W	2W > 6M
Fuji II LC	2W > 6M	2W > 6M	NS	2W > 6M
Reactmer	NS	2W > 6M	NS	NS
Dyract AP	NS	2W > 6M	6M > 2W	NS
Spectrum TPH	NS	6M > 2W	6M > 2W	NS

Results of Paired Samples T-Test ($p<0.05$), > Indicates significantly greater OLD mean lesion depths and significantly greater WA inhibition. NS indicates no statistical significance between materials. 2W=2 Weeks and 6M= 6 Months.

Table 4-10. Frequency Comparison of wall area patterns between time intervals.

Material	WA Patterns	
	Enamel	**Dentin**
Fuji II	-	-
Fuji II LC	-	-
Reactmer	-	-
Dyract AP	2W > 6M	NS
Spectrum TPH	NS	NS

Results of Paired Samples T-Test ($p<0.05$), > Indicates significantly higher frequency of WA lesion patterns. NS indicates no statistical significance between time intervals. 2W=2 Weeks and 6M= 6 Months. [-] indicates no frequency of wall area patterns were obtained.

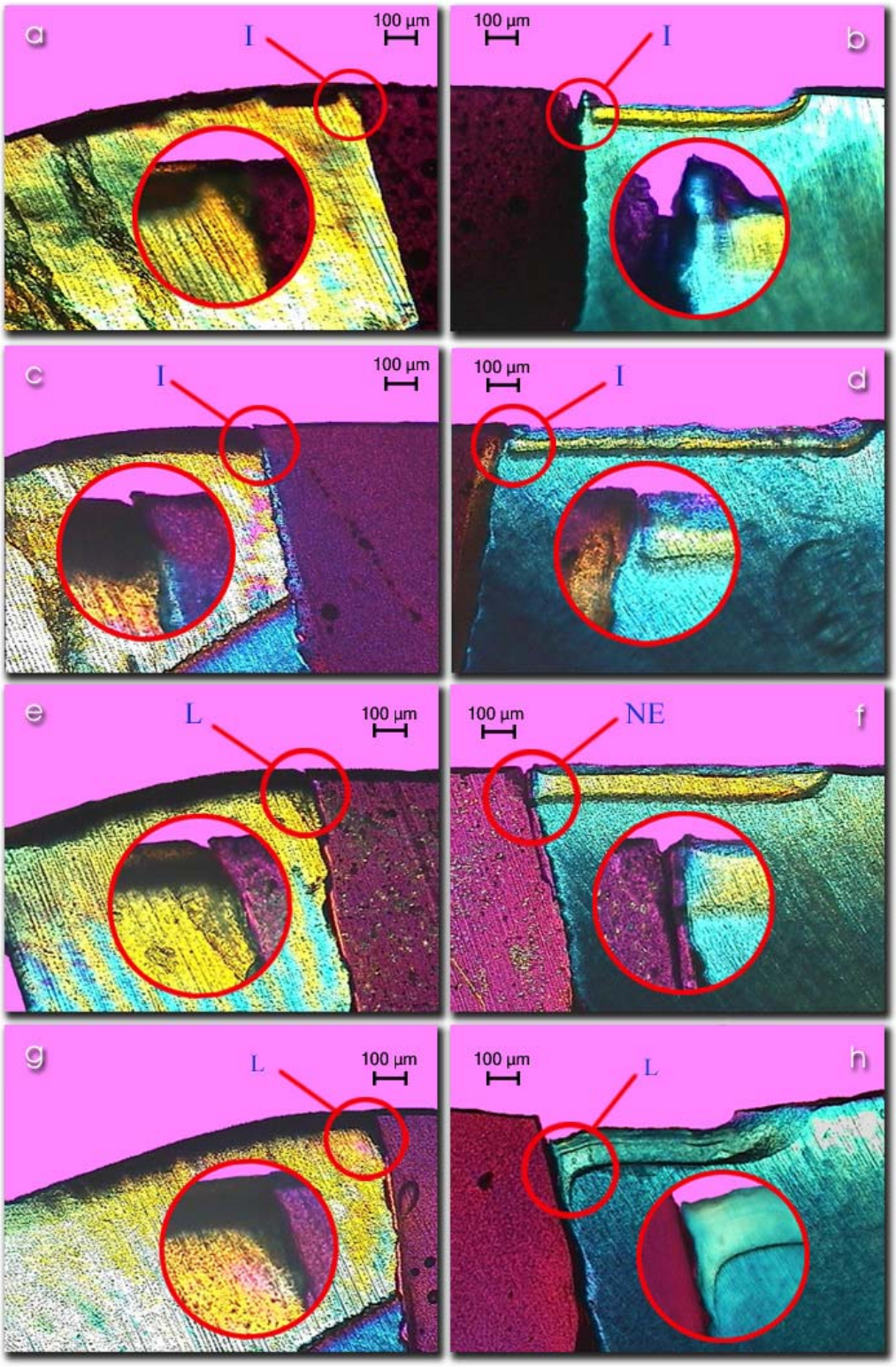

Figure 4-1. PLM pictures of materials at 2 weeks at 40x of: a) Fuji II and Fuji II LC in enamel, b) Fuji II and Fuji II LC in dentin, c) Reactmer in enamel, d) Reactmer in dentin, e) Dyract in enamel, f) Dyract in dentin, g) Spectrum TPH resin in enamel, h) Spectrum TPH resin in dentin. Restoration margin pictures at 100x are shown in circles. Note the presence of wall inhibition areas (I), no effect lesions (NE) and wall lesions areas (L).

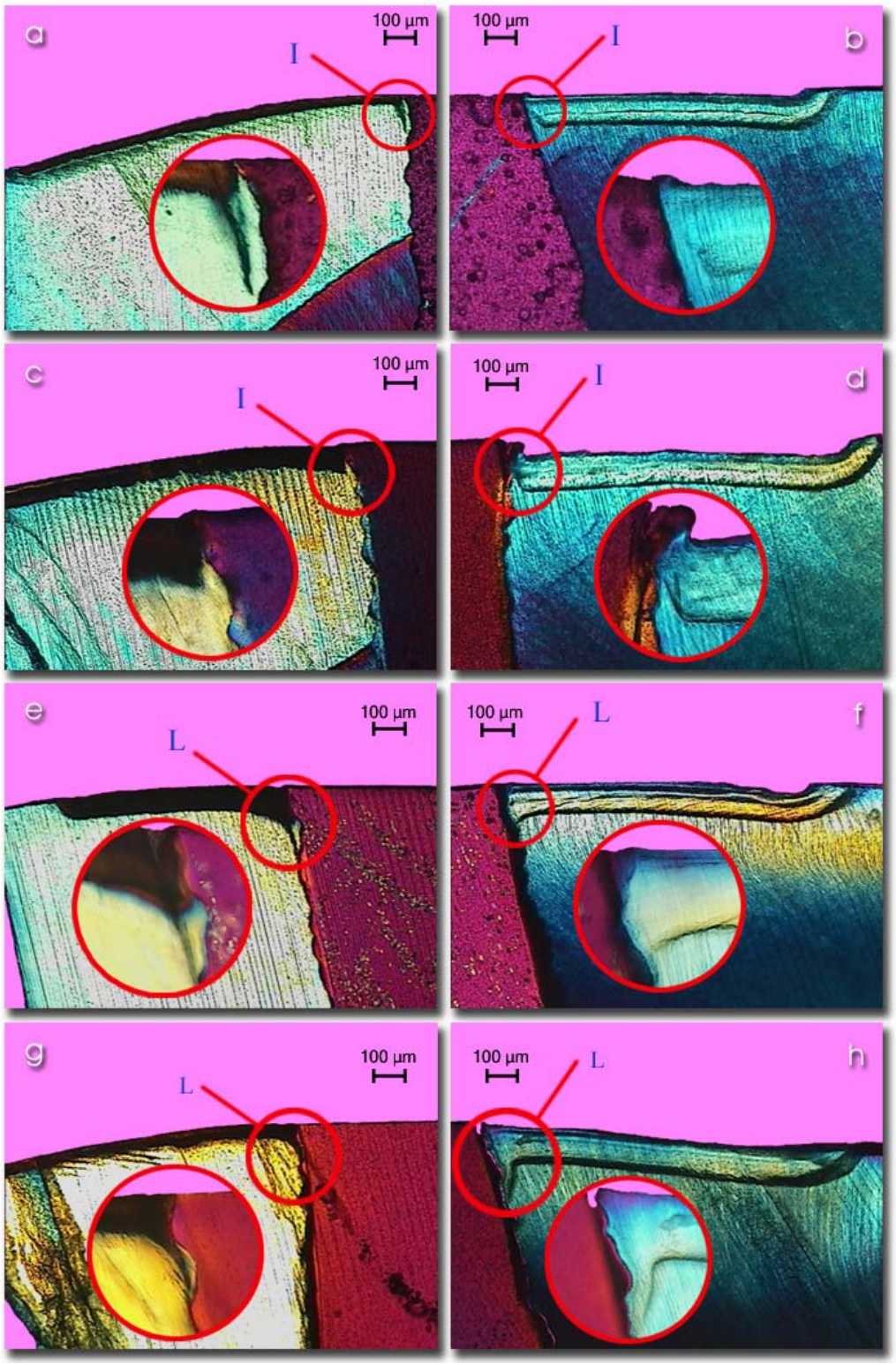

Figure 4-2. PLM pictures of materials at 6 months at 40x of: a) Fuji II and Fuji II LC in enamel, b) Fuji II and Fuji II LC in dentin, c) Reactmer in enamel, d) Reactmer in dentin, e) Dyract in enamel, f) Dyract in dentin, g) Spectrum TPH resin in enamel, h) Spectrum TPH resin in dentin. Restoration margin pictures at 100x are shown in circles. Note the presence of wall inhibition areas (I) and wall lesions areas (L).

5 DISCUSSION

The aim of this study was to compare the ability of the continuum of direct tooth-colored restoratives to prevent recurrent caries formation. The wall lesions along the cavity wall and the outer lesions away from the restoration interface were measured and compared among different materials at two time periods. The outer lesion represents a primary attack to the surface of the tooth. This demineralization is similar to the primary caries formation. In the presence of microleakage, demineralization is similar to the secondary caries formation and occurs along the interface between the cavity wall and the restoration interface.

In this experiment, the outer lesion is formed by a primary acid attack from the artificial caries media bathing the tooth surface. It is also influenced by the presence of cariostatic agents released to the media by the restorative material. On the other hand, the demineralization of the wall lesion is due to the primary acid attack from the artificial caries media and the secondary acid attack by microleakage. In fact, the wall lesion is influenced by the presence of cariostatic agents and the degree of microleakage in addition to primary acid attack. If there is higher presence of cariostatic agents in the media, it is possible to reduce or eliminate caries by inhibition of demineralization. The higher the concentration of these agents usually results in greater inhibitory effect on demineralization, thus, resulting in shallower lesions. This phenomenon occurs in both the outer lesion and the wall lesion. The presence of microleakage along the cavity wall will allow the penetration of acid into the restoration interface and enhance the demineralization along the cavity wall. If there is no microleakage there will be no wall lesion (Dérand, Birkhed & others, 1991). Therefore, the presence of microleakage is necessary for wall lesion formation.

The primary acid attack in addition to the secondary acid attack produced by the microleakage extends and causes the demineralization going downward along the cavity wall. This will result in greater demineralization, different from that of the outer lesion, which only receives the primary acid attack. In this study this additional demineralized area is defined as the wall area lesion, different from the lesion on what is known as wall resulting from the primary acid attack only. The presence of fluoride near the cavity wall may have an inhibitory effect on the secondary acid attack similar to that shown on the primary acid attack. Therefore, a higher concentration of fluoride will result in a smaller demineralized area along the cavity wall. The variance between the shallower lesion and that caused by the primary acid attack only in this study will be defined as inhibition area.

As it will be discussed further below, the presence and local concentration of cariostatic agents would mainly depend on three conditions: the ability of the tooth structure to act as a reservoir, the materials and the bonding agents used and the aging process (Hsu & others 1998).

The degree of microleakage and the cariostatic agents released on the bathing media might compensate each other and play an important role in lesions on the cavity wall. For the outer lesions, which of course have no direct contact with restorative materials, only the topical concentrations of the cariostatic agents in the bathing media are essential (Dijkman & Arends, 1992). In this case the cariostatic agent in the restorations is fluoride (Attar & Önen, 2002; Dionysopoulos, Kotsanos & others, 1998a; Dunne, Goolnik & others, 1996; Gilmour, Edmunds & others, 1997; Hicks & Flaitz, 2000; Nagamine, Itota & others, 1997; Torii, Itota & others, 2001), which at the same time is the major controlled variable in this study.

The ability of a restorative material to release fluoride and the ability of adjacent tooth structure to act as a reservoir are both important aspects regarding the cariostatic effect of fluoride. From the continuum of direct tooth-colored restorative materials releasing fluoride, the ranking of fluoride release from the highest to the lowest of the materials used in this study is the following: Fuji II > Fuji II LC > Reactmer > Dyract AP (Yap, Tham & others, 2002). The composite resin Spectrum TPH (SP) group has no fluoride release and therefore was used as our control group for comparison.

The ability of tooth structure to take up fluoride, firm or loosely bound, is associated with the surface area of crystallites, porosities, hydration, etc. (Weatherell, Robinson & others, 1983). Compared with dentin structure, enamel has less porosity, larger crystallites and less hydration due to more inorganic and less organic components. Therefore, the dentin substrate may take up more cariostatic agents than enamel; which in this case is fluoride. Previously, studies reported that dentin accumulate more fluoride, more rapidly than enamel (Tveit & Hals, 1980; Tveit & Lindh, 1980; Weatherell & others, 1983). Healthy enamel takes up little fluoride compared with demineralized or carious enamel (Weatherell & others, 1983). Also, it has been indicated that the demineralization process results in an enhanced ability to absorb the fluoride. However, dentin have been shown to be more vulnerable to acid attack than enamel (Marshall, Staninec & others, 1989; Phankosol, Ettinger & others, 1985). Therefore, at lower pH the dentin structure is demineralized before enamel structure and the available fluoride will be absorbed more rapidly into dentin than enamel. This phenomenon may explain why the lesion/inhibition effect of fluoride on demineralization was usually greater on the dentin surface than enamel, especially if the local concentration of fluoride was low.

5.1 Model Assessment

A variety of *in vitro* methods have been developed to produce artificial caries lesions for use in demineralization and remineralization studies. These include the use of acidified gels (Attar & Önen, 2002; Damato, Strang & others, 1988; Dunne & others, 1996; Hicks & Flaitz, 2000; Ingram & Silverstone, 1981; Millar, Abiden & others, 1998), buffered solutions (Damato & others, 1988; Donly & Ingram, 1997; Donly & Grandgenett, 1998; Forss & Seppa, 1990; Heilman, Jordan & others, 1997; Hsu, Jordan & others, 2000; Kerber & Donly, 1993), exposure to acid vapor (Weatherell & others, 1983), and incubation with natural plaque (Gilmour & others, 1997; Hsu, Donly & others, 1998; Nagamine & others, 1997; Torii & others, 2001) to demineralize tooth structure around restorations and determine if the restoration material will decrease demineralization in tooth structure. However, if a dental material is to be tested for its fluoride efficacy, the pH-cycling model provides a good tool to do this (Featherstone, 1994; Staninec, Giles & others, 1988). Besides, the chemical buffer models are preferred over the acidified gel model as less time is required for lesion formation and the system is easier to prepare.

In vitro demineralization / remineralization using a pH-cycling model have been employed to examine any cariostatic effect imparted by fluoride containing restorative materials. This model simultaneously measures the net result of the inhibition of demineralization and the enhancement of remineralization. Samples are immersed in demineralizing and remineralizing solutions for several hours and the procedure is then "cycled" for several days. Acid attack in tooth structures, results in the dissolution of the HAP crystals and the subsequent exposure of the collagen matrix in dentin. It is important to have well characterized solutions with known calcium, phosphate and fluoride concentrations, as well as carefully adjusted pH. Either lactic acid or acetic

acid or both should be used (Featherstone & Rodgers, 1981). Citric or hydrochloric acids are not relevant to the caries process, as they do not diffuse into the subsurface in the same way as the weak organic acids (Featherstone, 1996). Calcium and phosphate should be present in the solution, as well as some surface dissolution inhibitor to mimic the salivary pellicle (White, 1987).

It is well accepted that the bacterial enzymes, as well as organic acids, play an important role in the development of caries. Acid attack primarily results in the dissolution of the HAP crystals and the subsequent exposure of the collagen matrix in dentin. In an *in vitro* chemical dissolution system, whether gel or buffered solution, there is less destruction of collagen matrix due to the absence of bacterial enzymes. The chemical dissolution system, therefore, simulates only the physico-chemical dissolution process involving the mineral component on the dentin surface.

Without proteolytic enzymes, collagen would not be destroyed by an acid, but would be simply left unsupported and would collapse (Phankosol & others, 1985). However, when the collagen matrix is dissolved along with the HAP crystals it might be possible to result in an erosive surface. As discussed above, dentin has less HAP crystals than enamel due to the difference in organic structure. It may be the case that more collagen were exposed and dissolved in dentin than enamel, this could be the reason why our study often exhibited the erosive outer lesions on the dentin surface and the depth of the erosive part is proportional to that of the whole lesion in each group, while no erosive lesions were obtained in enamel surfaces. The measured outer lesion depth in dentin was only the half of the whole demineralized area. Therefore the actual outer lesion depth in enamel is less than that in the dentin as seen in Tables 4-1 and 4-2 in Chapter 4.

The method of lesion measurement in the present study is an adaptation of that of Hsu & others (1998). However, it is different from others both in outer lesion and wall lesions. The methodologies used in other studies for measuring outer lesions included measuring the length of the outer surface and the largest distance between the inner and outer border of the lesion (Attar & Önen, 2002), averaging 8 to 10 measurements at intervals of 0.2mm from the surface to the depth of the lesion (Dunne & others, 1996), measuring the maximum depth of a visible lesion from the surface (Gilmour & others, 1997; Millar & others, 1998; Tam, Chan & others, 1997), measuring the area in μm^2 from the restoration margin to 100µm away from the margin (Donly & Grandgenett, 1998), measuring the mean surface lesion depth (Hicks & Flaitz, 2000), measuring the depth at 50µm away from the restoration (Nagamine & others, 1997) and measuring at 100µm away from the restoration (Itota, Nakabo & others, 2001; Torii & others, 2001) .

Different methodologies also were employed in quantifying wall lesions, such as measuring the length from the surface to the innermost extended portion towards the DEJ (Attar & Önen, 2002; Dionysopoulos, Kotsanos & others, 1998b), measuring from the "edge" of the visible lesion to the cavosurface/restoration margin (Millar & others, 1998), measuring the thickness of the "radio-opaque layer" adjacent to the gingival wall at a depth of 250µm under the surface of the restorative material (Itota & others, 2001; Torii & others, 2001) and measuring the thickness of the "acid-resistant layer" adjacent to the gingival wall at a depth of 300µm under the surface of the restorative material (Nagamine & others, 1997).

Different methods of measurement will inevitably lead to different results just because the lesion formation is not uniform in the tooth structure, especially that along the cavity wall and the enamel surface. Regarding the outer lesions on enamel surface,

the inner border of the demineralization lesion is not straight and fluctuates according to the orientation of enamel rods. Thus, the measurement of area of the lesion is more accurate than that of length because of this fluctuating pattern. Regarding wall lesions, the proximal portion of the inner border of the lesion may curve in different degrees towards the cavity wall, depending upon the amount of the cariostatic agents and microleakage. In the same way the measurement of area is more accurate than that of length. We measured the “wall lesion” area defined by three peripheral lines, as mentioned in chapter 3, to rule out the effect of primary acid attack from the outer surface. This principle was also applied to the measurement of inhibition areas, as mentioned in chapter 3. Therefore, the exact influence from the cariostatic agent and microleakage can be more precisely expressed and compared with each other. In this way, the preventive effect of different restorative materials on recurrent caries can be revealed and quantified more accurately.

In this study, several sections restored with glass ionomers groups, whether aged or not, demonstrated a subtle line between the inner and outer borders of the outer lesion in dentin surfaces. When mineral is released from the advancing front of demineralization, the various mineral phases may re-precipitate along the previously demineralized collagen matrix and result in remineralization of an area with decreased mineral content, where the crystals may be changed, enlarged, or elongated by this phenomenon (Phankosol & others, 1985). It is possible that different adjacent restorative materials could have different influence on remineralization. In future studies, it may be useful to use different imbibition media, such as quinoline or Thoulet’s solution with different refractive index than water, to compare the remineralized area between groups. This may reveal and evaluate more accurately the remineralization effect of different materials.

5.2 Material Effect

Caries resistance and formation of the inhibition zone appears to be associated with the level of fluoride release from glass ionomer restorations (Dionysopoulos, Kotsanos & others, 1990; Donly, 1994; Swift, 1989). Featherstone (1994) emphasized that fluoride enhanced the remineralization of enamel caries and produced mineral at the surface that was more resistant to subsequent demineralization. However, previous studies indicated conflicting results regarding the amount of fluoride released from conventional and resin-modified glass ionomer cements. Diaz-Arnold & others (1995) observed that a conventional glass ionomer cement released greater amounts of fluoride than a resin-modified glass ionomer cement. Takanashi & others (1993) found no statistical significant differences in fluoride release between Fuji II and Fuji II LC. Forsten (1995) observed that fluoride levels released by a resin-modified glass ionomer cement were higher or the same as that of the conventional glass ionomer cement. An explanation for the variations in results obtained may be the different methods used to determine fluoride release. Moreover, other factors such as material composition and release of other elements from the glass ionomer materials may be more significant and may have greater influence on artificial caries inhibition than fluoride release alone.

Conventional glass ionomers releases the most fluoride amongst all direct tooth-colored restoratives. Hence, they have major effects of caries inhibition on tooth structures compared with other direct restoratives. Secondary caries initiation and propagation were found to be significantly reduced when glass ionomer restorations were placed (Donly, Segura & others, 1999; Hicks & Flaitz, 2000; Retief, Bradley & others, 1984; Torii & others, 2001). As expected, the mean depths of dentin lesions for

all groups were deeper than the maximum recorded depth for the enamel lesions whether aged or non-aged groups, similar to those reported by Tam & others (1997). Reasons of this phenomenon were previously detailed in Section 5.

No statistically significant difference among the depths of the enamel outer lesions for the five materials were found in the non-aged groups. These results are similar to those reported by Skartveit & others (1991) who compared the depths at the middle points of the lesions and found no significant differences between groups studied. The same results were found by Dunne & others (1996) who concluded that there was no significant difference in depth of the outer lesion among fluoride containing and non-fluoridated materials.

Regarding the wall inhibition area, Fuji II produced a greater area compared with those produced by Fuji II LC. Because of differences in the formulations of these materials, a difference in their respective capacity to inhibit artificial caries may also exist. Tam & others (1997) concluded that all glass ionomers produced an “acid-resistant inhibition zone” at the cavity margin and the dimensions of this “zone” were material dependent. In the present study conventional and resin-modified glass ionomers and giomer restorations exhibited significantly more enamel and dentin wall inhibition areas than compomer and non-fluoride composite resin restorations. This is not surprising since conventional and resin-modified glass ionomers have been shown to inhibit in vitro demineralization adjacent to restoration margins (Attar & Önen, 2002; Gilmour & others, 1997; Hicks & Flaitz, 2000; Torii & others, 2001). Tam & others (1997) also observed the presence of narrow zones of non-carious dentin between the margin of the restoration and the body of dentin decay. In an ultrastructural study, Tay and others (2001) demonstrated that glass ionomer phases were readily observed in these materials while no evidence of glass ionomer phase were noted in the compomer

after 24 hrs of aging. Compomers behaved more like composite resin (Meyer, Cattani-Lorente & others, 1998; Tay & others, 2001) showing more wall lesion patterns, while giomers behaved more like resin-modified glass ionomers (Tay & others, 2001) showing more wall inhibition patterns. Moreover, Xu and others (2000) reported that pre-reacted GIC powder incorporated into ceramic-whisker-containing experimental composites has a cumulative fluoride release of about 20% of the original GIC. Tay & others (2001) proposed that this decrease might be partially attributed to the presence of silane coupling in the pre-reacted fillers versus non-silanized glass particles in the original GIC. This may explain the smaller mean WA inhibition areas in giomers in comparison to those seen in glass ionomers.

The enamel margins of composite restorations had wall-lesion incidence. This is not surprising, since it may be expected that enamel margin, which is a butt/etch margin finish, would have an incidence of wall lesions of approximately 4% (Gilmour, Edmunds & others, 1993). It would appear that the bond to the enamel was not effective. This may have been because of structural differences in cervical enamel, in particular, the thinness of enamel and the increased incidence of prismless enamel in this region (Gilmour & others, 1997; Mejare, Mejare & others, 1987). Another possible explanation of wall lesions adjacent to resin composite materials is because recent adhesive resin systems may not be sufficient to inhibit secondary caries (Pereira, Inokoshi & others, 1998). Similar findings reported previously that a superior marginal seal may not be sufficient to prevent recurrent wall lesions under plaque conditions where there is no material fluoride release (Tam & others, 1997).

In the present study, Spectrum TPH had significantly greater dentin outer lesion depth than Fuji II, Fuji II LC and Reactmer. This finding is in agreement with that of Nagamine & others (1997), who evaluated the caries inhibitory effect of three RM-

GICs, one GIC and a composite resin in dentin. They found no significant difference in OLD between the GICs and RM-GICs and significant differences with composite resin.

Attar & Önen (2002), however, found significant differences in enamel and no significant differences in dentin OLD between a conventional glass ionomer and two compomers. As with the present study deeper lesions were found in dentin than enamel. Findings of the present study also corroborated those of Torii & others (2001) who found no difference in dentin OLD compomers and non-fluoride releasing composite resins. Dyract generally lacked the inhibitory beneficial properties of glass ionomers resulting in a high frequency of wall lesions adjacent to restoration margins (Donly & Grandgenett, 1998; Millar & others, 1998; Torii & others, 2001). Itota & others (2001) previously evaluated the effect of adhesives on the inhibition of secondary caries around compomer restorations *in vitro,* and indicated that the type of adhesive used with compomers might play a major role in fluoride release. They suggest that applying an adhesive without Bis-GMA resin to compomer restoration will not have a suppressive effect on the fluoride release and therefore might be beneficial for inhibiting secondary caries *in vitro*. However, much more work has to be done to improve the role of compomers on the continuum of direct tooth-colored restoratives.

5.3 Aging Effect

Most of the materials were reported to release a smaller amount of cariostatic agents after aging. In this study, we changed the distilled water and the de/remineralization solutions every other day as mentioned in Chapter 3. In the case of the outer lesion, the fluoride released into the media may be lost if not firmly absorbed or bound. The loosely bound fluoride may be lost during the media exchange through the six-months

aging period. In the case of the lesion along the cavity wall, the fluoride may diffuse into the cavity wall and may be retained due to the close contact with tooth structure. As early mentioned in chapter 4, the aging effect on the lesion along the cavity wall is both an increase in the wall lesion area and decrease in inhibition area due to the decrease in fluoride releasing ability of the materials as discussed before.

It is well accepted that the fluoride-releasing ability of GICs decrease with aging. However, the rate of decrease in fluoride release is still being debated. As reviewed in section 5.2.1, different materials showed various results regarding the aging effect on the materials' fluoride releasing ability. In the present study, the lesion depths on the outer surface of both enamel and dentin specimens restored with Fuji II showed no significant differences after aging, while specimens restored with Fuji II LC showed significantly deeper lesions for non-aged specimens (Table 4-9). Regarding the WA area inhibition, it may be the case that a perfect seal ability of Fuji II LC due to their enhanced characteristics in composition may lead to an increased protection to recurrent caries rather than the brittle Fuji II. Likewise, Crim (1993) showed perfect marginal adaptation of Fuji II LC after a six-month aging process. This implied that the good sealing ability of GIC may prevent the outlet of the released fluoride and help retain the accumulated fluoride to inhibit the demineralization of the cavity wall adjacent to GIC. An interesting observation is that enamel specimens restored with Fuji II showed greater wall inhibition at six months than 2 weeks, while in dentin Fuji II showed greater inhibition at 2 weeks than 6 months. However, no significant differences in wall inhibition were seen in enamel restored with Fuji II LC, while in dentin Fuji II LC showed greater wall inhibition at 2 weeks than 6 months.

The wall inhibition areas or absence of wall lesion adjacent to GIC restorations have been observed in other studies (Attar & Önen, 2002; Dionysopoulos & others, 1998b;

Donly & Grandgenett, 1998; Dunne & others, 1996; Gilmour & others, 1997; Hicks & Flaitz, 2000; Hsu & others, 1998; Millar & others, 1998; Nagamine & others, 1997; Tam & others, 1997; Torii & others, 2001).

Regarding the new pre-reacted glass ionomer composite material (Reactmer), no significant differences were observed in enamel outer lesion, while in dentin the specimens showed deeper lesions at 2 weeks than 6 months. This phenomenon may suggest that Reactmer has a similar cariostatic effect compared with glass ionomers and confirmed that giomers behaved more like resin-modified glass ionomers than compomers. Likewise, no significant differences were seen in wall inhibition whether aged or non-aged in both enamel and dentin and no wall lesion were seen in specimens with giomer restorations.

In summary, the cariostatic effect of the conventional and resin-modified glass ionomer (Fuji II LC), and the pre-reacted glass ionomer composite (Reactmer) is not degraded after the six-month aging process and even increases the inhibition area on the wall, as long as the marginal seal remains intact (Hsu & others, 1998).

Theoretically, the fluoride released in the compomer group slowly increase with the aging process as well as the microleakage of the composite resins. However, it is well accepted that even with the increase in fluoride release, compomers does not exhibit inhibition areas (Attar & Önen, 2002; Tay and Others, 2001; Millar, Abiden & others, 1998; Donly & Grandgenett, 1998).

In the present study, the outer lesions of the Dyract group had no significant difference in enamel after aging, while in dentin the 2 weeks period showed deeper lesions than 6 months. The outer lesions of the Spectrum TPH group had no significant difference in enamel after aging, while in dentin the 6 months period had deeper lesions than 2 weeks. These different manifestations of aging effect may result from the different

microstructure of the enamel and dentin surface. As mentioned above, the enamel structure is more resistant to the acid attack and has smaller capacity to take up fluoride than dentin structure during acid attack (Retief & others, 1984; Tveit & Hals, 1980; Weatherell & others, 1983). That may be the reason why the aging effect was observed on the dentin surface but not the enamel surface in both Dyract and Spectrum TPH groups.

For the wall area along the cavity wall, no significant difference was seen in dentin, while less demineralization with the aging process were obtained in both Dyract and Spectrum TPH groups. This can be explained by temporarily balancing the effect on microleakage possibly due to the hygroscopic expansion and the hydrolytic degradation. However, this inhibitory effect in both materials did not result in inhibition areas as those obtained with glass ionomer and giomer materials. In fact, a true wall lesion remained after the aging process in most of the specimens restored with Dyract and Spectrum TPH groups.

Interestingly, Dyract showed an increased number of tooth sections with both wall lesions and inhibition areas after aging. Even in the same tooth sample, albeit not often seen, some sections may have inhibition areas but others have wall lesions or “no effect”. In addition to this complex situation, an insufficient bonding might enhance the diffusion of fluoride through the tooth-restoration interface while an intact bonding might hinder or diminish it.

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6 CONCLUSION

The present study investigated the effects of materials as well as the effect of aging on demineralization inhibition and found significant advantages in favour of the glass-ionomer materials and giomers. However, due to the extensive limitations of this *in vitro* study, it is difficult to extrapolate a definitive conclusion regarding the demineralization inhibition effects of giomers in the clinical situation. Therefore, clinical trials on demineralization inhibition effects using giomers as well as different methodologies, warrants further investigation.

In summary, under the conditions of this *in vitro* study:

1. Dentin is more susceptible to demineralization than the enamel.
2. The threshold concentration of cariostatic effect on dentin surfaces is lower than that of enamel.
3. Dentin outer surfaces might be more sensitive to the low concentration of preventive agents than enamel.
4. At the margins of the restorations, the demineralization inhibition effects of all materials were significantly greater in dentin than in enamel with the exception of the composite material.
5. The demineralization inhibition effect of giomers, conventional and resin-modified glass ionomer cements appear to be more evident at the margins of restorations.
6. The demineralization inhibition effect of materials was tissue and time dependent.
7. At both time intervals, FJ & RM had similar enamel and dentin OLD.

8. At both time intervals, enamel and dentin WA inhibition by glass ionomers and giomer was significantly greater than the compomer and composite.

In future, it may be worthwhile to study the fluoride reservoir capability of the giomers; the ability of the dental tissues to take up fluoride from the giomers and the distribution of fluoride in the surface in contact with the giomer restoration. Inferences of the above studies might prove giomers to be a more structural and cariostatic restorative material. Further, tests conducted on these materials for longer periods of storage in distilled water or artificial saliva (1 year, 5 years) would help in better understanding of the effects of aging on these materials. As the initial *in vitro* trials of new or experimental materials do not always reveal their full limitations or assets, clinical data is essential to prove the success of these materials.