TOKYO MEDICAL AND DENTAL UNIVERSITY
Faculty of Dentistry
Department of Operative Dentistry

GLASS IONOMERIC RESTORATIVES: CONCEPTS ON SECONDARY CARIES INHIBITION AND ADHESION

Patrícia Nóbrega Rodrigues Pereira

Promotor: Professor Junji Tagami

Thesis submitted in partial fulfillment of the requirements for the degree of Doctor in Dental Sciences.

1998
Tokyo - Japan

List of original publications

This study was based on the following publications referred in the text by the respective chapters.

Chapter 2. P.N.R. Pereira, S.Inokoshi, J. Tagami: *In vitro* secondary caries inhibition around fluoride releasing materials. *J Dent* (in press).

Chapter 3. P.N.R. Pereira, S.Inokoshi, T. Yamada, J. Tagami: Microhardness on *in vitro* caries inhibition zone adjacent to conventional and resin-modified glass ionomer cements. *Dent Mater* (accepted).

Chapter 4 P.N.R. Pereira, T. Yamada, R. Tei, J. Tagami: Bond strength and interface micromorphology of an improved resin-modified glass ionomer cement. *Am J Dent* 10:128-132, 1997.

Chapter 5. P.N.R. Pereira, T. Yamada, S. Inokoshi, M.F. Burrow, H. Sano, J. Tagami: Adhesion of resin-modified glass ionomer cements using resin bonding systems. *J Dent* (in press).

CONTENTS

Acknowledgments

This work was carried out at the Department of Operative Dentistry, Tokyo Medical and Dental University. This thesis is the result of efforts of a whole group of people, rather than of one individual, from different departments and different universities. I wish to express my deepest gratitude to all who have contributed to this final product and to those who are not mentioned below.

This work is respectfully submitted to Professor K. Eto, Dean of the Faculty of Dentistry, Tokyo Medical and Dental University (TMDU), and to Professor T. Ohyama, Director of Dental Hospital, TMDU.

I wish to express my sincere gratitude to my supervisor Professor J. Tagami, Professor and Chairman of the Department of Operative Dentistry, TMDU, who gave me the opportunity, guidance, and facilities to undertake this research, and was always willing to spare of his time to render me with valuable advises.

I am deeply grateful to my first supervisor, Professor T. Takatsu, Professor and Chairman of the Department of Operative Dentistry, Ohu University, Japan, who received me warmly when I first arrived in Tokyo, instructed and supported me during the first two years.

I wish to acknowledge my sincere gratitude to my other supervisors, Dr. T. Yamada, actual Director of the Dental Clinic, Toranomon Hospital, Tokyo, and Dr. S. Inokoshi, actually dedicated to private practice. Their guidance, scientific experience, generosity, and dynamism were essential for the development, process, and conclusion of the studies herein.

I am particularly grateful to Professor H. Sano, Professor and Chairman of the Department of Operative Dentistry, Hokkaido University, for all his generosity in teaching and his constant supportive attitude towards my work. Also for thoroughly revising my thesis many times in a very short period of time, giving many significant critical comments. I also appreciated our many plentiful discussions about research and other aspects of life. I would like to thank him for always believing in me.

I am greatly indebted to Dr. T. Nikaido, Lecturer, Department of Operative Dentistry, TMDU, who critically reviewed this work numerous times in a short period of time. His scientific knowledge, constructive criticism, and suggestions were valuable for this work. Without his effort and critical advises, I would not have been capable of accomplishing this thesis.

I also owe special thanks to Dr. M. Otsuki, Lecturer, and to Dr. N. Inai, Lecturer, Department of Operative Dentistry, TMDU, for their encouragement towards my work and for their endless patience regarding my troubles with the computers and Internet.

I am very grateful to Dr. M. Nakajima, research associate, of the Department of Operative Dentistry, TMDU, for reading this work in a short period of time and for his important critical comments, which were valuable to the content of this thesis.

I want to thank the entire staff members of the Department of Operative Dentistry, TMDU, for always being friendly and ready to help me with anything I needed, and for producing a pleasant working environment in the Department.

My warm and sincere thanks to Dr. Y. Nakaoki and Dr. M. Ogata, research associates, Department of Operative Dentistry, TMDU, to Dr. N. Harada, colleague and graduate student, and to Dr. M. Okuda, graduate student, TMDU, for always being there when I needed a friend to listen to my problems, needed extra hands when I needed help in the experiments, and needed a translator when I had problems with the Japanese language.

My sincere thanks to Dr. H. Sonoda, hospital staff, for his help with the documents in Japanese and to Dr. Y. Kitasako, hospital staff, for our discussions about research. I also want to express my special thanks to my colleagues Dr. S. Horiguchi, graduate student, for his friendship and helping me with the translation of part of this thesis to Japanese, and Dr. M. Kataumi, graduate student for always being friendly and willing to help me during our graduate course.

I am much obliged to Dr. M. Burrow, Senior Lecturer, University of Melbourne, Australia, for instructing me the basics of research and encouraging me while he was here at Tokyo Medical and Dental University and I was still in my first year. Since then he has supported and believed in my work, and was always ready to proof-read my manuscripts, correcting my English and giving his critical and stimulating comments.

I am deeply grateful to Professor D.H. Pashley, Medical College of Georgia, USA, to Professor D. Ruse, University of British Columbia, Canada, to Professor C.F. Cox, Department of Restorative Dentistry, University of Alabama, USA, for their generous hospitality when I visited their departments, and for their constructive and inspiring discussions and advises.

I wish to thank Professor F. Garcia-Godoy, Department of Pediatric Dentistry, University of Texas, USA; Professors G.W. Marshall, Chair of the Division of Biomaterials Science, and S. Marshall, Vice-Chair of the Department of Restorative Dentistry, University of California in San Francisco, USA; Professor P. Lambrechts, Department of Operative Dentistry, Catholic University of Leuven, Belgium; Professor

J.M. ten Cate, Department of Cariology and endodontology, University of Amsterdam (Acta), The Netherlands; Dr. J. Perdigao, Associate Professor, Department of Operative Dentistry University of North Carolina Chapel Hill, USA; S. Suzuki, Associate Professor, Department of Restorative Dentistry, University of Alabama, USA; Dr. M. Yoshiyama, Assistant Professor, Department of Operative Dentistry, University of Tokushima, Japan; Dr. B. Ciucchi, Department of Restorative and Endodontic Dentistry, University of Geneva, Switzerland; Dr. R.M. Carvalho, Assistant Professor, Department of Operative Dentistry, Bauru School of Dentistry, Brazil; Dr. M.F. de Goes, Associate Professor, Department of Dental Materials, University of Campinas, Brazil; Dr. L. Perinka, Associate Professor, Charles University, Czech; Dr. S. Uno, Lecturer, Department of Operative Dentistry, Hokkaido University, Japan; and Dr. Y. Momoi, Lecturer, Department Operative Dentistry, Tsurumi University, Japan, for their interest in my study, constructive and stimulating comments, and encouragement to conclude my work.

I am very thankful to Dr. K. Hirota, Director of the Research and Development Department, GC Corp, Japan, for his interest and technical assistance in this work, and for providing the major financial support to cover the expenses of this thesis.

My appreciation to the researchers Dr. R.L. Erickson, USA, Dr. J. Yamauchi, Japan, Dr. K. Ikemura, Japan, Dr. N. Honda, Japan, and to Dr. T. Kobayashi, Japan, for their encouragement and significant contribution to science.

I would like to thank all the manufacturers that generously supplied us with the dental materials that were used in the studies included in this thesis.

I am also obliged to the Japanese Ministry of Education (Monbusho) for funding me with the scholarship during the five years I have lived in Japan.

My dearest thanks to Dr. W. Duarte, Japan, Dr. K. Piquero, Japan, Dr. S. Belli, Turkey, Dr. P. Albert, Germany, Dr. T. Yamashita, Japan, Mrs. C. Nishio, and the late Mr. Y. Nishio, who encouraged me with their loyalty, understanding, empathy and friendship.

Finally I would like to thank all my friends throughout the world who supported and encouraged me during these last five years. I want to especially thank my parents and brothers, who constantly encouraged me to pursue with my studies, and gave me all their love from such a long distance and during long period of time absent from home.

Introduction

Dental caries, by definition, is a multifactorial and contagious disease of the mineralized tissues of the teeth. It is caused by bacterial activity on carbohydrates resulting in fermentation, and is identified when the mineral content is demineralized followed by disintegration of the organic content. Basically, dental caries prevention can be assessed by eliminating plaque, reducing consumption of sugars, and increasing resistance of the tooth. However, secondary caries is still one of the main factors that afflict the longevity of the restorations, therefore prevention and assessment of secondary caries has become of great importance.

The development of new bonding restorative systems has revolutionized the concept of cavity preparation and restorative procedures. Extensive cavity preparations have been replaced by conservative ones by removing the carious tissue only, producing minimal reduction of the sound tooth tissue (Fusayama, 1979) and permitting an adequate surface for adhesion of restoratives to the tooth tissue. Adhesive restoratives are expected to promote enhanced short- and long-term bond strengths to tooth substrate, effectively seal the cavity margins while protecting against formation of secondary caries.

The advent of fluoride in the preventive dentistry as well as in restorative dentistry has yielded the development of a new concept of dental therapy. The role of fluoride in preventive dentistry is well known and has been widely reported in literature. Both systemic ingestion (e.g., fluoridated water, salt, or milk, fluoride tablets or drops) and topical applications (e.g., rinses, toothpaste, topical solutions and gels) are efficient for caries prevention. Restorative dentistry has also been revolutionized by the incorporation of fluoride ions to many of the recent adhesive and restorative materials, in the attempt to increase the longevity of restorations by protecting the restoration against secondary caries. The glass ionomeric materials, which include the conventional and resin-modified glass ionomer cements, and the so-called compomers, have been reported to promote a anti-cariogenic effect and an ion-exchange reaction with the tooth tissue (Mount, 1994).

Fluoride has also been incorporated to several recent adhesive resin systems and resin composites, with the intention to provide long lasting restorations with durable bonds, sealing of the cavity margins, and protection against secondary caries. However, consistent levels of fluoride release of these materials are still low, and extensive research is being conducted to improve these levels without weaken the physico-mechanical properties of these materials.

Since the development of the first glass ionomer cement (Wilson and Kent, 1972), a great variety of glass ionomeric materials have been marketed with purposes of restorative dentistry. Because of their potential anticariogenic capacity and bonding physico-chemically with the tooth tissue, these materials have become popular as esthetic

filling materials to restore carious lesions or for patients with a high caries risk. The conventional glass ionomer cements were initially placed directly into the cut cavity, without any prior surface treatment. However, the smear layer that covers the cut dental surfaces (Pashley, 1984), may break cohesively and fail during polymerization shrinkage (Tao and Pashley, 1988). Since bond strengths without pre-treatment were reported to be inconsistent (Hewlett *et al.*, 1991) impairing clinical retention (Ngo *et al.*, 1986), pre-treating surface with acid solutions became routine before restoring with a glass ionomer or a resin-modified glass ionomer cement. Polyacrylic acid is an effective pre-treatment agent, however, to date there is little evidence reporting to what extent the resinous monomers can penetrate the demineralized dentin.

Volumetric shrinkage of light-cured restoratives (e.g., resin composites, resin-modified glass ionomers, and compomers) during polymerization can be detrimental to the bonding properties of these materials to dentin (Feilzer *et al.*, 1988). Similar to resin composites, resin-modified glass ionomers shrink approximately 3% in volume during setting (Feilzer *et al.*, 1988). Although this intrinsic stress can be relieved by later water sorption (Davidson and De Gee, 1984; Feilzer *et al.*, 1988), the initial polymerization shrinkage of the RmGICs may lead to adhesive failure at the tooth-filling interface compromising the longevity of the restoration (Ciucchi *et al.*, 1997). Therefore, the use of adhesive bonding systems prior to filling with glass ionomeric materials could be a good alternative to increasing bond strengths to the tooth tissue. Studies are still required to determine if the anti-cariogenic potential of these materials and fluoride release are affected by the application of bonding systems.

With the increase of life expectancy and retained teeth in the elderly, as well as periodontal problems in later life, root surfaces become exposed to the oral environment increasing the risk of root surface caries. Root caries present specific operative difficulties such as retention and access of the restoration. Resin-modified glass ionomer cements are the materials indicated for restoration of these lesions, due to their ion-exchange adhesion to tooth tissue (Hinoura *et al.*, 1991; Pawlus *et al.*, 1994), and because of their anticariogenic potential to inhibit secondary caries by fluoride release.

Ideally, the restorative adhesive materials should be able to promote strong short- and long term bonding to the tooth tissues, undergo minimal contraction during light-curing, and possess anticariogenic properties by releasing and uptaking fluoride into and from the oral environment.

Chapter 1.

Introduction to glass ionomer materials

Conventional glass ionomer cements

The first glass ionomer cement for dental use was developed by Wilson and Kent in the early 1970s (Wilson and Kent, 1972). The conventional glass ionomer cement (glass polyalkenoate cement) contains a powder of fluoroaluminosilicate glass with high concentrations of aluminum, fluoride, calcium, sodium, and silica (Sidhu and Watson, 1995; Smith, 1992; Wilson and McLean, 1988); and a liquid that is normally polyalkenoic acid but may contain polymers and copolymers of polyacrylic acid, itaconic, maleic, or vinyl phosphonic acid (McLean, 1992; Smith , 1992). When the powder and the liquid are mixed together, the ion-leachable glass and the polyacid react forming overlapping acid-base reactions. In the first phase, the fluoroaluminosilicate glass particles in the powder are attacked by the hydrogen ions (H^+) from the polyalkenoic acid, resulting in a limited degradation of the glass surface and releasing the simple metal ions calcium (Ca^{2+}), aluminum (Al^{3+}), sodium (Na^+), and fluoride ions (F^-). Silicic acid is also released and a layer of silica gel is slowly formed around the surface of the unreacted glass powder. With rapid increase of the pH of the mixture, the metallic ions are progressively lost until complete decomposition of the glass particles occurs (Burgess, Norling, Summitt J, 1994). When the free calcium and aluminum ions reach saturation in the silica gel, they diffuse into the liquid and cross-link with two or three ionized carboxyl groups (COO^-) of the polyacid to form a gel. The cross-linking increases through calcium ions being replaced by aluminum ions, hydrating sufficiently the gel, resulting in a precipitation of the cross-linked polyacrylate salt until the cement is solid (Burgess *et al.*, 1994; Van Meerbeek *et al.*, 1996). During the acid diffusion phase, sodium fluoride ions are released, however, because these are not a matrix forming species, it does not deplete the cement from its physical properties. As the cement matures over the first 24 hours, progressive cross-linking occurs possibly by hydrated Al^{3+} ions since the sensitivity to water decreases and the percentage of bound water and glass transition temperature increase (Wilson and McLean, 1988). The rate of reaction can be controlled by the powder/liquid ratio and the surface area of the powder, availability of fluoride ions and types of acids used (Walls, 1986; Wilson and McLean, 1988; Wilson, 1989).

The main advantages of the conventional glass-ionomer cements over other esthetic restorative materials are short and long term release of fluoride with cariostatic potential, ion-exchange reaction with enamel and dentin, low setting shrinkage and thermal

expansion similar to the tooth structure (Burguess *et al.*, 1994; Mount, 1994; Sidhu and Watson, 1995; Dunne *et al.*, 1996; Forsten, 1991). However, practical difficulties such as a short working time and a slow setting reaction, high sensitivity to water and susceptibility to desiccation compromising the physical integrity and esthetic properties, and low adhesive properties compared with the resin composites have demanded modifications of the cement composition.

Resin-modified glass ionomer cements

In order to overcome the disadvantages of the conventional glass ionomer cements yet preserving the benefits of these materials, a new type of material that combines the acid-base reaction with the methacrylate resin technology has been developed (Antonucci *et al.*, 1988). The resin-modified glass ionomer cements are set by both photoinitiator systems and conventional acid-base reaction. Light irradiation of the mixed cement results in fast initial hardening of the cement due to free radical polymerization of HEMA, forming a poly-HEMA matrix (Mitra, 1994; Tosaki and Hirota, 1994; Yoshikawa *et al.*, 1994). The acid-base reaction starts slowly as the powder and liquid are mixed. This acid-base reaction continues up to 24 hours after the material has been light cured because the pH values of the surface of the light-cured cement gradually increases to 24 hours similarly to a conventional glass ionomer cement (Tosaki and Hirota, 1994).

The systems that undergo both acid-base reaction and free radical polymerization by light-curing are the so called dual-cure resin-modified glass ionomer cements (e.g., Fuji II LC, GC Corp.). One commercial product, 3M Vitremer™ has been reported to be a tri-cure system, since curing of the methacrylate group also occurs with reaction of redox initiator systems (e.g., amine/peroxide; ascorbic acid/persulfate) in the dark (Mitra, 1994).

The resin-modified glass ionomers provide a longer working time and improved esthetics, immediately hardens when light-cured, are less sensitive to water, possess improved mechanical adhesive properties, are radiopaque, and are biocompatible with the tooth structure (Momoi and McCabe 1993; Swift *et al.*, 1995; Shono, 1995; Sidhu and Watson, 1995; Gladys *et al.*, 1997). Furthermore, they preserve the clinical advantages of the conventional cements, which are potential chemical reaction with the tooth substance, fluoride release, and thermal expansion similar to the tooth structure (Burguess *et al.*, 1994; Mount, 1994; Swift *et al.*, 1995).

Because of their simple clinical application, the resin-modified glass ionomer cement has become very popular among the general practitioners. Although bond strengths were improved by applying conditioners/primers, bond-strengths of these materials are still low compared to the resin composite systems.

Compomers

Recently, a new class of hybrid resinomers (compomer) that combines the resin composite and glass ionomer technology has been developed (e.g., Dyract, DeTrey/Dentisply; Xeno, Sankin Kyogo). They are a one component system, containing basically aluminofluorosilicate glass, Bis-GMA, photoinitiators, and a reactive acid monomer. The compomers come in the anhydrous form preventing the initial acid/base reaction from taking place. These materials contain shorter chain monomers carrying both acidic and methacrylate groups, and are initially cross-linked through the methacrylate groups due to light activation. However, when it is placed as a restoration and the material becomes hydrated, the ionic reaction between the acid groups on the polymer and glass occurs. (Hammesfahr, 1994; Blackwell and Käse, 1996)

Differently to the resin-modified glass ionomers, the one component compomers have practically unlimited working time and are not sensitive to water. Their chemical properties are similar to those of the resin composites, however posses lower flexural strength and lower abrasion resistance of occlusal restorations. The compomers are less soluble in water than the ionomer cements, but have shown to be susceptible to degradation at low pH, releasing fluoride, metal ions, and silica (Blackwell and Käse, 1996; Watts, 1996).

A great variety of current resin-modified glass ionomer cements and compomers are available in the market, including novel two or single-step bonding systems (Nikaido *et al.*, 1997). Although the future of these materials is still unclear, compomers may become a strong competitor to resin composite systems in preventive and general dentistry; for fissure pit sealing and for replacement of natural tooth structure, with anti-cariogenic properties and simple clinical application.

Release of fluoride and other elements from glass ionomeric materials

The release of fluoride from conventional and resin-modified glass ionomer cements has been extensively studied since the development of these materials (Forsten, 1977; Tveit and Gjerdet, 1981; Cranfield *et al.*, 1982; Swartz *et al.*, 1984, Meyron and Smith, 1984; Wilson *et al.*, 1985; Forsten 1990; Mitra, 1991; Diaz-Arnold *et al.*, 1995). Many studies have been conducted to compare the fluoride release from conventional and resin-modified glass ionomers (Forsten, 1977; Tveit and Gjerdet, 1981; Cranfield *et al.*, 1982; Swartz *et al.*, 1984, Meyron and Smith, 1984; Wilson *et al.*, 1985; Forsten 1990; Mitra, 1991; Takahashi *et al.*, 1993; Diaz-Arnold *et al.*, 1995; Forss, 1995). However, the results varied when comparing both types of cements, leading to the conclusion that the release of fluoride can be easily affected by the measuring conditions and dissolving medium.

It is known that the formulation of the glass ionomer, initial fluoride content of the glass, pH changes of the environment, type and amount of resin incorporated to the material, differences in powder:liquid ratio, and mixing and setting times can influence the fluoride release (Thorton *et al.*, 1986; Swift, 1988; Swift *et al.*, 1990; Rezk-Lega *et al.*, 1991; Forss, 1993; Momoi and McCabe, 1993; Takahashi *et al.*, 1993). The pattern of fluoride release has been proposed (Forsten, 1977), and it was shown that after the initial fluoride 'burst' that occurs in the first 24 hours, the release rate will drop to lower levels by the end of 3 months, and traced up to 2 years (Forsten, 1990; Diaz-Arnold, 1995). The initial burst of fluoride that is released is probably due to initial 'wash-off' of fluoride from the surface of the cement. After this, the release rate seems to be controlled by dissolution of fluoride from the cement. This dissolution rate may dependable on the surface area available for dissolution rather than shape of the sample or of the immersion solution (Fukuzawa *et al.*, 1987).

It has been reported that glass ionomer materials also release different chemical elements other than fluoride (Crisp *et al.*, 1976; Wilson *et al.*, 1985; Fukuzawa *et al.*, 1987). This probably occurs because modified glasses have been included, and part of the calcium has been replaced by strontium in order to improve the radiopacity of the resin-modified glass ionomers. Crisp *et al.*, (1976) have reported that the release of matrix forming elements such as calcium, strontium and aluminum leached in lesser amounts than the non-matrix forming elements silica, sodium and fluoride. It was further shown that the calcium released by the ionomeric materials may protect the adjacent enamel from demineralization and accelerate formation of calcium fluoride (Sëppa *et al.*, 1992). It has also been shown that release of these elements can be affected by the changes in pH (Forss, 1993), saliva proteins, and buffer systems (Rezk-Lega *et al.*, 1991).

The compomers were reported to release fluoride and metal ions, as well as silica once the acid-base reaction takes place after water sorption (Watts, 1996; Hickel, 1996). This fluoride release mechanism is a result of the reaction between acid and the amino-fluoro-silicate glass during setting. The compomers do not release as much fluoride as the conventional or resin-modified glass ionomers, however, to date the quantity of fluoride necessary to provide a suitable cariostatic effect has not been established. Therefore *in vitro* and *in vivo* studies are still necessary to clarify the effectiveness of fluoride-releasing materials.

Uptake of fluoride by glass ionomer materials

The concept that glass ionomers could also uptake fluoride as well as release it, was initially suggested (Walls, 1986) and it was later confirmed that these materials could act as rechargeable fluoride sources (Forsten, 1991). Previous studies have reported that

regular application of topical fluoride and regular use of fluoride toothpaste could result in uptake of fluoride into the conventional glass ionomer, and that this fluoride could be subsequently released to the adjacent tooth structure (Hatibovic-Kofman and Koch, 1991; Seppä *et al.*, 1993). Another study has reported that resin-modified glass ionomer materials could also act as a short-term rechargeable fluoride sources (Diaz-Arnold *et al.*, 1995). They reported that fluoride release after exposure to fluoride gels could only represent a wash-out of ions adsorbed to the surface of the material, rather than a diffusion into the matrix. Nevertheless, they recommended a 6 min daily use of fluoride gel for caries-prone patients, since most fluoride released after exposure to the gel occurred within two days.

The potential for surface degradation should also be considered after application of fluoride gels, since a decrease in microhardness of resin-modified glass ionomers has been reported (Diaz-Arnold *et al.*, 1995; Billington *et al.*, 1987) after long exposures to fluoride gels. This may surpass the cariostatic benefits of recharging the glass ionomeric restoration with fluoride gels.

Uptake of fluoride by enamel and dentin

The fluoride functions in several ways to increase resistance against enamel and dentin caries. The fluoride ion enhances remineralization of demineralized early lesions of enamel (Koulourides *et al.*, 1961; Iijima and Koulourides, 1988; Featherstone *et al.*, 1990), and increases enamel resistance to subsequent acid attack when incorporated to the crystals (Koulourides and Cameron, 1980; ten Cate and Duijsters, 1990a/b; Featherstone *et al.*, 1990). The fluoride ion influences remineralization of enamel by acting as a catalyst. The size and electrostatic charge of the fluoride ion permits a favorable stereoscopic arrangement of calcium and phosphate on the crystal surface (Hatibovic-Kofman *et al.*, 1997), and diminishes the energy activation required for the hydroxyapatite crystal growth.

Fluoride uptake by dentin is similar to that of the enamel, but may be greater because of its greater porosity, greater water content, and smaller size of the apatite crystallites (Mellberg and Singer, 1997, Scott and Symons, 1982). A low pH increases the uptake of fluoride by dentin, possibly due to the initial demineralization of the tissue and to deposition of greater amounts of fluoride into the hydroxyapatite crystals (Tveit *et al.*, 1983). The possibility of secondary caries inhibition and remineralization of early carious lesions by fluoride release are the main advantages of glass ionomeric restoratives over other restoratives.

Topical applications of solutions containing high fluoride concentration promote the formation of calcium fluoride (CaF_2) on the sound tooth surface or on the surface of initial lesions. The CaF_2 will later dissolve and release the fluoride ions that will react

with the hydroxyapatite converting to fluorohydroxiapatite. This process may increase remineralization or decrease the demineralization process, depending on the pH, calcium, and phosphate concentrations of the environment (Rølla and Saxegaard, 1990). However, it has also been suggested that constant low levels of fluoride ions for at least 24 hours (Arends and Schuthof, 1975) were a favorable condition for formation of fluoroapatite, and that multiple applications of agents with low fluoride concentrations were more effective than a single, high concentration approach (Koulourides *et al.*, 1975).

Dental caries

Dental caries, by definition is an infectious disease that afflicts the enamel, dentin, and cementum, caused by the production of acids as a result of the fermentation of carbohydrates by microorganisms. The caries lesion can be classified according to the anatomical site, to the severity of the lesion, or rapidity of the attack. When divided according to the anatomical region, the initial lesion can be a pit and fissure or smooth surface lesion, starting on enamel or root surfaces. It can also be secondary caries when located at a margin of a restoration. The classification for caries according to the rapidity of the attack is broader, since there is the individual variation of the teeth, and different degrees of carious challenge. The two extremes are namely the rampant caries, which occur as a result of a rapid destruction of numerous teeth, and the arrested caries, which is a carious lesion that does not progress due to changes in the environment (hygiene habits, fluoride applications, etc).

Enamel caries lesion

The histological appearance of the early human enamel carious lesion has been previously described (Darling, 1956; Gustafson, 1957; Darling, 1958; Silverstone, 1968), and divided into zones according to the histological appearance under the light microscope. The classical lesion has been divided into four zones (Silverstone, 1973). From the outer surface they are the surface zone, body of the lesion, dark zone, and translucent zone. The surface zone is a relatively unaffected region that is superficial to the lesion. The body of the lesion is markedly demineralized and is the greatest area of demineralization (Darling *et al.*, 1961; Silverstone, 1970). As the caries lesion progresses, the body of the lesion increases in extension and cavitation of the surface occurs. Once the lesion becomes cavitated, it can no longer be remineralized (Limeback, 1996). The dark zone lies just under the advancing front of the body of the lesion. When examined under the light polarized microscope with quinoline as imbibing medium, the dark zone shows positive birefringence in contrast to the negative birefringence of the sound enamel (Hörsted-

Bindslev and Mjör, 1996). Studies have reported that the dark zone contained micropores that were easily penetrable to small molecules (Darling et al., 1961). The pores that were not penetrated with nonaqueous media remained filled with air, disclosing the positive birefringence zone characterized by a dark color. In contrast when observed with water as imbibing medium, the dark zone is not apparent. The translucent zone lies under the dark zone, at the advancing front of the lesion. This zone is a result of mineral loss (Hörsted-Bindslev and Mjör, 1996), and although well demarcated from sound enamel, it is not always present on the entire extension of the advancing front.

Dentin caries lesion

The dentin caries lesion in an early stage occurs when the caries progression invades the amelodentinal junction spreading the disease laterally. As the lesion progresses it follows the curvature of the dentinal tubules towards the dental pulp. At this early stage of lesion development, the caries lesion is still free of bacteria, however, the acids can easily diffuse to the underlying dentin because of the extremely porous enamel at this stage. At this stage, the dentin lesion can be classified microscopically in two zones (Hörsted-Bindslev and Mjör, 1996) from the pulp outwards: translucent zone and body of the lesion. The translucent zone (or zone of sclerosis) is formed by physicochemical precipitation of mineral salts within the tubules (Hörsted-Bindslev and Mjör, 1996). It is produced as result of mild stimulation of the dentin/pulp complex, and serves as a mineralized barrier against acids, enzymes and bacteria towards the pulp. The body of the lesion (also referred as 'dead tract') in this stage of lesion progression has not been yet affected by bacteria. However, the superficial peritubular dentin and intertubular dentin are partly demineralized, and the tubules possibly confine remnants of the odontoblastic process and air. This will disclose a dark zone when observed under a polarized light microscope (Hörsted-Bindslev and Mjör, 1996).

When the enamel lesion becomes cavitated, and bacterial invasion occurs, the progression speed of the lesion increases. The primarily acidogenic bacteria produce acid, demineralizing the dentin tissue as deep as the translucent zone (Hörsted-Bindslev and Mjör, 1996). In this stage of caries progression, the lesion can also be divided microscopically in two zones: translucent zone and body of the lesion. However, because the pulp may show some inflammatory reaction to chemical stimuli, the irregular dentin may increase in thickness. Since the tissue changes at this stage are more complex, the body of the lesion can be subdivided microscopically in three other zones leading to a total of four zones: zone of demineralization, zone of penetration, and the zone of destruction (Hörsted-Bindslev and Mjör, 1996). The translucent zone lies deepest to all, separating

the body of the lesion from the sound dentin and is bacteria-free. The zone of demineralization is the deepest part of the body of the lesion, and is usually bacteria-free. The zone of penetration contains bacteria and is situated in the middle part of the body of the lesion. The intertubular dentin is markedly demineralized, however the collagen fibers still present the typical banding (Hörsted-Bindslev and Mjör, 1996). The zone of destruction is the outermost zone, and lies at the amelodentinal junction. This zone has been described as the 'outer carious dentin' (Fusayama, 1979), since the dentin substance is completely destroyed and the bacteria spread throughout the entire zone. It is clinically recognized by a greatly softened and discolored dentin, and is stainable by fuchsin or acid red dye. The ultra-morphological analysis of this lesion showed a demineralized intertubular dentin with few collagen fibers, and absence of peritubular dentin and odontoblastic processes (Ohgushi and Fusayama, 1975). The hardness of this zone of destruction or outer carious dentin has been reported to be approximately 4.4 Knoop Hardness Number (KHN) in acute lesions, and approximately 61.0 KHN in chronic lesions (Fusayama *et al.,* 1966).

What was microscopically described as the body of the lesion may correspond to the 'inner carious dentin' described by Fusayama et al. (1972). Ultramicroscopic observations of the inner carious dentin disclosed a partly demineralized intertubular dentin with apatite crystals bound to collagen fibers, that possessed apparently normal crossbands (Ohgushi and Fusayama, 1975). It was observed that the peritubular dentin was partially demineralized and with sound odontoblastic processes (Ohgushi and Fusayama, 1975). Furthermore, biochemical studies have reported that the crosslinks and precursors of the outer carious dentin were dramatically lesser than those of the inner carious dentin or sound dentin (Ohgushi and Fusayama, 1975).

Root caries lesion

With the increase of life expectancy and retained teeth in the elderly, as well as periodontal problems in later life, root surfaces become exposed to the oral environment increasing the risk of root surface caries. The process of root caries formation appears to be similar to that of enamel caries, in which alternating cycles of demineralization and remineralization occur constantly (Wefel, 1994). Root surface lesions initially occur at or below the cementoenamel junction with exposition of the root surfaces to the oral environment. It may involve cementum only, cementum and dentin, or dentin alone when the cementum has been lost.

As for enamel carious lesion, the root lesions have been also divided into zones under the microscope. The surface zone lies superficial to the lesion, and can be apparently intact or eroded. The body of the lesion represents the considerably demineralized dentin, and lies under the surface zone. The translucent zone of enamel

has been termed frontal zone for root surface lesions, and it lies deep in the body of the lesion denoting the advancing front of the lesion (Featherstone *et al.*, 1987).

More advanced lesions have shown loss of surface contour and cementum, or a radiopaque surface layer (Wefel, 1994). This radiopaque surface layer may be a sign of an inactive root surface lesion, since a hard mineral surface is the requirement for a clinical classification of inactive root caries (Schüpback *et al.*, 1992; Nyvad and Fejerskov, 1987). It has been also hypothesized that inactive root surface caries often show surface loss and later rehardening of the remaining exposed dentin, by means of oral hygiene and fluoride treatments (Nyvad and Fejerskov, 1986).

Enamel and dentin as adhesive substrates

Because restorative materials do not adhere effectively to dental tissues, bonding to tooth structure has become one of the greatest expectations of the general practitioner in the last decade. The development of the bonding technology and new bonding systems have caused great advances in adhesive dentistry and changes in the concept of cavity preparation. Box-shaped extensive cavity preparations have been replaced by conservative ones, cutting the carious tissue only while preserving the intact tooth structure (Fusayama *et al.*, 1986).

The enamel is basically a dry tissue that comprises approximately 96% by weight of hydroxyapatite crystals [$Ca_{10}(PO_4)_6(OH)_2$] that are arranged in a rod structure, and 4% water and organic material (Scott and Symons, 1982). It was initially proposed that etching the enamel surface with phosphoric acids would increase adhesion of acrylic filling materials (Buonocore, 1955; Hotta *et al.*, 1992). Since then, phosphoric acid has been widely used to etch enamel and successfully bond restorative materials. When the enamel surface is etched by acids, the hydroxyapatite crystals are selectively removed increasing the surface porosity and energy, allowing the monomers of the adhesive systems to provide appropriate wettability and to penetrate into the microporosities. With the polymerization of these monomers and formation of resin tags in the enamel microporosities, a strong micromechanical bond is formed and is the predominant mechanism of enamel bonding (Gwinnett and Matsui, 1967; Hotta *et al.*, 1992). However, recent milder etching agents have been introduced as a substitute to the phosphoric acid, and several *in vitro* reports exist in literature demonstrating that bond strengths of different strong and mild etchants differ only slightly. However to date there are little clinical studies proving clinical efficacy of the mild etchants.

On the other hand, bonding to dentin is a greater challenge and has been extensively studied because of the difficulty of a less reliable substrate (Fusayama, 1988; Douglas, 1989). The dentin is composed of a heterogeneous structure containing

approximately 70% hydroxyapatite, 18% organic material (mainly type I collagen) and 12% water (Linde, 1985; Scott and Symons, 1982). The organic and inorganic components are unevenly distributed in the inter- and peritubular dentin. Moreover, the dentin is a highly permeable tissue with numerous dentinal tubules that extend radially from the pulp throughout the entire thickness of the dentin (Gaberoglio and Brännström, 1976). Therefore, several factors account for the difference in bonding mechanism of enamel and dentin.

When the tooth structure is cut by rotary instruments, a cutting debris is created over the dentin surfaces and is called the smear layer. This layer covers the dentin surface and occludes the entrance of the dentinal tubules. The thickness of this smear layer normally varies from 1 to 5 μm and depends on the type of instrument used and the irrigation condition (Eick *et al.*, 1970; Pashley, 1984). Although the smear layer diminishes the dentin permeability, it may interfere impeding the direct contact of the bonding material with the dentin (Pashley, 1984; Pashley, 1990). In order to obtain a good adhesion to dentin, the smear layer should be removed or modified with conditioners, such as acid solutions or EDTA.

When the smear layer is removed and the superficial dentin is demineralized by acids, the resin monomers of the adhesive resin infiltrate and polymerize *in situ,* producing a hybrid structure of collagen fibrils surrounded by resin and residual hydroxyapatite crystals (Nakabayashi, 1982; Wang and Nakabayashi 1991; Sugizaki, 1991; Gwinnett and Kanca, 1992; Van Meerbeek *et al.*, 1992; Eick *et al.*, 1993). Several dentin bonding mechanisms have been proposed, however, the hybridization theory, which consists of micromechanical interlocking between the adhesive resin monomers and the network of exposed collagen fibrils, has been generally accepted (Bowen *et al.*, 1982; Nakabayashi *et al.*, 1982; Erickson, 1989; Inokoshi *et al.*, 1990; Pashley, 1990; Asmussen *et al.*, 1991; Harniratissai *et al.*, 1991; Erickson, 1992; Van Meerbeek *et al.*, 1993).

Adhesion of glass ionomeric materials to enamel and dentin

Although the exact mechanism of bonding conventional glass ionomer cements to enamel and dentin is still unknown, it is believed that it involves the wetting capacity of the surface by these materials and subsequent formation of ionic bonds by ion exchange with the hydroxyapatite (Wilson and McLean, 1988). It has been proposed that the polyacrylic acid will soften the surface of the tooth and the polyacid chains will diffuse into the tooth structure displacing the calcium and phosphate ions (Akinmade and Nicholson, 1993). The ion exchange has been traced by employing a fluorescent dye technique and confocal microscopy (Watson et al., 1991). It has also been suggested that there is a degree of

adhesion of the glass ionomer cement to the collagen of the dentin in addition to the ion exchange reaction (Akinmade, 1994).

For many years, the glass ionomer restoratives have been used as direct bonded materials without prior acid etching of the cavity. However, surface conditioners have been found to improve the bond strengths. Although many types of acids have been tested as conditioners, the polyacrylic acid has been found to be the most adequate, because it acts as a weak etching agent removing the smear layer, but does not remove completely the smear plugs from the dentin tubules (Powis *et al.,* 1982). The polyacrylic acid will also alter the surface energy of the tooth surface, exposing the highly mineralized substrate to the diffusion of the acid and exchange of ions (Mount, 1994). In order to define these two actions, and to distinguish the behavior of the conditioner from that of the acid, the term "conditioning" was originally used by McLean and Wilson (1977). Therefore, it was suggested that the polyacrylic acid conditioner acts as a self-etching primer, because it demineralizes and probably penetrates the surface promoting a surface receptive for the cement. It is also chemically compatible with the components in the liquid phase of the cement, and may as well improve the wetting ability and surface integrity of the cement (Erickson and Glasspoole, 1994).

Although the exact bonding mechanism of the resin-modified glass ionomer cement is still unclear, adhesion of these materials probably occurs both chemically and micromechanically. As the conventional glass ionomer cements, they undergo an acid-base reaction as soon as the powder and liquid are mixed to later form ionic bonds. Additionally, a mechanism similar to those of the adhesive systems has been suggested, because they contain monomers such as HEMA that penetrate the exposed collagen network or enamel, bond micromechanically when light-cured (Carvalho *et al.,* 1995), and are influenced by different light-curing intensities (Erickson and Glasspoole, 1994). It has been shown that light-curing directly influences the bond strengths of resin-modified glass ionomer cements, since bond strengths have decreased when the material thickness was increased or when the light-curing intensity was decreased (Erickson and Glasspoole, 1994). Because the resin bonds tend to decrease over time (Burrow *et al.,* 1993), and probably due to degradation of the resin (Sano *et al.,* 1998), the bonding mechanism of the resin-modified glass ionomer cements may be advantageous because ions may diffuse from the cement filling the spaces between the collagen fibrils. Long term studies on bonding properties of glass ionomer cements are still limited in literature, and are essential for a full understanding of the bonding mechanism.

Chapter 2

In vitro secondary caries inhibition around fluoride releasing materials

Introduction

Clinical dentistry aims to provide long lasting restorations that produce durable bonds and sealing of the cavity margins. However, the longevity of a restoration may be compromised due to many factors such as secondary caries, microleakage, and failure of the bond at the tooth-restoration interface. Volumetric shrinkage during light curing of the restorative material may stress and break the bond with the tooth, forming a marginal gap (Feilzer *et al.,* 1988; Ciucchi *et al.,* 1997). One of the main factors that induce secondary caries and failure of a restoration is microleakage. This gap between the restoration and the cavity wall permits invasion of fluids and bacteria leading to secondary caries.

With the increase of life expectancy and retained teeth in the elderly, as well as periodontal problems in later life, root surfaces become exposed to the oral environment increasing the risk of root surface caries. Root caries present specific operative difficulties such as retention and access of the restoration. Adhesive restorative materials which release fluoride such as resin-modified glass ionomer cements are the materials indicated for restoration of these lesions, because of their good adhesion to both dentine and enamel substrates (Hinoura *et al.,* 1991; Pawlus *et al.,* 1994; Pereira *et al.,* 1997) and because they possess an anticariogenic effect and the capacity to inhibit secondary caries by fluoride release (Griffin *et al.,* 1992; Prado *et al.,* 1994; Souto and Donly, 1994).

Fluoride has been also incorporated in resin composites with the purpose of inhibiting secondary caries at the cavity margins by fluoride release. Additionally, newer bonding systems adhere much better to the cavity walls preventing separation of the restorative material from the cavity walls. Therefore it would be reasonable to speculate that restorations with recent bonding systems would prevent formation of artificial secondary caries especially when restored with a fluoride releasing resin composite.

The aims of this study were to compare the capacity of two resin-modified glass ionomer cements, a conventional glass ionomer cement, and a fluoride-releasing adhesive resin composite to inhibit *in vitro* secondary caries, as well as to measure the width and height of the inhibition zone and the depth of the demineralized outer lesion.

Materials and Methods

Sample preparation for artificial caries lesions

Bovine incisors from 20-24 month old cattle were used within 24 hours after extraction. Oral tissues and the cementum were manually removed using scalers to expose the underlying dentine. The roots were obtained by separating them from the crowns at the cementum-enamel junction with a low-speed diamond saw (Bronwill, NY, USA) under water spray coolant. In order to prevent dehydration of the dentine during the restorative procedure, the pulp tissue was left *in situ*, and the cut surface and root apex sealed with wax and coated with two layers of nail varnish.

Two box-shaped cavities approximately 3 mm long, 2 mm wide, and 1.5 mm deep were prepared on both buccal and lingual dentine surfaces of each root, using a diamond bur (ISO # 106) mounted in high speed turbine with air-water coolant. The cavity margins were finished with a straight fisher steel bur (ISO # 010) in a slow speed handpiece under copious water spray, to achieve a cavosurface angle as close as possible to 90°. Twelve cavities were prepared for each material.

The materials used in this study were two resin-modified glass ionomer cements (Fuji II LC and Vitremer), a conventional glass ionomer cement (Fuji II), and a fluoride releasing adhesive resin system (Clearfil Liner Bond II, Protect liner F, and Clearfil AP-X) (Table 2.1). The cavities were treated according to the manufacturer's instructions. The cements were mixed at room temperature, transferred into a C-R syringe tip (Centrix™, Connecticut, USA) and injected into the cavity. Fuji II LC and Vitremer were light cured for 60 s each, and Fuji II was allowed to set for 15 min in room temperature. The glass ionomer cement and resin-modified glass ionomer cement restorations were coated with the respective varnishes, and then stored for one week in tap water at 37 °C, because it has been shown that the greatest release of fluoride occurs within the first week (Takahashi *et al.*, 1993). For the resin composite specimens, after application of the adhesive system, Protect Liner F was painted to the cavity walls, and light cured for 20 s. The cavity was then bulk filled with a resin composite, and light cured for another 60 s. Like the glass ionomer specimens, the resin composite specimens were stored in tap water for one week at 37 °C. All restorations were then finished and polished flat with polishing disks (Rainbow polishing Kit, Shofu Inc. Kyoto, Japan) under running water to expose the cavity margins. The integrity of each cavosurface margin was examined under a light microscope at 20x magnification. Two coats of acid-resistant nail varnish were then applied to the entire specimen surface, leaving a 1 mm-window around the cavity margins. Each specimen was stored for three days at 37 °C in individual bottles in 20ml of a buffered demineralizing solution previously described by Wefel *et al.*, (1995), containing 2.2

mmol/L $CaCl_2$, 2.2 mmol/L NaH_2PO_4 and 50 mmol/L acetic acid adjusted to pH = 4.5.

Specimen preparation for Polarized Light Microscopy

The specimens were then removed from the demineralizing solution and thoroughly rinsed in running water. Longitudinal sections of approximately 150 μm thick were cut through each restoration, perpendicular to the long axis of the root by means of a water-cooled diamond saw microtome (Leitz 1600 Microtome, Wetzlar, Germany). The sections were reduced to approximately 100 μm thick by grinding and polishing on coarse and fine wetstones. The sections were then dehydrated in varying grades of ethanol up to 100% and then immersed in quinoline for 20 min. The specimens were observed under a polarized light microscope (PM-10AK, Olympus Inc., Tokyo, Japan) with quinoline as the imbibing medium (refractive index: 1.62), and photomicrographs of the cavity margins were taken at 40x magnification.

In both conventional- and resin-modified glass ionomer cement-cavity margins, a zone exhibiting the same birefringence as normal dentin was observed adjacent to the restoration. This zone was defined as an inhibition zone. Adjacent to this inhibition zone, a demineralized lesion with positive birefringence was defined as outer lesion.

The resulting photomicrographs were projected through a profile projector (V-16C Nikon, Tokyo, Japan), and evaluated for features such as: lesion depth and shape, and presence of an acid-resistant zone adjacent to the cavity wall. The contour of each outer lesion was traced to identify a possible correlation between depth and form of the lesion with tubule orientation and material. The depth of the outer lesion was measured from the top of the lesion to the deepest demineralized front, i.e., the limit between positive and negative birefringence fronts (Fig. 2.1a). Existence of wall lesion was determined when the demineralized front extended from the top of the dentin surface deeper than the outer lesion along the cavity margin (Fig. 2.1b). The inhibition zone was traced to determine differences in width and height of this zone according to the different restorative materials. The height of the inhibition zone was measured from the top of this zone to the deepest demineralized front of the outer lesion; the width of the inhibition zone was measured at the midpoint of this zone (Fig. 2.1a).

The depths of outer lesions, and widths and heights of the inhibition zones were analyzed by one-way analysis of variance (ANOVA) and Fisher's PLSD test at 95% level of confidence.

Results

The outer lesions for all specimens showed similar features, which could be clearly

observed due to changes in birefringence (Fig. 2.2a-d). The top surfaces of the outer lesions were slightly concave due to loss of mineral and therefore surface shrinkage. Both conventional and resin-modified glass ionomer restorations showed a small separation at the cavity margins. This could have occurred during light-curing or setting of the cement due to volumetric shrinkage of the materials, or artificially created during the sectioning process by mechanical stress and material loss, or during dehydration process for microscopic observation by contraction of the demineralized outer lesion away from the restoration. Separation of the material from the cavity margin was not noted for the adhesive resin composite specimens.

The resulting mean values of the depths of the outer lesions and heights and widths of inhibition zones for the four materials are shown in Fig. 2.3a-c. The mean depths of the outer lesions did not show dependency on the direction of the dentinal tubules or differences among the four materials ($p>0.05$).

Nevertheless, the widths and heights of the inhibition zones were material dependent. The width of the inhibition zone adjacent to Fuji II was significantly greater than those created adjacent to Fuji II LC and Vitremer ($p<0.001$). The height of the inhibition zones created by Fuji II and Vitremer were statistically similar, and were significantly higher than the zone created adjacent to Fuji II LC ($p<0.001$). No inhibition layer was observed for the fluoride releasing low viscosity resin composite. In contrast, wall lesions were observed at the cavity margins in 80% of the resin composite restorations. A Wall lesion was not observed adjacent to the conventional or resin-modified glass ionomer cement restorations.

Discussion

Polarized light microscopy is a method used for assessment of mineralized dental tissues and of secondary caries inhibition. Estimation of the demineralized lesions and existence of inhibition layers have been previously reported using this method (Hicks and Silverstone, 1984; Griffin *et al.*, 1992; Souto and Donly, 1994; Dionysopoulos *et al.*, 1994). However, little is known about the consistency i.e., height and width of the inhibition zone adjacent to the restorative materials. This may be an important factor to the long-term protection against secondary caries, and longevity of a restoration. Therefore, in order to permit accurate quantitative analyses of the inhibition zones through a polarized light microscope, we traced all cavity margins and measured the outer lesions and inhibition zones.

No statistically significant differences among the contours and depths of the outer lesions for the four materials were found. These results are similar to those reported by Skartveit *et al.*, (1991) who compared the depths at the midpoints of the lesions and found no significant differences between groups studied. The same results were found by

Dunne *et al.*, (1996) who concluded that there was no significant difference in depth of the body of the outer lesion among fluoride containing and non-fluoridated materials.

Regarding the inhibition zones, Fuji II produced a zone with greater width and height when compared with those produced by Fuji II LC and Vitremer. Because of differences in the formulations of these materials, a difference in their respective capacity to inhibit artificial caries may also exist. Caries resistance and formation of the inhibition zone appears to be associated with the level of fluoride release from the glass-ionomer restorations (Swift, 1989; Dionysopoulos *et al.*, 1990; Donly, 1994).

However, previous studies indicated varying results concerning the amount of fluoride released from conventional and resin-modified glass ionomer cements. Diaz-Arnold *et al.*, (1995) observed that a conventional glass ionomer cement released greater amounts of fluoride than a resin-modified glass ionomer cement. Takahashi *et al.;* (1993) found no statistically significant differences in fluoride release between Fuji II and Fuji II LC. Forsten (1995) later 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 variation in results may be the different methods used to determine fluoride release. Moreover, other factors such as material composition and release of other elements from the ionomeric materials may be more significant and may have greater influence on artificial caries inhibition than fluoride release alone.

The current study also evaluated the caries inhibition capacity of a fluoride releasing low viscosity resin composite. An inhibition layer was not identified adjacent to the Clearfil Liner Bond II system restoration. Similar results to ours have been presented regarding the lack of an inhibition layer adjacent to resin composites incorporated with fluoride (Griffin *et al.*, 1992; Glasspoole and Erickson, 1993; Dionysopoulos *et al.*, 1994). Although these materials release fluoride ions, the very low concentrations or slow release may not be sufficient to inhibit acid-attack demineralization.

Purton and Rodda, (1988) have reported that the demineralized outer lesion showed contraction away from the resin composite restorations. Because of the development of the dentin adhesive resins, the recent bonding system and fluoride releasing low viscosity resin that were used in this study bonded well to dentine and prevented separation of the material from the cavity margin (Kemp-Scholte *et al.*, 1990 Burrow *et al.*, 1994). The polarized light microscopy observations showed that the resin composite restoration was adhered to the artificially demineralized dentin even after the sectioning for sample preparation. Nevertheless, wall lesion was observed in 80% of the specimens. This suggests that wall lesion might occur even when separation at the cavity margin is not microscopically observed.

Dentine bonding is enhanced with the formation of a hybrid layer that is produced by demineralizing the dentine surface and later applying monomers that will infiltrate between the collagen fibers and will be polymerized within the subsurface of the

demineralized dentin (Nakabayashi, 1984). Sano *et al.*, (1995) reported that newer bonding systems that promote good adhesion to dentin showed penetration of silver nitrate through nano-sized spaces in the base of the hybrid layer (nanoleakage). Although the aim of this study was not to investigate leakage, it is possible to speculate that the demineralizing solution used in this study could have infiltrated through the base of the hybrid layer and be responsible for the formation of the wall lesions observed in this study (Figs. 2.1b and 2.2d). Therefore, recent adhesive resin systems may not be sufficient to inhibit secondary caries.

Because the current study is an *in vitro* evaluation, further *in vivo* studies on secondary caries inhibition around restorations with recent resin bonding systems are needed to clarify the relationship between adhesion, fluoride release, and caries inhibition.

Conclusions

Although all ionomeric restorative materials tested in this study produced an acid-resistant inhibition zone at the cavity margin, the dimensions of this zone were material dependent. The conventional glass ionomer cement produced the thickest zone, followed by the resin-modified glass ionomer cements. The recent bonding system that release fluoride failed to produce an inhibition zone and disclosed wall lesion at the cavity wall.

Table 2.1. Restorative materials employed

Material	Brand name	Content	Batch	Manufacturer
Conventional glass-ionomer cement	Fuji Ionomer Type II	Conditioner:10% polyacrylic acid P: Fluoro-aluminosilicate glass L:Acrylic-maleic acid copolymer, Polybasic carboxylic acid, Water P/L ratio = 2.7(g/g)	#071141 #300771 #250621	GC Corp. ,Tokyo, Japan
Resin-modified glass-ionomer cement	Fuji Ionomer Type II LC	Conditioner: 10% polyacrylic acid P: Fluoro-aluminosilicate glass L: Acrylic-maleic acid copolymer, HEMA, Water, CQ P/L ratio = 3.0(g/g)	#071141 #071241 #291141	GC Corp. , Tokyo, Japan
Resin-modified glass-ionomer cement	Vitremer	Primer: 46% HEMA, 39% Ethyl Alcohol, P: Fluoro-aluminosilicate glass, potassium persulfate, ascorbic acid L: 50% Polycarboxylic acid copolymer 20% HEMA, Water, 13% carboxylic acid copolymer	#3303P #3303A3 #3303L	3M Dental Products, MN, USA
Adhesive Resin	Clearfil Liner Bond II	Primers: Phenyl-P, 5-NMSA, HEMA,CQ, water Adhesive: Bis-GMA, MDP, HEMA, microfiller, CQ Protect Liner F: low viscosity intermediate F-releasing resin, Bis-GMA, microfiller	#001 #0002 #0007	Kuraray Co. , Osaka, Japan
Resin composite	Clearfil AP-X	Hybrid resin composite	#0029	

Chemical Names for Abbreviations: HEMA: 2-hydroxyethylmethacrylate; CQ: Camphorquinone; NMSA: N-methacryloyl-5-aminosalicylic acid; Bis-GMA: Bisphenyl-glycidyl-methacrylate; MDP: 10-methacryloyloxy decyl dihydrogenphosphate

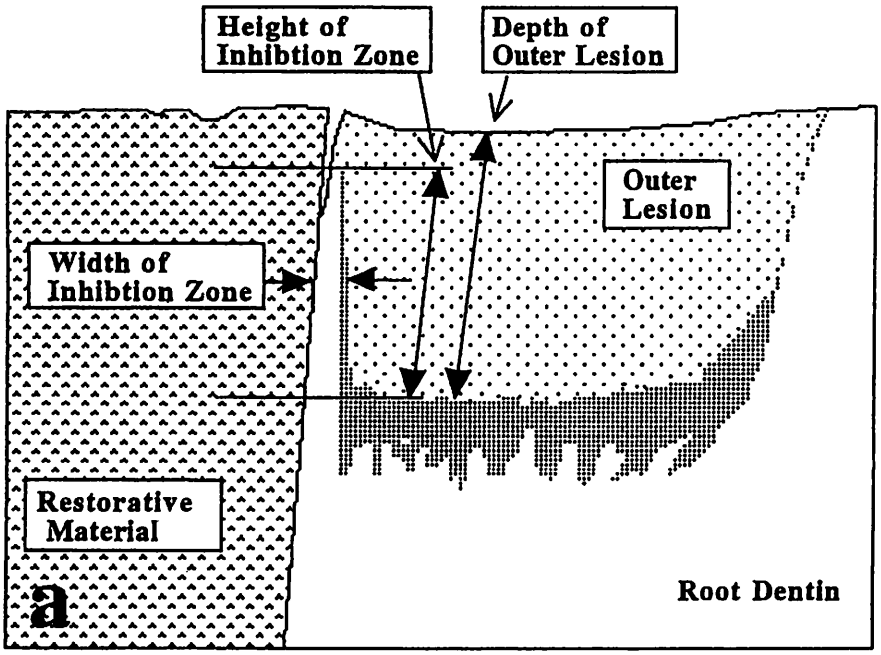


Figure 2.1a. Schematic representation of the caries-like lesion and the inhibition zone around the conventional or resin-modified glass ionomer restoration

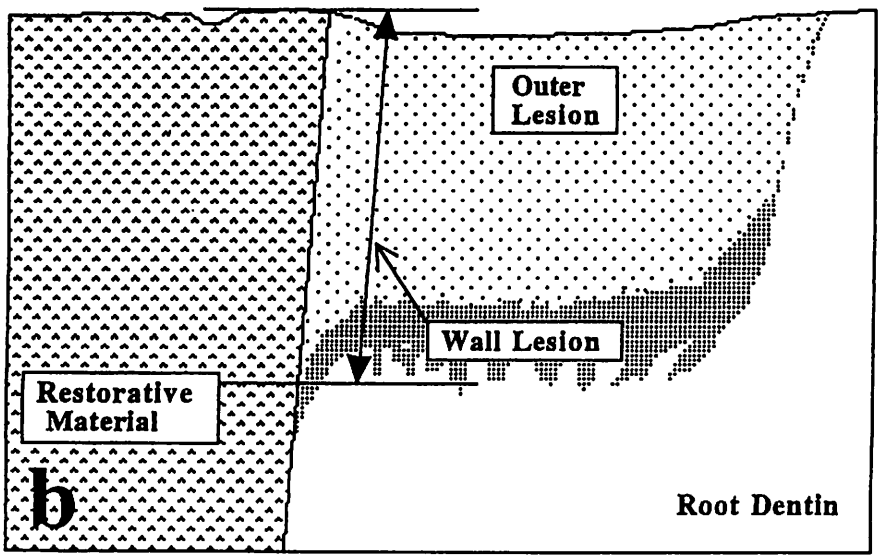


Figure 2.1b. Schematic representation of the caries-like lesion adjacent to the fluoride-releasing adhesive composite restoration.

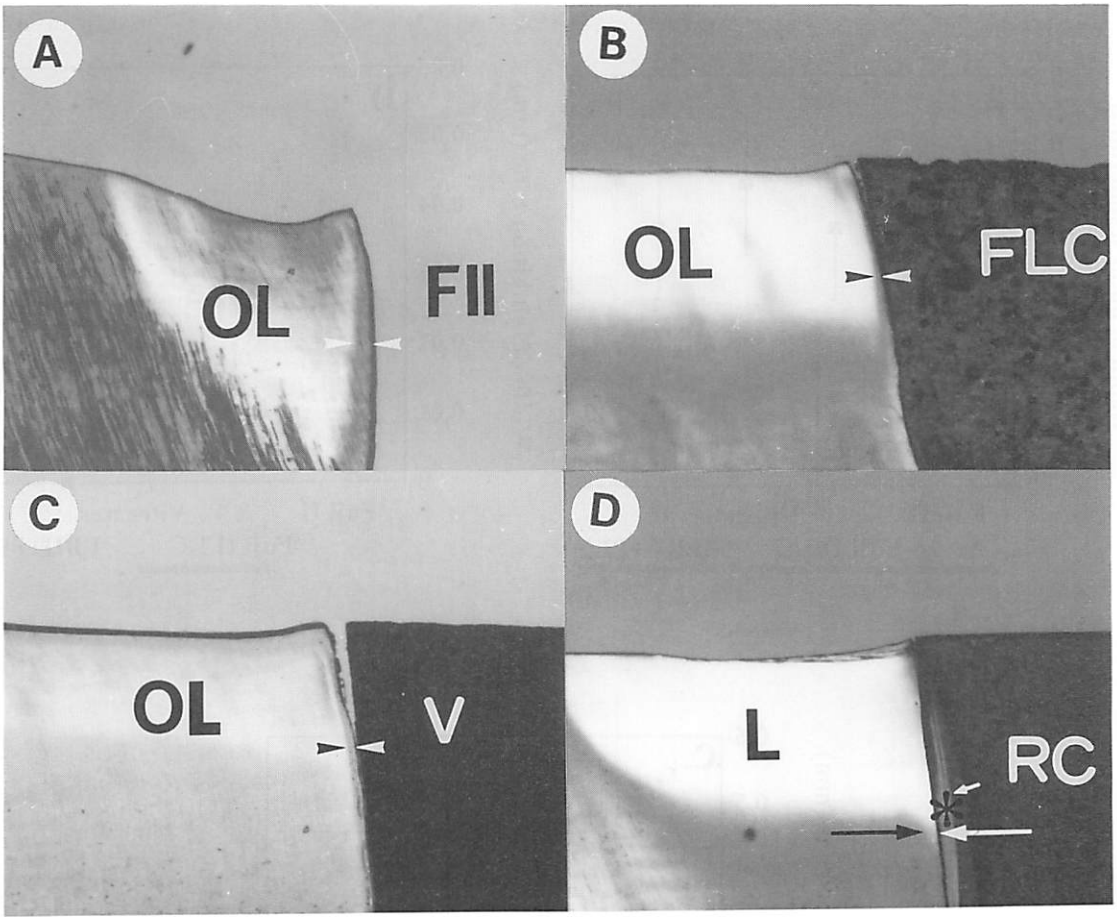


Figure 2.2a, b, c, d. **a:** Photomicrograph of a caries-like lesion and the inhibition zone formed adjacent to the conventional glass ionomer cement (Fuji II). Note that a thick inhibition zone (arrows) was formed despite the outer lesion (OL). Fuji II was lost during specimen preparation for polarized light microscopy. **b:** Photomicrograph of a caries-like lesion depicting the outer lesion (OL) and the inhibition zone (arrows) formed adjacent to Fuji II LC (FLC). **c:** Photomicrograph of a caries-like lesion and a thin inhibition zone (arrows) formed adjacent to Vitremer (V). In this sample the outer lesion (OL) seems shallower than the other samples, but not significantly different. Inhibition zone, OL = Outer lesion. **d:** Photomicrograph of a caries-like lesion and wall lesion (long black and white arrows) adjacent to the fluoride releasing adhesive resin composite (RC). Note that demineralization along the cavity wall extends beyond the length of the outer lesion (L) despite absence of gap at the cavity margin. Asterisk and white small arrow indicates bonding resin, and white long arrow indicates Protect Liner F.

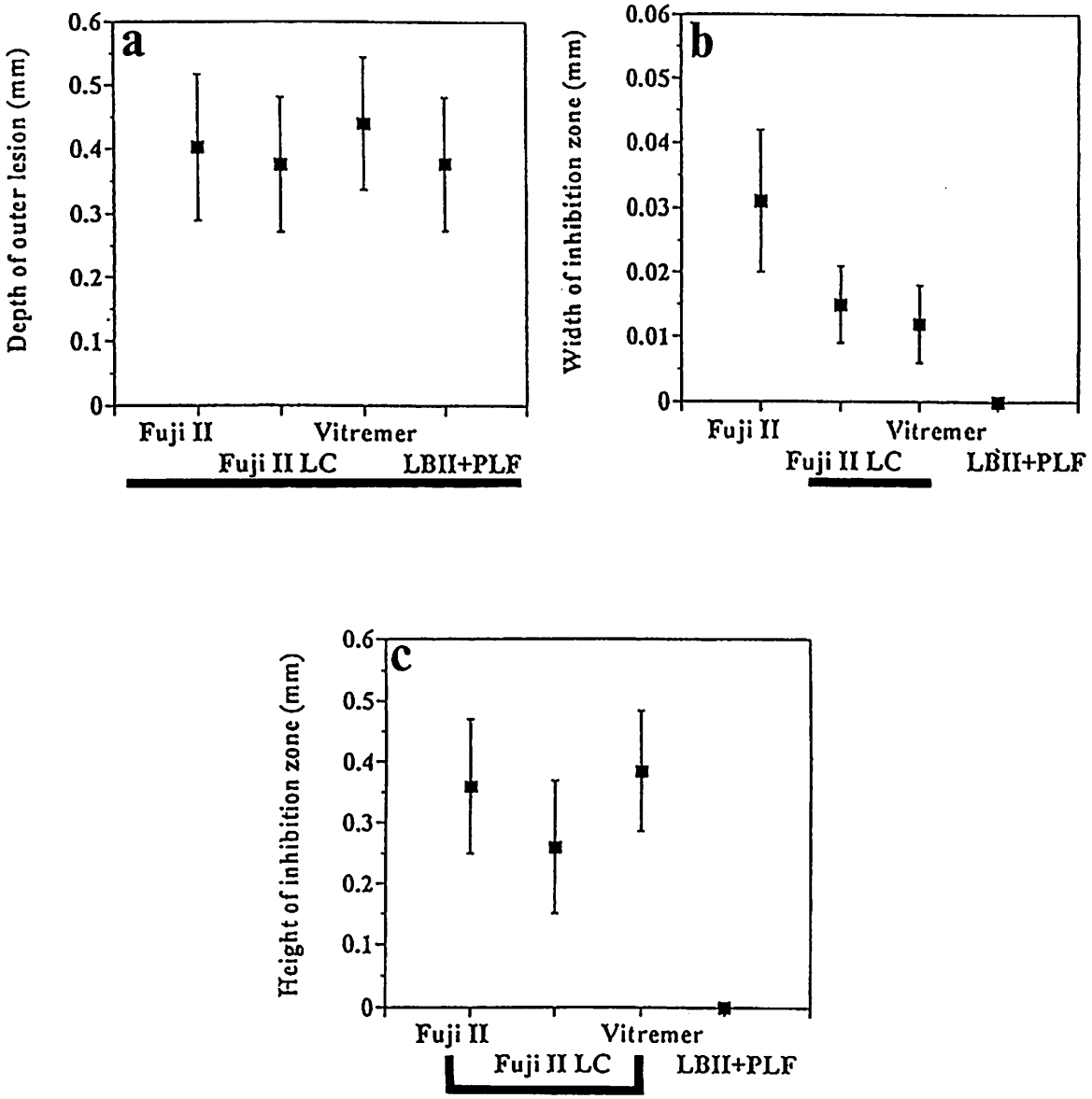


Figure 2.3a, b, c. Graphs indicate mean depths of outer lesions, widths and heights of the inhibition zones, respectively. Bars indicate values that are not significantly different ($p>0.05$).

Chapter 3

Microhardness of *in vitro* caries inhibition zone adjacent to conventional and resin-modified glass ionomer cements

Introduction

The increase of retained teeth, particularly in the elderly, has accounted greatly for the development of dental materials designed for the treatment of root surface caries. Glass-ionomer cements are usually one of the restorative materials indicated for root surface lesions, due to their good adhesion to both enamel and dentin (Triana *et al.*, 1994), as well as fluoride release (Forsten, 1991; Takahashi *et al.*, 1993). However, due to poor mechanical and esthetic properties of conventional glass ionomer cements, these materials have been combined with resin components in order to improve these properties while still releasing fluoride (McCaghren *et al.*, 1990; Mitra and Kedrowski, 1994; Mount, 1994). The resin-modified glass-ionomer cements (McLean *et al.*, 1994) promote better esthetics, adhere better to tooth structure (Hinoura *et al.*, 1994; Pawlas *et al.*, 1994; Proado *et al.*, 1994), and possess the capacity to inhibit both *in vivo* and *in vitro* secondary caries (Souto and Donly, 1994; Griffin *et al.*, 1992).

Surface softening is one of the initial signs of dental caries. Hardness changes of the tooth surface may indicate the degree and extent of the carious lesion. Various studies have measured the microhardness of sound, demineralized, or remineralized enamel and dentin (Caldwell *et al.*, 1958; Craig and Peyton, 1958; Ryge *et al.*, 1961; Hegdal and Haebö, 1972; Davidson *et al.*, 1974; Arends *et al.*, 1979; Arends *et al.*, 1980; Herkströter *et al.*, 1989). However, micro hardness of the acid-inhibited zone in dentin adjacent to fluoride containing materials has not yet been reported.

The quality of the inhibition zone seems to be related to its degree of mineralization. Since mineral content contributes mainly to hardness of dental substrates (Davidson *et al.*, 1974), the micro hardness of this acid resistant zone may indicate the level of mineralization which could be an important factor in prevention of secondary caries. However, this hardness has not yet been measured, probably because of the minute width of the acid-inhibited layer, and large size of conventional hardness indenter tips. Since glass ionomer cements are the materials of choice for restoring root surface lesions, we focused on measuring the triangular hardness of the inhibition zones and lesions around these materials and correlating these with Knoop hardness numbers.

Materials and methods

Sample preparation for artificial caries lesions. Bovine incisors from 20-24 month old cattle were used within 24 hours after extraction. The cementum was manually removed using scalers to expose the underlying dentin. The roots were obtained by separating them from the crowns at the cementum-enamel junction with a low-speed diamond saw (Bronwill, NY, USA) using water coolant. In order to prevent dehydration of the dentin during the restorative procedure, the pulp tissue was left in situ, and the cut surface and root apex were sealed with wax and coated with two layers of nail varnish.

Two box-shaped cavities approximately 3 mm long, 2 mm wide, and 1.5 mm deep were prepared on both the buccal and lingual dentin surfaces of each root, using a diamond bur (ISO # 106) mounted in high-speed turbine with air-water coolant. The cavity margins were finished with a straight fisher steel bur (ISO # 010) in a slow-speed handpiece under copious water spray, to achieve a cavosurface angle as close as possible to 90°. Twelve cavities were prepared for each material.

The materials used in this study were a conventional glass ionomer cement (Fuji II, GC Corp., Tokyo, Japan), and two resin-modified glass ionomer cements (Fuji II LC, GC Corp., Tokyo, Japan; and Vitremer, 3M Co., St. Paul, MN, USA) (Table 3.1). The cavities were conditioned according to each manufacturer's instructions. The cements were mixed at room temperature, transferred into a C-R syringe tip (Centrix™, Connecticut, USA) and injected into the cavity. Fuji II was allowed to set for 15 min, and Fuji II LC and Vitremer were light cured for 60 s each. The glass ionomer cement and resin-modified glass ionomer specimens were then coated with the manufacturer's recommended varnish. Because greatest release of fluoride has been shown to occur within the first week (Takahashi *et al.*, 1993), the specimens were stored for one week in tap water at 37 °C. All restorations were then finished and polished flat with polishing disks (Rainbow polishing Kit, Shofu Inc., Kyoto, Japan) under running water. The integrity of each cavosurface margin was examined under a light microscope at 20x magnification. Two coats of acid-resistant nail varnish were then applied to the entire specimen surface, leaving a 1 mm-window around the cavity margins. Each specimen was stored in individual bottles, in which 20 mL of an acid buffer solution previously described by Wefel *et al.* (1995) was placed. The acid buffer contained 2.2 mmol/L $CaCl_2$, 2.2 mmol/L NaH_2PO_4 and 50 mmol/L acetic acid adjusted to pH = 4.5. Specimens were stored in the solution for three days at 37 °C.

The specimens were removed from the demineralizing solution, and thoroughly rinsed in running tap water. Longitudinal sections of approximately 150 μm thick were cut through each restoration, parallel to the long axis of the root by means of a water-cooled diamond saw microtome (Leitz 1600 Microtome, Wetzlar, Germany). · The sections were ground to approximately 100 μm thick using coarse and fine wet stones.

All sections were dehydrated in gradations of ethanol, immersed in quinoline, and observed under polarized light microscope (PM-10AK, Olympus Inc., Tokyo, Japan) in order to confirm formation of outer lesions and inhibition zones.

Procedures for Micro Hardness Determination.
Five dentin slabs for each material were randomly selected and embedded in epoxy resin. Special care was taken so that the surface to be evaluated would remain free from the embedding material. The samples were polished manually to a high gloss using diamond pastes of successively smaller (6, 3, 1 and 0.4 μm) grits. Between each step, the samples were cleansed in ultrasonic bath for 1 min, in order to remove remnants of polishing debris and paste.

First, Knoop hardness number (KHN) of the outer lesion and underlying normal dentin was determined. Then, because the size of the Knoop indenter was greater than the width of the inhibition zone, triangular hardness (HT) of this layer was measured. The HT of the demineralized dentin adjacent to the knoop indentations was also measured (Fig. 3.1).

Knoop Hardness. The specimens were mounted on the stage of the Knoop Hardness apparatus (MXT70, Matsuzawa, Tokyo, Japan) in a horizontal position. The indentation process was made perpendicular to the surface with a 5 g force load for 15 s. The first indentation was made 100 μm from the top of the demineralized lesion. Subsequent indentations were made at 50 μm intervals parallel to the cavity margin and towards the normal underlying dentin. The surface area of the plastic deformation of the indentation was measured by means of a video control connected to the light microscope, and converted to KHN automatically. Fifteen indentations were performed on each lesion totaling 75 indentations for each material.

Triangular Hardness. A micro-hardness tester (DUH-200 Shimadzu, Kyoto, Japan) was used to determine the triangular hardness of both the demineralized lesion and inhibition zone. The micro-hardness tester apparatus consists of three major components: a triangular pyramidal indenter with an apex angle of 115°, an optical microscope with a maximum magnification of 500x, and a X-Y test piece stage that transports the specimen between the microscope and the indenter with a high lateral precision of 0.01 μm (information provided by the manufacturer).

In order to obtain homologous hardness at similar points (Fig. 3.1), the triangular micro indenter was positioned and loaded precisely adjacent to the knoop indentations (n = 15) on the demineralized lesion and on the underlying sound dentin. To measure the hardness of the inhibition zone, the indenter was first placed at a distance 100 μm from the top of this layer. Subsequent indentations (n = 15) were performed at 50 μm intervals

parallel to the long axis of the restoration wall. A load of 2 gf was applied to the triangular indenter tip, at a loading speed of 0.029 g/s. During the indentation process, the load, as well as the loading rate were continuously monitored. The diagonal length of the triangular indentation was determined through the microscope device, and the triangular hardness obtained according to the formula: $HT = 1569.7\ p/l^2$ HT = triangular hardness, p= test load gf, and l= height of indentation (μm).

Specimen preparation for SEM and EDS Line-Analyses. Following hardness measurements, the samples were gold-coated, and the micromorphology of the inhibition layer and outer lesion were observed under a Scanning Electron Microscope (SEM; JXA-840 JEOL, Tokyo, Japan). The plastic deformation of dentin caused by the triangular indenter on the inhibition layer and outer lesion was recorded photographically.

For analyses of calcium and phosphorous in the inhibition zones and outer lesions, the specimens were again polished with diamond pastes, and then carbon coated. They were line-analyzed with an energy-dispersive x-ray spectrometer (EDS) (SED800, Seiko EG&G Co., Tokyo, Japan) attached to the SEM.

Statistical Analysis. Triangular and Knoop hardness were analyzed by correlation coefficient. Hardness measurements at corresponding depths along the inhibition zone and demineralized lesion were analyzed using one-way analysis of variance (ANOVA) and Fisher's PLSD Test at the 95% level of confidence.

Results

Polarized light microscopy. Demineralized lesions (outer lesions) and inhibition zones along the cavity walls were observed in all samples (Figs. 3.2a and b).

Hardness measurements. The relationship between KHN and HT was analyzed by correlation coefficient. The correlation was highly significant ($r^2 = 0.81$, $p < 0.05$), and a linear relation was found between Knoop and triangular hardness numbers (Fig. 3). The equation was $y = 0.93x + 2.40$, indicating that triangular hardness number was almost identical to Knoop hardness number in the case of softened and normal dentin.

Mean values and standard deviations of the triangular hardness of the outer lesions and inhibition layers created by the different materials are depicted in Fig. 3.4. The geometry of both Knoop and triangular indentations on the inhibition zone and on the outer lesion, are presented in Fig. 3.5.

Microhardness of the demineralized lesion was of 28.7 ± 5.9 HT, whereas hardness of the underlying normal dentin 52.4 ± 5.7 HT. The mean triangular hardness of the inhibition zone adjacent to the Fuji II restoration was of 59.2 ± 3.8 HT, values which were not significantly different from those of the sound dentin. The hardness of the inhibition layer created by Fuji II was identical throughout the depth of this layer. Fuji II LC produced an inhibition layer with an average HT number of 48 ± 3.5 and like Fuji II,

the hardness of this layer was constant at all depths. Vitremer produced an inhibition layer with the initial hardness being similar to that of the outer lesion (28.8 ± 8.8 HT and 26.9 ± 3.8 HT, respectively). Nonetheless, this hardness gradually increased as the distance from the top increased, and at 300 μm, it was identical to that of Fuji II LC.

Calcium and Phosphorous content. Line-analyses indicated the existence of calcium and phosphorous in the inhibition layer adjacent to Fuji II, as well as Vitremer and Fuji II LC (Fig. 3.6). This correlates well with the triangular hardness findings.

Discussion

Bovine teeth were selected as a substitute for human teeth because of the large number of teeth required and human teeth are scarce. The size of bovine teeth simplifies the experimental procedures being beneficial for screening different products.

An acidic buffered solution (pH = 4.5) was used in this study, which does not simulate the oral environment where the pH tends to fluctuate. However, it permitted rapid and consistent formation of outer lesions and inhibition zones that are exemplified in Figs. 3.2a and b. The resulting lesions and inhibition zones were comparable to previous studies which used different acidic solutions and pH (Silverstone, 1967; Swift, 1989; Almqvist and Lagerlof, 1993).

Considerable research has been carried out investigating demineralization, remineralization, and fluoride uptake by the enamel and dentin (Souder and Schoonover, 1944; Swartz and Phillips, 1952; Caldwell *et al.*, 1958; Craig and Peyton, 1958; Hegdahl and Haebö, 1972; Davidson *et al.*, 1974; Arends *et al.*, 1979; Arends *et al.*, 1980; Herkströter *et al.*, 1989). However, microhardness of the inhibition zones created by various fluoride-releasing materials has not been measured. Arends *et al.*, (1980) reported that an empirical linear relationship exists between the lesion depth of artificial enamel carious lesions and Knoop microhardness indentations. Their findings, although for enamel, corresponded with our results for dentin considering the extent of the demineralized lesion.

Because the size of the Knoop hardness indentation on sound bovine dentin was approximately 40 μm at the minimal load (5g) of the apparatus, and it is larger than the width of the inhibition layer (maximum 30 μm), we also utilized a triangular micro indenter with an apex angle of 115° to measure the hardness of the inhibition zone. Indentations were also performed on the outer lesion, adjacent to the Knoop indentations to correlate the two measuring methods. The Knoop hardness results showed a linear correlation with those for triangular hardness ($r^2 = 0.81$, $p < 0.05$) (fig. 3.3), indicating that triangular hardness testing is a good alternative for measuring narrow surfaces.

The mean values of inhibition zones created around Fuji II LC and Vitremer were 48.3 ± 3.5 HT and 44.0 ± 7.6 HT, respectively (Fig. 3.4). These observations suggest that

within our testing parameters, the resin-modified glass ionomer cements produced a softer inhibition zone compared with that of the conventional glass ionomer cement. Our speculations are that the incorporation of resin components into the glass ionomer cement may reduce uniform release of fluoride and other components to the adjacent cavity wall. This could result in a weaker inhibition zone with areas within this zone being more susceptible to demineralization by secondary caries. Line analysis for calcium and phosphorous content confirmed the microhardness results (Fig. 3.6). A greater content of calcium and phosphorous was found in the inhibition layer, but failed to appear in the outer lesions. Fuji II produced an inhibition zone with a greater microhardness and width compared to those formed adjacent to Fuji II LC and Vitremer (Fig. 3.4). Because of the differences in the formulations of these materials, a difference in their respective capacity to inhibit caries may also exist. Although formation of this layer appears to be associated with the level of fluoride release from the restorative material (Swift, 1989; Donly, 1994), conflicting results regarding the amount of fluoride release and caries inhibition from conventional and resin-modified glass ionomer cements have been presented (Forsten, 1991; Forss, 1993; Takahashi *et al.*, 1993; Dunne *et al.*, 1996; Diaz-Arnold *et al.*, 1995; Forsten, 1995). Takahashi *et al.*, (1993) found no statistically significant differences in fluoride release between Fuji II and Fuji II LC, and Dunne *et al.*, (1996) observed that Fuji II LC provided similar in vitro caries inhibition to a conventional glass-ionomer cement. However, Diaz-Arnold *et al.*, (1995) observed that a conventional glass ionomer cement released greater amounts of fluoride than a resin-modified glass ionomer cement after 24 h storage. Forsten (1995) later observed that fluoride levels released by a resin-modified glass ionomer cements were higher or the same as that for a conventional glass ionomer cement. An explanation for the variation in these results may be the different content of fluoride in the powder and different methods used to determine fluoride release.

Another report has suggested that glass-ionomer cements may promote hypermineralization of carious lesions by possibly depositing minerals, therefore increasing acid resistance (ten Cate and van Dunned, 1995). In the oral environment in addition to pH, saliva proteins and buffer systems may affect fluoride release and dissolution, as well as erosion of non-soluble components of glass ionomer cements (Rezk-Lega *et al.*, 1991; Forss, 1993). Thus, factors such as material composition and release of other elements from the glass ionomers may be more significant and may have a greater influence on formation of the inhibition zone, other than fluoride release alone.

In conclusion, a linear correlation between triangular and Knoop hardness numbers was obtained. The triangular hardness test was demonstrated to be excellent for examining very narrow areas. Hardness of the inhibition zone adjacent to the conventional glass ionomer cement was significantly higher than that of the inhibition zones adjacent to the resin-modified glass ionomer cements.

Table 3.1. Restorative materials employed

Material	Brand name	Content	Batch
Conventional Glass-ionomer Cement	Fuji Ionomer Type II	Conditioner: 10% polyacrylic acid Powder: Fluoro-aluminosilicate glass Liquid: Acrylic-maleic acid copolymer, Polybasic carboxylic acid, Water P/L ratio = 2.7(g/g)	071141 300771 250621
Resin-modified Glass-ionomer Cement	Fuji Ionomer Type II LC	Conditioner: 10% polyacrylic acid Powder: Fluoro-aluminosilicate glass Liquid: Acrylic-maleic acid copolymer, HEMA, Water, CQ P/L ratio = 3.0(g/g)	071141 071241 291141
Resin-modified Glass-ionomer Cement	Vitremer	Primer: 46% HEMA, 39% Ethyl Alcohol, Powder: Fluoro-aluminosilicate glass, potassium persulfate, ascorbic acid Liquid: 50% Polycarboxylic acid copolymer, 20% HEMA, Water, 13% carboxylic acid copolymer	3303P 3303A3 3303L

Chemical Names for Abbreviations: HEMA: 2-hydroxy ethylmethacrylate; CQ: Camphorquinone

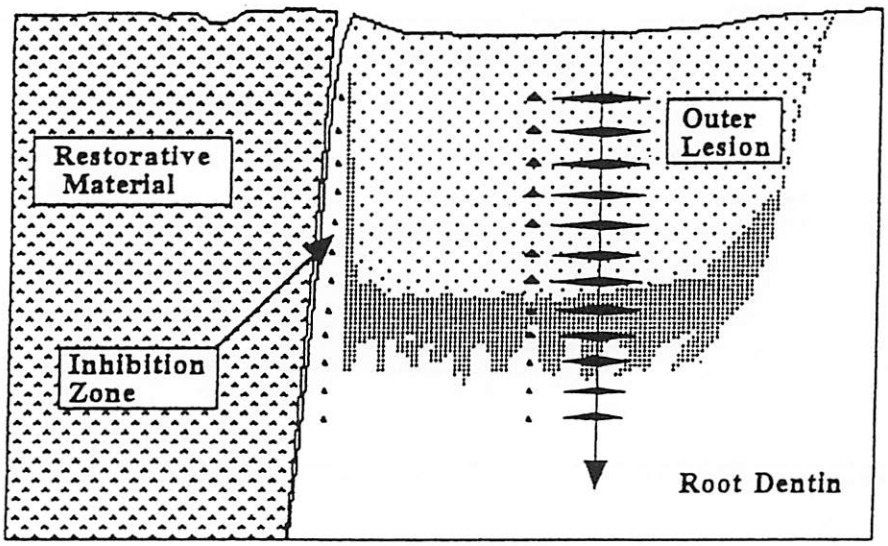


Figure 3.1. Schematic diagram of specimens subjected to acid demineralization. It represents a specimen with an inhibition layer adjacent to the restoration. Large indentations represent KHN measured on the outer lesion and intact dentin. Small triangular indentations represent HT measured both on outer lesion and inhibition zone.

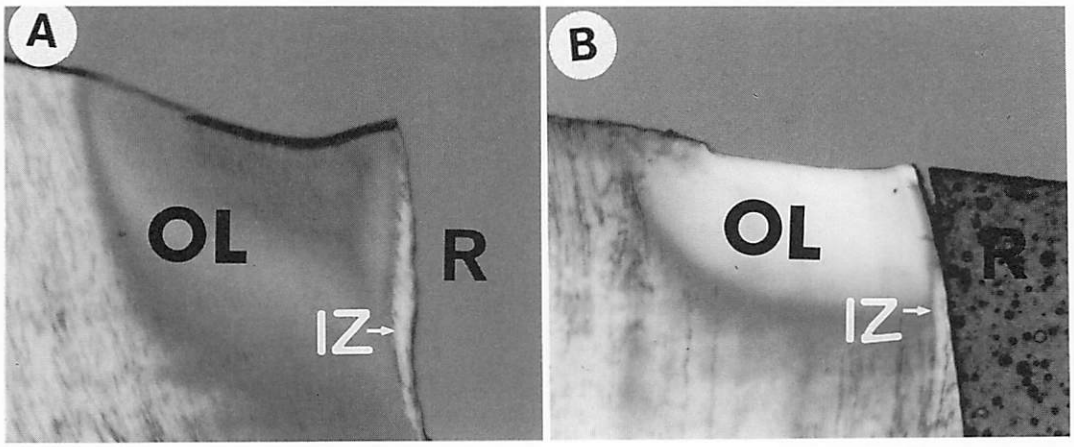


Figure 3.2. Polarized light microscope photographs of artificial caries lesions and inhibition layers adjacent to (A) Conventional GIC and (B) Resin-modified GIC. The conventional GIC was lost during slicing procedure. R= Restorative Material, OL= Outer Lesion, IZ= Inhibition Layer. Magnification x 20.

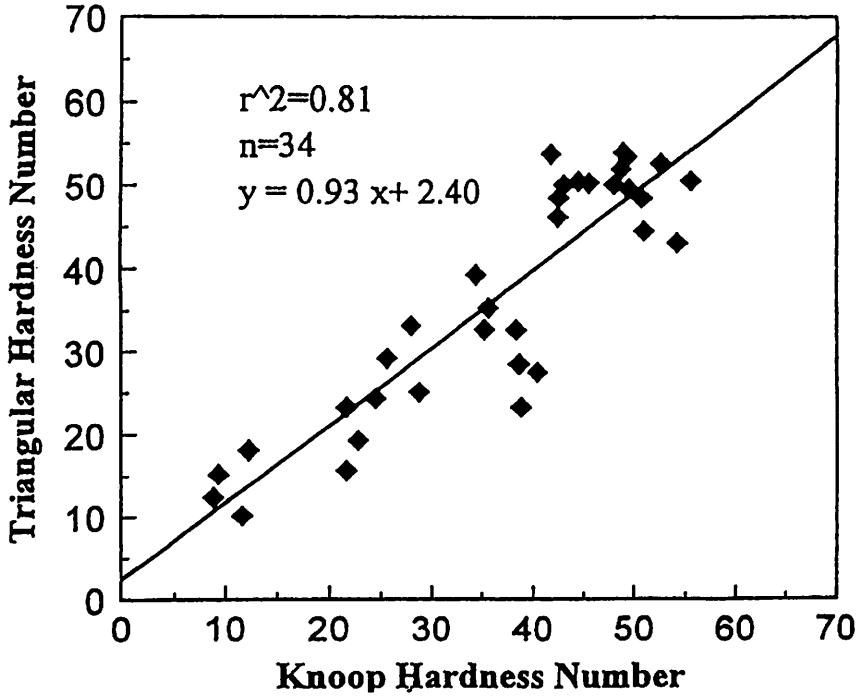


Figure 3.3. Relationship between Knoop and triangular hardness numbers.

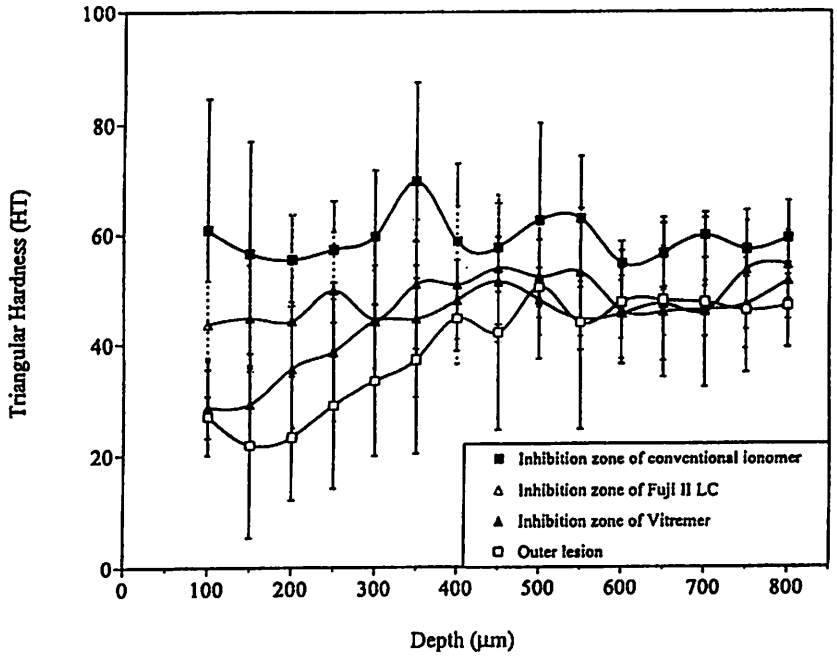


Figure 3.4. Mean values of HT measured along the depth of the inhibition layers and outer lesions. Note that differences between hardness of IZ for the different materials and outer lesion became insignificant beyond 400μm.

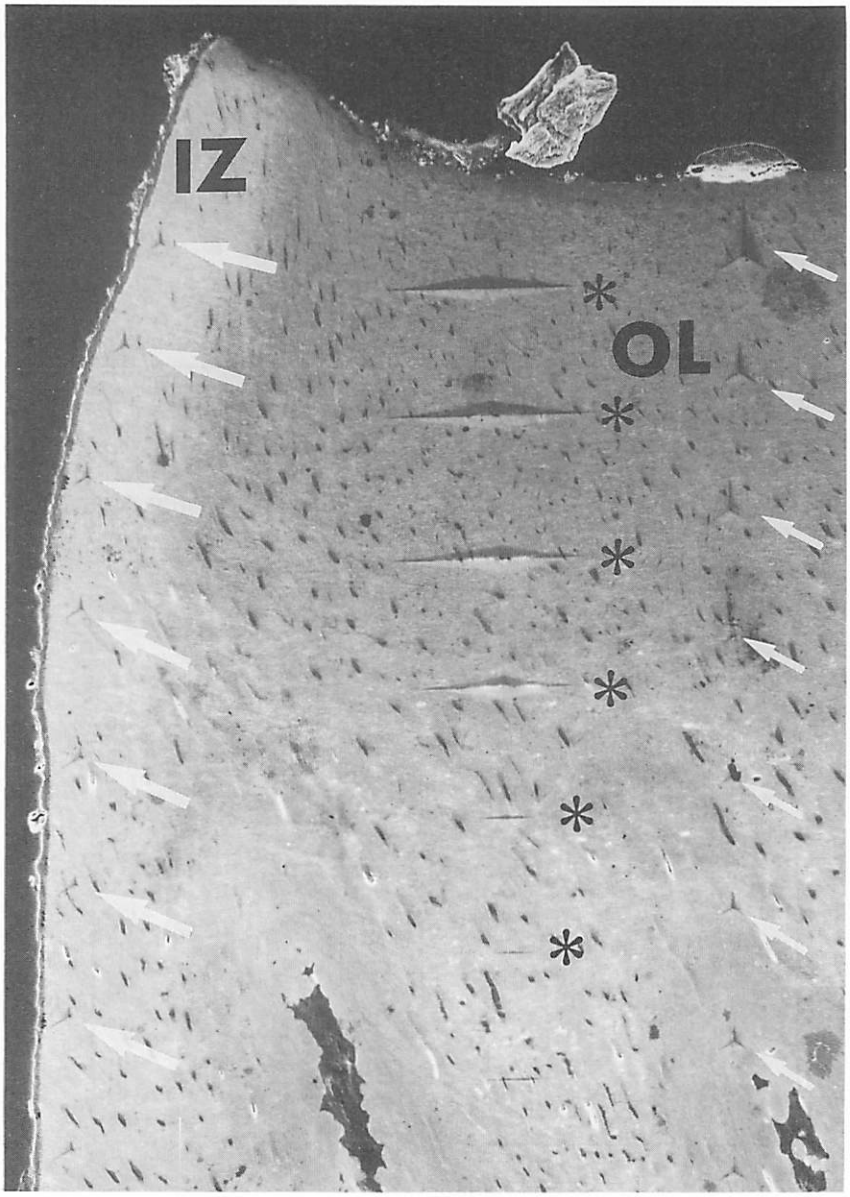


Figure 3.5. SEM photograph of outer lesion an inhibition layer. The arrows indicate triangular hardness indentations. Asterisks indicate Knoop hardness indentations. IZ = Inhibition zone, OL = Outer lesion. Magnification x 300.

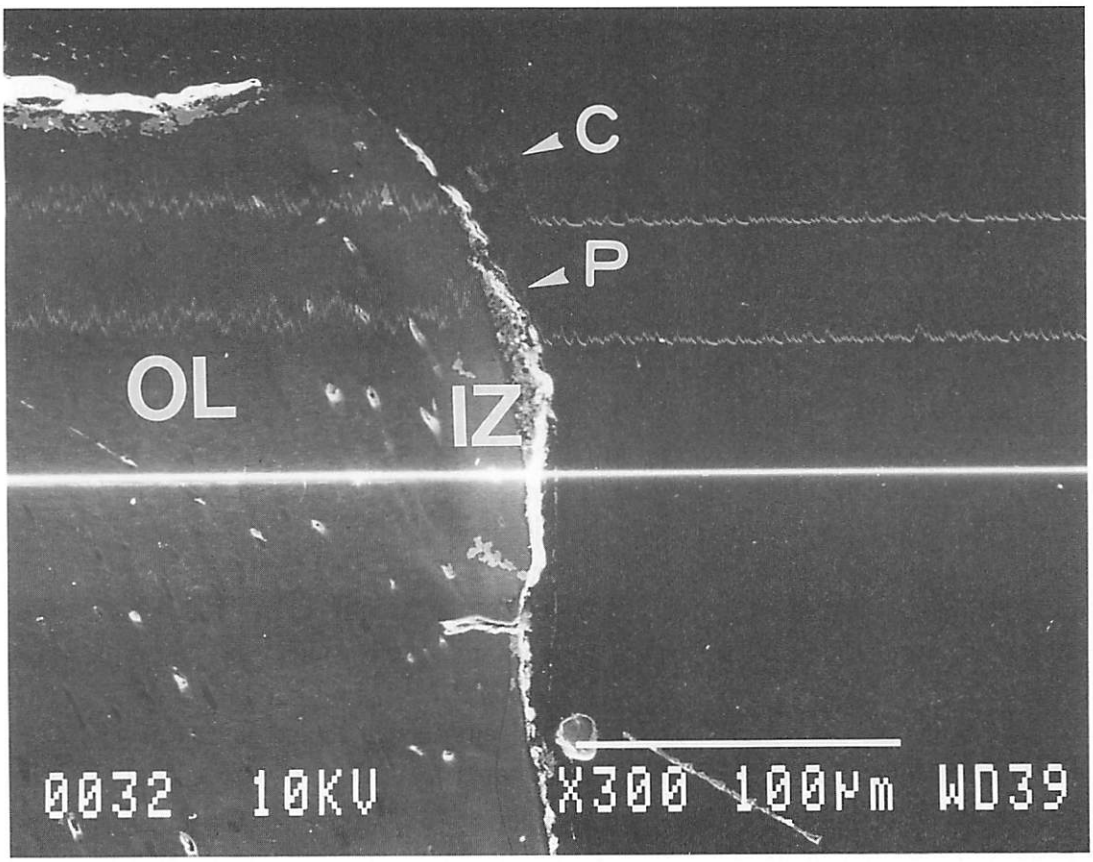


Figure 3.6. SEM photograph and line-analysis of calcium and phosphorous contents in the inhibition layers produced by Fuji II. Note existence of calcium and phosphorous on the inhibition layer and absence in the outer lesion. IZ = Inhibition layer, OL = Outer lesion. Magnification x 300.

Chapter 4

Bond strength and interface micromorphology of an improved resin-modified glass ionomer cement.

Introduction

Glass ionomer cements possess unique properties as a restorative material, leaching fluoride and bonding dynamically to tooth structure through ion exchange (McLean, 1988). However, they are very technique sensitive, due to water uptake and loss, they lack toughness, and they exhibit a high degree of porosity (McLean and Wilson, 1977).

Recently, by mixing the glass ionomer cement constituents with resinous components, a new generation of restorative cements has been developed, namely resin-modified glass ionomer cements (RmGIC). The RmGIC undergoes a two-type setting reaction. First, an acid/base reaction occurs by mixing the glass powder and the polyalkenoic liquid. Then, with exposure to visible light, polymerization takes place, due to the incorporation of methacrylate groups and light activators (Watson, 1990; Mitra, 1991). The RmGIC retains the beneficial properties of the conventional glass-ionomer cements, such as releasing fluoride and physicochemical bonding to dental tissue, but with improved mechanical properties, working time, esthetics, and decreased sensitivity to water (Mitra, 1991; Walls, 1986; Mount, 1993). Therefore, these materials have become popular as an esthetic filling material to restore carious lesions or for patients with a high caries risk.

Since the conventional glass ionomer cements can adhere physicochemically to the tooth structure, they were initially placed directly into the cut cavity, without any prior surface treatment. However, the smear layer which covers the cut dental surfaces (Pashley, 1984) may break cohesively and fail during polymerization shrinkage (Tao and Pashley, 1988). It was reported that bond strength to dental tissue without pre-treatment was inconsistent (Hewlett *et al.*, 1991; Ngo *et al.*, 1986) and that clinical retention (Ngo H *et al.*, 1986) and shear bond strength to dentin could be improved by removing the smear layer (Powis *et al.*, 1982). Since then, pre-treating the cavity walls with acid solutions became routine before restoring with a glass ionomer or a resin-modified glass ionomer cement.

Polyacrylic acid is an effective pre-treatment agent, however, to date there is no consensus as to the optimal concentration and application time. Fuji II LC requires dentin conditioning with a 10% polyacrylic acid solution (DC) for 20 seconds to achieve greater bond strengths. With the attempt to shorten operating time and create prompt bond

strengths to both enamel and dentin, a 20% polyacrylic acid conditioner containing 3% $AlCl_3$ to be applied for 10 s was developed, along with an improved version of Fuji II LC which is superior in polishability by minimizing powder particle size. The purposes of this study were to evaluate the effect of the new cavity conditioner (CC) and improved Fuji II LC (Fuji II LC-I), on shear bond strength to dentin and enamel, and to analyze the micromorphology appearance of the cement/tooth interface.

Materials and Methods.

Ninety-six crowns of stored frozen bovine incisors were cut from their roots at the cementum-enamel junction, taking care not to remove the pulp tissue. The teeth were divided into enamel and dentin substrates for bonding groups, and then each group subdivided into three subgroups: a non-treatment group (control)(n = 12), a group treated with 10% polyacrylic acid (DC+Fuji II LC) (n = 36), and a group treated with 20%polyacrylic acid containing 3%$AlCl_3$ (CC+Fuji II LC-I) (n = 36). The enamel and dentin surfaces were ground flat, and finished with # 600-, 800- and 1000- grit silicon carbide paper in order, under running water. To prevent the embedding medium from entering the pulp chamber, the entrance was covered with wax. The teeth were then embedded in plaster taking care not to contaminate the prepared surface which stood several millimeters above the plaster. A double-sided adhesive vinyl tape with a hole of 3 mm diameter to demarcate the area for bonding was placed on the prepared surface. No surface treatment was performed for the control group. The surfaces were treated either with DC for 20 seconds or CC for 10 seconds, rinsed and air dried. Acrylic tubes of 4 mm diameter x 2 mm height were placed over the demarcated area. The RmGICs and conditioners used in this study, manufacturer, and compositions are listed in Table 4.1. Fuji II LC and Fuji II LC-I were mixed following the manufacturer's instructions and while the surface was still glossy, it was transferred to a C-R syringe tip (Centrix™, Connecticut, USA) and bulk filled into the respective tubes. Excess material was pressed into the tubes with a glass slide and light cured from the top for 60 seconds. Except for the specimens (dentin: n = 12; enamel: n = 12), which were tested 5 minutes after light curing to test early bond strength, the samples were stored 24 hours or 1 week in tap water at 37^{0}C. Before testing the specimens, the vinyl tape was carefully removed so that the shear blade could be positioned closer to the junction of the tooth and cement interface. The jig used to for the shear bond test (ISO standard TR 110405) was mounted in an universal testing machine (Autograph AG-500B, Shimadzu Co., Kyoto, Japan), and the specimens were loaded at a crosshead speed of 1 mm/min. The debonded surfaces were examined visually, and with a stereomicroscope at 20x magnification. The results were analyzed by two-way analysis of variance (ANOVA) and Fisher's PLSD Test.

For the ionomer-tooth interface SEM observation, erupted non-carious human

third molars which had been stored frozen were used. The bonded specimens were prepared using the sandwich technique, described by Inokoshi *et al.*, (1990). The specimens were stored in water for 1 day at 37^{0}C. The bonded 'sandwiches' were then sectioned perpendicular to the flat dentin surface, into approximately equal halves. The two-halves were embedded in a self-curing epoxy resin. After overnight cure, the surfaces of the cut specimens were polished to high gloss with abrasive discs and diamond pastes successively up to 0.25 μm grit size. The specimens were gold-sputter-coated and the polished surfaces observed under an SEM (JXA-840, JEOL, Tokyo, Japan).

After SEM observation, the polishing steps were repeated and the specimens were etched with argon-ion-beam for 270 seconds (Inokoshi *et al.*, 1990) (E1S-1E, Elionix Ltd., Tokyo, Japan). The samples were then gold-sputter-coated and observed again under an SEM.

The specimens were then re-polished with diamond pastes and were subjected to 10% phosphoric acid treatment for 3 to 5 seconds (Gwinett and Kanca, 1992; Sano *et al.*, 1995) followed by 5% sodium hypochlorite immersion for 5 minutes (Wang and Nakabayashi, 1991) for dissolution of the mineral content and removal of exposed collageneous material of the dentin. After being thoroughly rinsed in water, the treated specimens were air-dried, gold-sputter-coated, and observed with the SEM.

For analysis of the effect of dentin pre-treatment with both acid solutions, human dentin disks of approximately 2 mm thick were cut, and finished with 1000-grit silicon carbide paper. The control specimen was observed without any pre-treatment. The remaining samples were treated actively with DC (10% polyacrylic acid) for 20 sec or CC (20% polyacrylic acid containing $AlCl_3$) for 10 seconds. The dentin disks were immersed in 2-Methyl-2-Propanol [c] for one hour, and freeze-dried at -20° C. The specimens were then immediately gold sputter-coated and observed with the SEM.

Results

The resulting shear bond strength values and standard deviations are shown in Tables 4.2 and 4.3. The control group results, which were measured at one day are shown in Table 4.4. The results of two-way ANOVA disclosed no statistically significant interaction between differences of conditioners and storage time for enamel and dentin. For the enamel specimens, no significant difference existed between bond strengths of the two RmGICs systems at 5 minutes. As expected, significant higher bond strengths were obtained for both materials at 1 day, and remained stable after 1 week. However, significantly higher bond strengths to enamel were obtained at 1day and 1week for CC+ Fuji II LC-I, compared to those obtained with DC+ Fuji II LC.

Interestingly, for the dentin specimens, the 5 minutes bond strength of CC+ Fuji II LC-I was significantly higher than that of DC+Fuji II LC. However, the bond

strengths of both materials were similar at 1 day and 1 week. What was initially thought by visual inspection to be adhesive failure, turned out being a mixture of adhesive and cohesive material failure, very close to the bonded area for most of the specimens, when observed with the stereomicroscope.

SEM observations of the polished and argon-ion etched interfaces of DC+Fuji II LC/dentin and CC+ Fuji II LC-I/dentin are shown in Figs. 4.1 and 4.2. In these cross-sections, a resin-rich layer and an indistinct zone (approximately 1μm thick) were noted between the RmGICs and the underlying dentin. Tags which were formed by the resinous part of the RmGICs and which included small glass particles were observed (Fig. 4.3). An indistinct zone beneath the CC+Fuji II LC restoration was also observed following argon-ion etching (Fig.4.4). When the specimens were exposed to phosphoric acid followed by sodium hypochlorite, the existence of a resin-rich layer overlying the demineralized dentin was confirmed (Figs. 4.5 and 4.6). All cross-sections, including the control group, showed resinous tag formation when subjected to phosphoric acid sodium hypochlorite treatment, suggesting that the polyacrylic acid incorporated in the cement, can easily penetrate the tubules (Figs. 4.5 and 4.6).

The morphological characteristics of the dentin surface followed by DC and CC pre-treatment are presented in Figs. 4.7 - 4.10. Both treatments succeeded to remove the smear layer, but varied slightly in the amount of opening of tubule orifices. A clear difference was not observed between the degree of demineralization between both conditioners.

Discussion

Because of the large number of teeth required, and the lack of plentiful human teeth, bovine teeth were selected as a substitute for human teeth for the bond strength portion of this study. It has been previously shown that the size of bovine teeth may eliminate some factors that may influence testing for bond strengths (Suzuki and Finger, 1988; Tagami *et al.*, 1993) and simplifies the experimental procedures. In addition, little or no difference was observed for bond strength tests when comparing human and bovine teeth (Nakamichi *et al.*, 1983; Fowler *et al.*, 1992). Therefore, the use of bovine incisors is beneficial for screening the experimental products.

The smear layer (Fig. 4.11) may cohesively break or fail during shear bond tests or during polymerization shrinkage of the restorative material (Hewlett *et al.*, 1991). As shown in Fig. 4.5, although resinous tags could be formed, the underlying dentin was susceptible to sequential acid/base treatment which removed the smear layer and some of the underlying dentin (Gwinnett and Kanca, 1992). The polyacrylic acid of the cement failed to penetrate into the intertubular dentin or to improve bond strengths. By modifying or removing the smear layer, Hinoura *et al.* (1991) showed that this problem

could be overcome. The results of this study correspond with previous reports, and confirm the fact that RmGICs provide considerable bond strengths, when they followed an acidic pre-treatment of dentin or enamel (Holtan *et al.*, 1990; Bell and Barkmeier, 1994; Triana *et al.*, 1994). Pre-treatment of dentin with acidic conditioner removes the smear layer and demineralizes the superficial dentin layer, allowing the HEMA in the RmGICs to penetrate the exposed collagen network (Friedl *et al.*, 1995; Titley *et al.*, 1996).

The shear bond strengths to dentin found in this study for DC+Fuji II LC at 1day and 1week (14.4 and 13.2 MPa, respectively) correspond to values previously reported ranging between 11 and 15 MPa (Bell and Barkmeier, 1994; Triana *et al.*, 1994; Friedl et al., 1995; Charlton and Havemann, 1994; Kato *et al.*, 1995; Fritz *et al.*, 1996). Our enamel bond strengths for 1day and 1week were 15.9 and 16.3 MPa respectively, compared to previously reported values (Charlton and Havemann, 1994; Kato *et al.*, 1995; Burgess and Burkett, 1993; Swift *et al.*, 1995) ranging from 9 to 14 MPa . On the other hand, the new system (CC+ Fuji II LC-I), showed greater bond strength to dentin 5 minutes after light curing, remaining statistically unchanged to 1day and 1week periods. This is possibly the greatest clinically-related advantage of this new system over DC/Fuji II LC, yielding higher early bond strengths after light curing, and shortening operating time. Regarding bonding to enamel, both bonding systems produced low early bond strengths (i.e. 5 minutes), which increased after 1 day. However, bond strengths for CC+ Fuji II LC-I were significantly greater at 1 day and 1week. The reason for the different results between early bond strengths at 5 minutes to enamel and dentin is still unclear, but may be due to the difference in nature and water content of the substrates.

Through visual inspection, most of the specimens appeared to show an adhesive fracture pattern. However, when examined under 20x magnification, the surfaces seemed to be covered with a fine film, suggesting a cohesive fracture close to the bonded surface within the cement. This film has been previously observed and classified by Berry and Powers (1994).

As an additional study (unpublished data), the RmGICs were exchanged, so that Fuji II LC was applied to the CC-treated surface, and Fuji II LC-I was applied to the DC-treated surface. These results indicated similar data as the ones obtained in this study, indicating that slight differences in bond strengths were due to the change in pre-treatment conditioner. The CC is composed of 20% polyacrylic acid containing 3% $AlCl_3$. It is thought that $AlCl_3$ strengthens the collagen fibers after demineralization (information provided by the manufacturer).

The interface micromorphology between Fuji II LC/dentin and Fuji II LC-I/dentin showed tags within in the dentinal tubules, formed by the resinous part of the material, which were also previously described (Kato *et al.*, 1995). Although it may be assumed that CC would produce a slightly greater depth of demineralization of the dentin surface (Fig. 4.2), the bond strengths of both materials to dentin were not significantly

different. Other than resin tags, a resin-rich layer and an indistinct zone were observed between the RmGICs and the underlying dentin. The addition of $AlCl_3$ may have avoided the complete collapse of demineralized collagen, enabling perhaps, an optimal permeability of HEMA, which is necessary for maximal bond strength (Van Dijken, 1992).

Microphotographs of Fuji II LC or Fuji II LC-I bonded to enamel showed similar interface appearance, although significantly different bond strengths were obtained at 1 day and 1 week. Carvalho *et al.* (1995) have suggested that micromechanical interlocking may exist at the interface of a RmGIC. Our SEM observations did not clarify if other mechanisms, besides micromechanical interlocking, are responsible for the higher bond strengths.

The new version of RmGIC with a cavity conditioner reduces operating time, and showed significant improvement in early bond strength both to enamel and dentin. Being by nature a hybrid material, which combines the beneficial properties of conventional ionomers and resin composites, release fluoride, and bonds efficiently to enamel and dentin, this new system may be an excellent restorative material for caries-prone patients.

Table 4.1. RmGICs, conditioners, compositions, batch numbers, and manufacturer.

Brand name	Abbreviation	Content	Batch	Manufacturer
Fuji Ionomer Type II LC	Fuji II LC	P: Fluoro-aluminosilicate glass L. Acrylic-maleic acid copolymer, HEMA, Water, CQ P/L ratio = 3.0(g/g) conditioner: 10% polyacrylic acid	#071241 #291141 #071141	GC Corp. Hasunuma,Tokyo, Japan
Fuji Ionomer Type II (Improved)	Fuji II LC-I	P: Fluoro-aluminosilicate glass L: Acrylic-maleic acid copolymer, HEMA, Water, CQ P/L ratio = 3.2g/1.0g conditioner: 20% polyacrylic acid containing 3% $AlCl_3$	#120161 #170161 #300651	GC Corp. Hasunuma,Tokyo, Japan

Table 4.2. Shear bond strength (MPa ± S.D.) of Fuji II LC and Fuji II LC-I to enamel.

	5 min	1 day	1 week
DC+Fuji II LC	4.0 (1.7)	15.9 (1.8)	16.2 (2.6)
CC+Fuji II LC-I	6.7 (3.1)	20.1 (6.6)	19.7 (1.5)

Bars indicate no statistically significant difference among figures.

Table 4.3. Shear bond strength (MPa ± S.D.) of Fuji II LC and I-Fuji II LC to dentin.

	5 min	1 day	1 week
DC+Fuji II LC	9.5 (4.0)	14.4 (3.0)	13.2 (1.3)
CC+Fuji II LC-I	13.4 (2.2)	14.4 (5.7)	16.4 (2.0)

Bars indicate no statistically significant difference among figures.

Table 4.4. Shear bond strength (MPa ± S.D.) of Fuji II LC and Fuji II LC-I to untreated enamel and dentin (1 day).

	Enamel	Dentin
Fuji II LC	8.7 (5.1)	5.8 (4.0)
Fuji II LC-I	3.5 (1.0)	5.1 (3.0)

Bar indicates no statistically significant difference among figures.

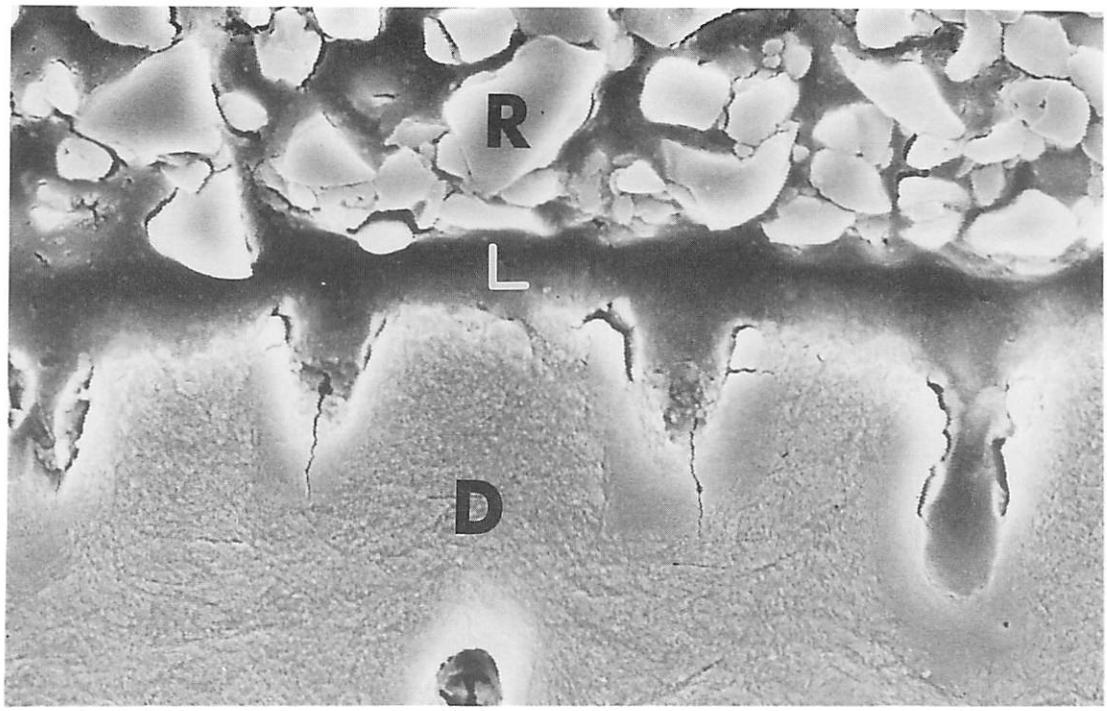


Figure 4.1. Polished surface of DC+ Fuji II LC/dentin interface. A resin rich layer (L) was observed between the RmGIC (R) and underlying dentin (D). SEM. Bar = 1μm.

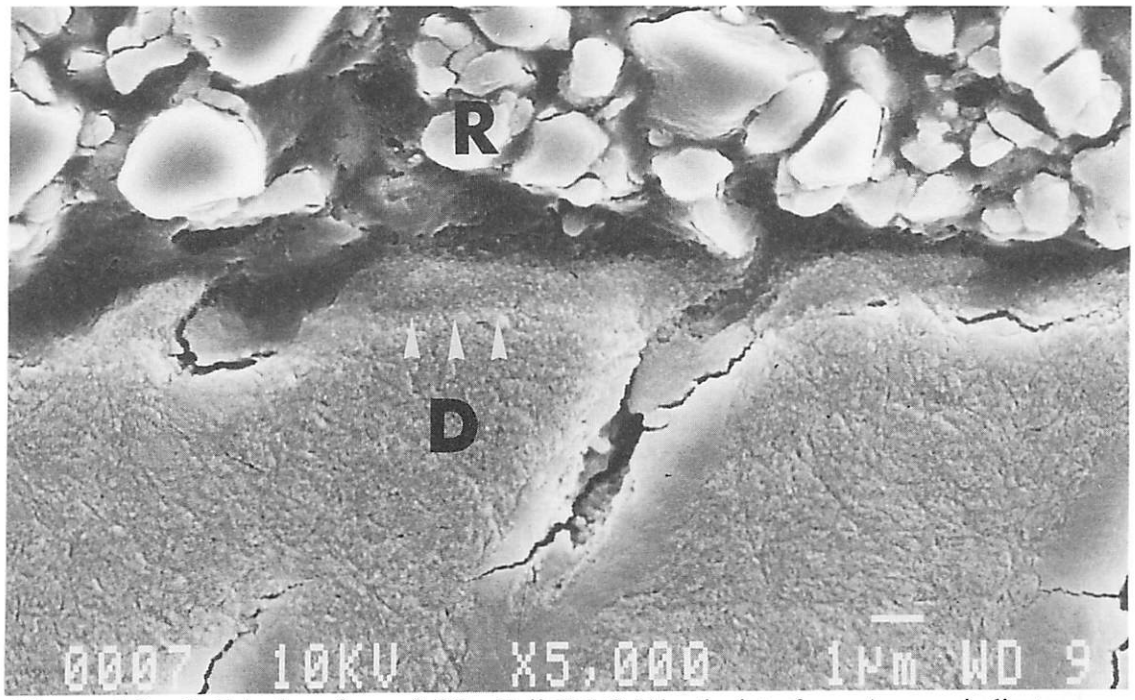


Figure 4.2. Polished surface of CC+ Fuji II LC-I/dentin interface. Arrows indicate an indistinct zone between the RmGIC (R) and dentin (D). SEM. Bar = 1μm.

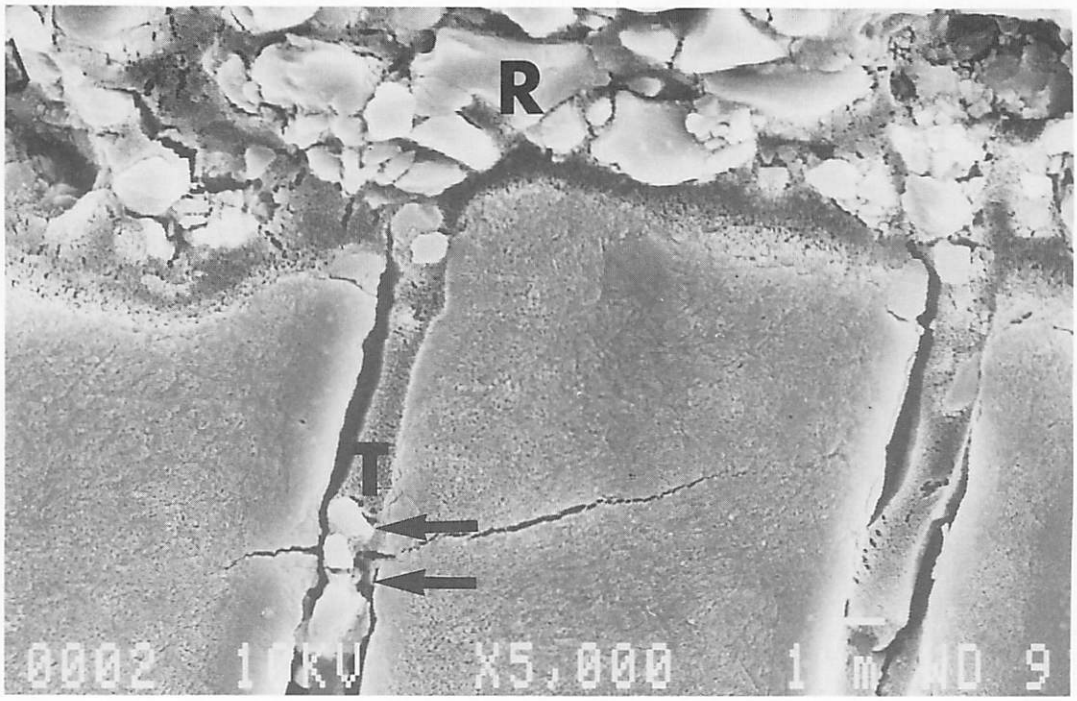


Figure 4.3. DC+ Fuji II LC/dentin interface after argon-ion etching. RmGIC (R) penetrated the dentin tubules (T). Arrows indicate glass particle within the tag. SEM. Bar = 1μm.

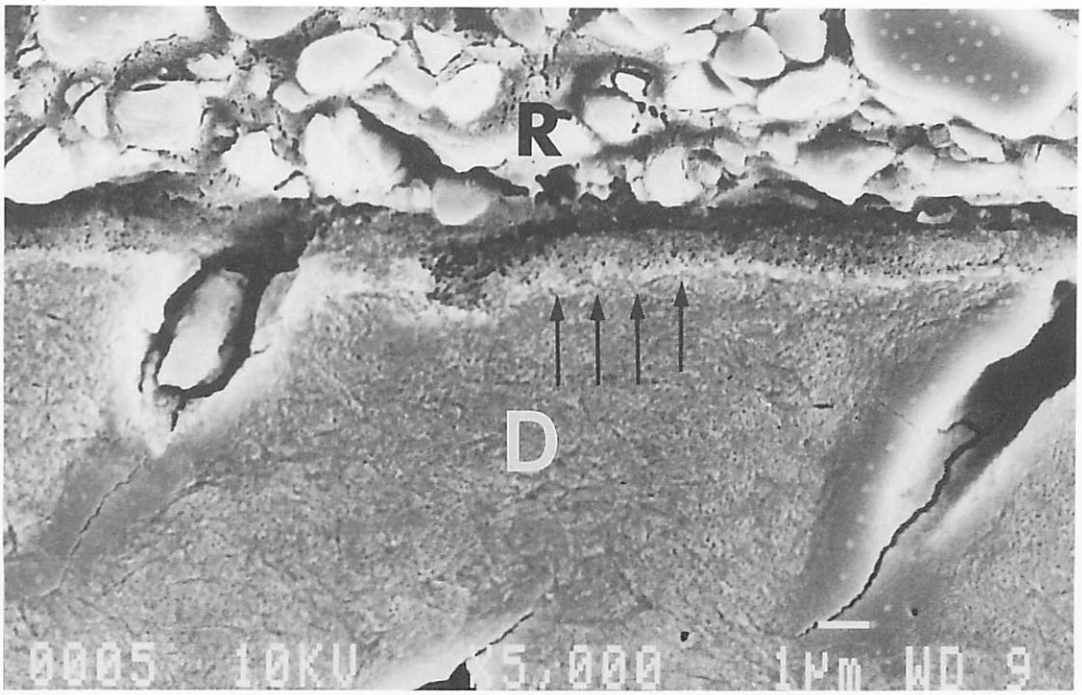


Figure 4.4. CC+ Fuji II LC-I/dentin interface after argon-ion etching. Arrows indicate an indistinct zone between the RmGIC (R) and the underlying intact dentin (D). SEM. Bar = 1μm.

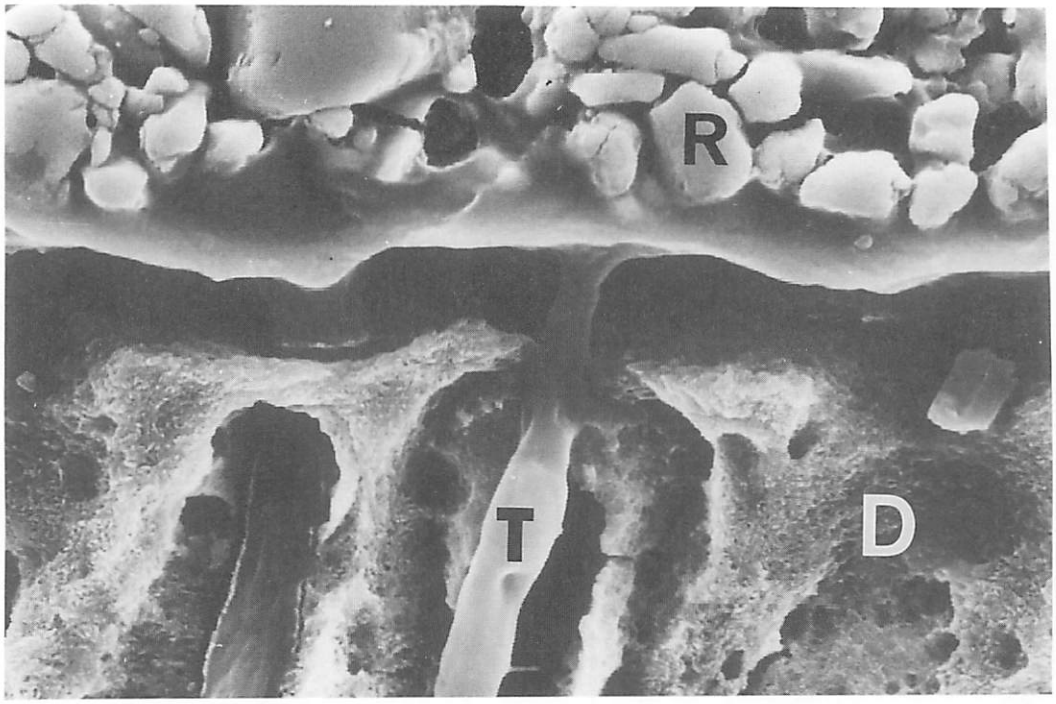


Figure 4.5. RmGIC/dentin interface of the control group (without surface pre-treatment) after treatment with 10% phosphoric acid and 5% sodium hypochlorite. Resinous tag formation (T) is observed, however, separation between the RmGIC (R) and the underlying dentin (D) has occurred. SEM. Bar = 1μm

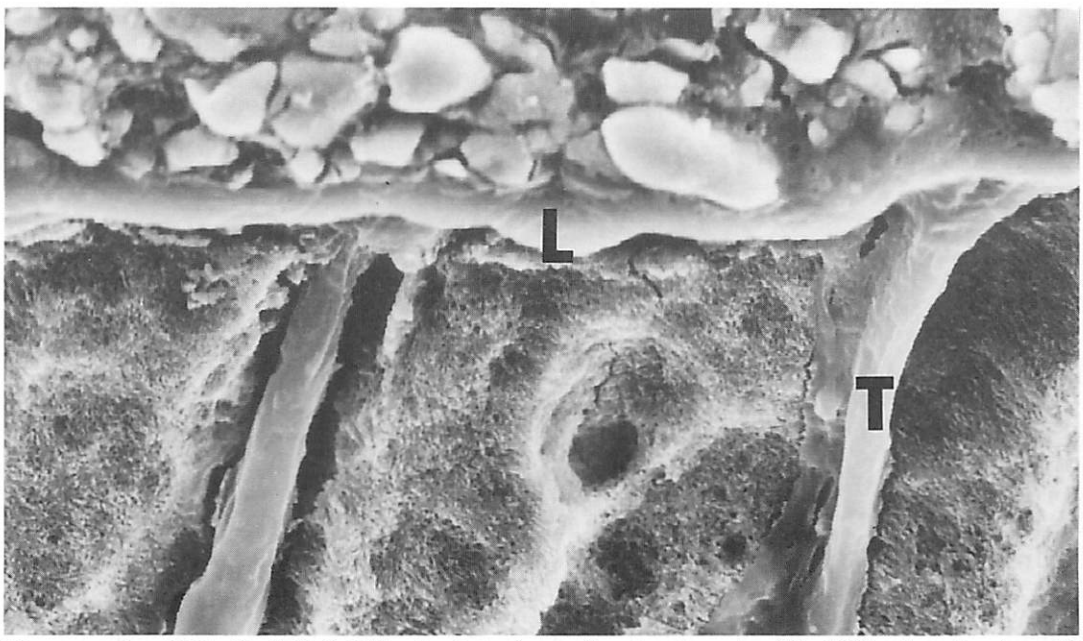
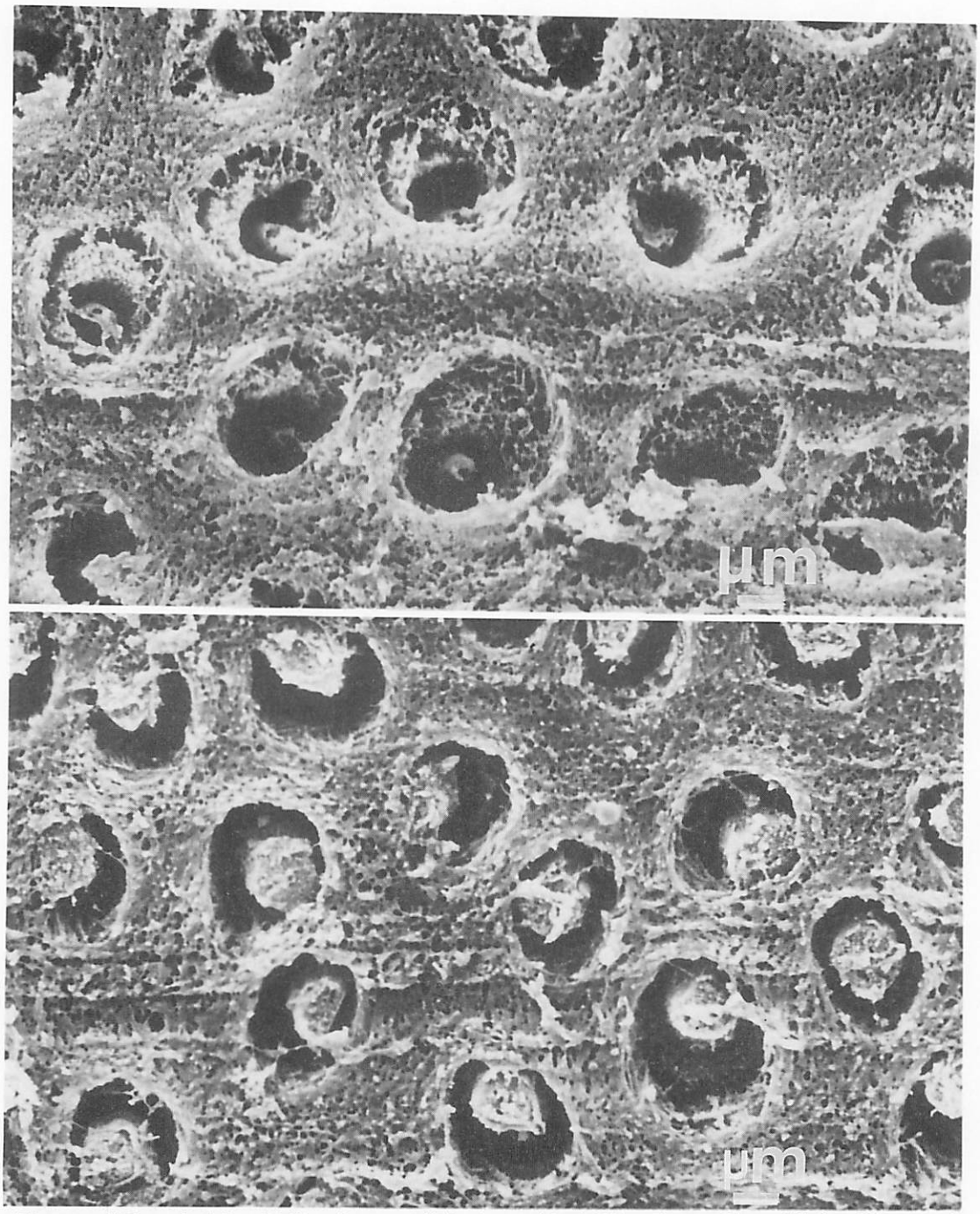
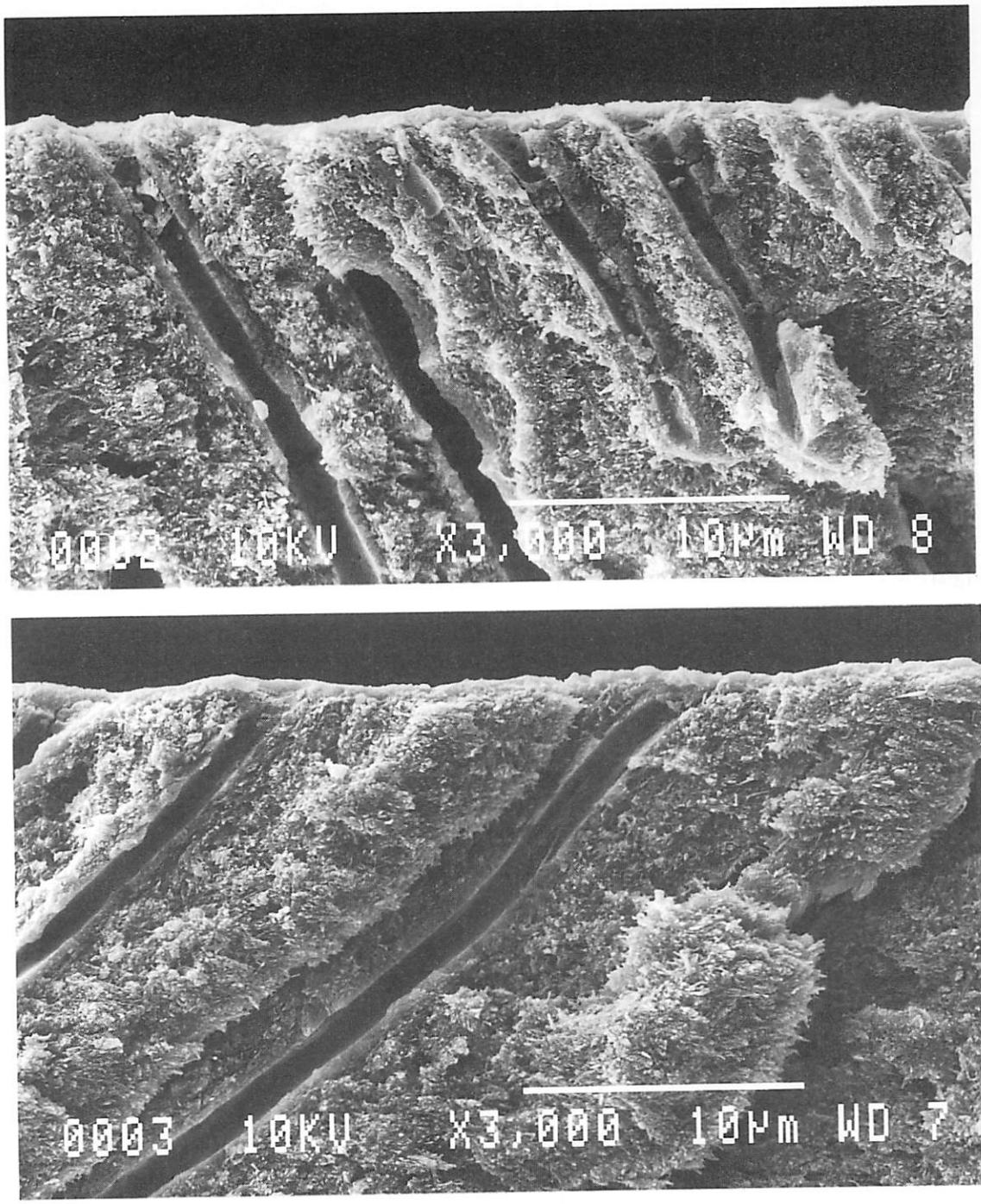


Figure 4.6. DC+ Fuji II LC/dentin interface after treatment with 10% phosphoric acid and 5% sodium hypochlorite. Resinous rich layer (L) and tag formation (T) are observed, without separation at the interface. SEM. Bar = 1μm.



Figures 4.7 and 4.8. Following conditioning with 10% polyacrylic acid for 20 s, and 20% polyacrylic acid for 10 s respectively, the collagenous intertubular network became evident. The tubules are superficially widened due to slight loss of peritubular dentin, however, no significant difference in demineralization degree was noted between both conditioners. SEM Bar = 1µm.



Figures 4.9 and 4.10. Following conditioning with 10% or 20% polyacrylic acid for 20 s and 10 s respectively, the dentin disks were freeze-dried and fractured. No significant difference of degree of demineralization was noted. SEM. Bar = 10µm.

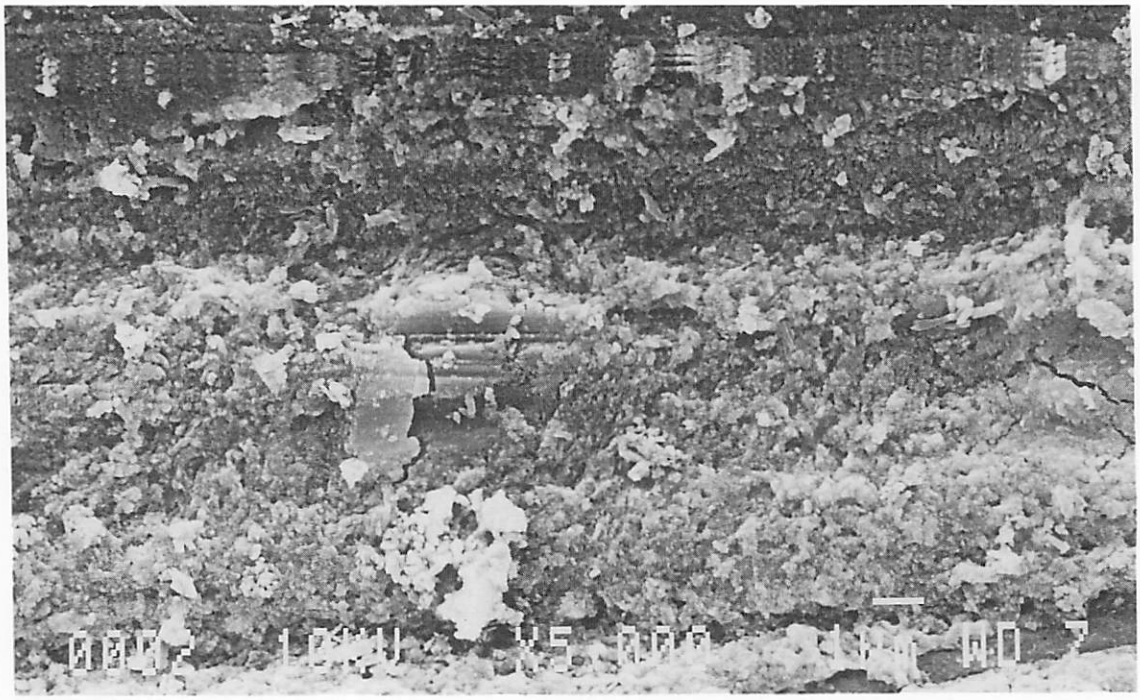


Figure 4.11. Dentin smear layer produced by grounding with sequentially finer abrasives up to 1000-grit silicon carbide paper. SEM. Bar = 1μm.

Chapter 5

Adhesion of resin-modified glass ionomer cements using resin bonding systems

Introduction

Since the development of glass ionomer cement by Wilson & Kent (1972), these materials have undergone many modifications and improvements. The incorporation of methacrylate groups to conventional glass ionomers improved handling and working characteristics (Antonucci *et al.,* 1988; Mitra, 1989), as well as reducing their brittleness, moisture sensitivity, and improving bond strengths to the tooth (Mathis and Ferracane, 1989; Holtan *et al.*, 1990; Wilson, 1990; Mitra, 1991). Resin-modified glass ionomer cement (RmGIC) is considered a good substitute for dentine in deep cavities, due to their physical properties, while resin composite is a good substitute for enamel because of their superior strength, surface integrity, and esthetics (McLean, 1996). Adhesion of these materials to enamel has been successfully achieved; however, with regard to dentine, forces of contraction of the RmGIC during light irradiation may lead to failure of the bond between the RmGIC and dentine.

Volumetric shrinkage during polymerization of the RmGIC (Feilzer *et al.*, 1988) can affect bond strength of this material to dentine. While initial set is rapid by light activation, the chemical setting reaction continues for up to 24 hours, and is responsible for the final physical properties of the cements (Bourke *et al.*, 1992). Similar to resin composites, RmGICs shrink approximately 3% in volume during setting (Feilzer *et al.*, 1988). Although this intrinsic stress can be relieved by later water sorption (McLean, 1996, Davidson and De Gee, 1984) the initial polymerization shrinkage of the RmGICs may lead to adhesive failure at the tooth-filling interface compromising the longevity of the restoration (Ciucchi *et al.*, 1997).

Bond strength of RmGICs to dentine is inconsistent when the smear layer is not removed (Helwett *et al.*, 1991), probably because the smear layer can break cohesively and fail as a result of the polymerization shrinkage (Tao and Pashley, 1988). Pre-treatment with polyacrylic acid removes the smear layer and improves the bond strengths of the RmGICs to dental tissue (Triana *et al.*, 1994; Carvalho *et al.*, 1995; Swift *et al.*, 1995). Other conditioners that demineralize the dentine surface have also shown improved bond strengths, suggesting the importance of micromechanical-bonding (Carvalho *et al.*, 1995; Smith, 1992; Titley *et al.*, 1996).

Increase of the bond strength of RmGIC to dentin and consequent prevention of immediate separation at the interface may be altered by first bonding the dentin with an adhesive resin, which has demonstrably greater bond strengths. This study aimed to

evaluate the effect of three adhesive resin systems on shear bond strength of RmGIC to dentine, and to analyze the interfacial micromorphology of the cement/adhesive/tooth interface.

Materials and methods

Specimen Preparation for Shear Bond Strength Test

The resin-modified glass ionomer cements, adhesive systems used in this study, and their manufacturers are listed in Table 5.1. The adhesive systems used in this study were from the same manufacturer and selected because Clearfil Photo Bond is an acid-etching system that does not require priming, Clearfil Liner Bond is an acid-etching system that requires priming, and Clearfil Liner Bond II is a self-etching primer system. The crowns of 96 bovine incisors, stored frozen before use, were used in this study. The crowns were cut from their roots at the cementum-enamel junction, taking care not to remove the pulp tissue to avoid dehydration of the specimen. Superficial flat dentine surfaces were prepared, and finished with 1000-grit silicon carbide paper under running water. The teeth were embedded in dental stone taking care not to contaminate the prepared surface, which protruded several millimeters above the stone. To prevent the embedding medium from entering the pulp chamber, the entrance was covered with dental wax. After the stone had set, a double-sided adhesive vinyl tape, in which a hole of 3 mm in diameter had been punched, was placed on the prepared surface to demarcate the bonding area. The teeth were then randomly divided into 4 groups for bonding:

Control group: Surface treatment was performed following each manufacturer's instructions. For the specimens to be bonded with Fuji II LC, the dentin substrate was treated with 10% polyacrylic acid for 20 seconds, rinsed with water and gently air-dried. For the specimens to be bonded with Vitremer, the primer was applied to the surface for 30 seconds, gently air-thinned, and light-cured (Optilux 400, Demetron/Kerr, Danbury, CT) for 20 seconds. Twelve specimens were prepared for each material.

Clearfil Photo Bond group: The surfaces were etched with 37% phosphoric acid gel for 30 seconds, rinsed with water and gently air dried. One drop of each Photo Bond adhesive resin base and catalyst were mixed, applied to the etched surface, gently air-blown to remove the alcohol solvent, and light-cured for 10 seconds.

Clearfil Liner Bond group: The surfaces were treated with a gel containing 10% citric acid with 20% calcium chloride for 40 seconds, rinsed with water and gently air-dried. SA Primer (3% N-methacryloyl-5-aminosalicylic acid in alcohol) was applied to the etched surface and gently air-dried until complete evaporation of the alcohol. A thin layer of Photo Bond adhesive resin was then applied to the surface, gently air blown, and light-cured for 10 seconds.

Clearfil Liner Bond II group: One drop each of Liner Bond II self-etching primers A and B were mixed, applied to the surface for 30 seconds and gently air-dried to remove excess of water and alcohol. A thin layer of LB Bond was painted on the surface and light-cured for 20 seconds.

Following the bonding procedures, acrylic tubes 4 mm diameter x 2 mm high were positioned over the bonded area. Fuji II LC or Vitremer were mixed following each manufacturer's instructions, respectively, transferred to a C-R syringe tip (Centrix™, Shelton, Connecticut, USA), and bulk-filled into the tubes. Excess material was pressed into the tubes by placing a glass slide over the tube, and then light cured from the top for 60 seconds. All specimens were then stored for 24 hours in tap water at 37°C prior to bond testing. Twelve specimens were prepared for each bonding system with Fuji II LC and Vitremer.

Before testing the specimens, the vinyl tape was carefully removed so that the shear blade could be positioned at the junction of the tooth and bonded interface. The samples were mounted on the jig for the shear bond test described in ISO standard TR110405 (ISO) and were stressed at a crosshead speed of 1 mm/minute in an universal testing machine (Autograph AG-500B, Shimadzu Co., Kyoto, Japan). The debonded surfaces were examined with a stereomicroscope at 20X magnification. The means and standard deviations were calculated from the bond test data. The results were analyzed by two-way analysis of variance (ANOVA) and Fisher's PLSD Test ($p<0.05$).

SEM Observation

Two erupted non-carious human third molars stored frozen were used for each experimental group and control, within two weeks following extraction. A pair of dentine disks, approximately 1-1.5 mm thick, was cut from each tooth using a low-speed diamond saw microtome (Leitz 1600, Wetzler, Germany) under running water. Disks with enamel remnants and exposure of pulp horns were discarded. One surface of each disk was ground with 600-grit silicon-carbide paper under running water to create a standardized smear layer. The dentine surfaces were bonded in the same manner as for the bond strength test. The specimens were stored in tap water for 24 hours at 37°C. The bonded assemblies were then sectioned perpendicularly to the flat dentin surface, into approximately equal halves disclosing 4 bonded surfaces for SEM observation, and were embedded in a self-curing epoxy resin (Epon 815, Thomide 245, Nisshin EM, Tokyo, Japan). The surfaces of the cut embedded specimens were polished to a high gloss with abrasive discs and diamond pastes successively, down to 0.25 μm particle size. The samples were gold-sputter-coated and observed under the SEM (JXA-840, JEOL, Tokyo, Japan).

After SEM observation, the coated surfaces of the specimens were again polished

and then etched with an argon-ion-beam for 270 seconds (E1S-1E, Elionix Ltd., Tokyo, Japan) to better determine the structures at the bonded interface. The samples were again gold-sputter-coated and observed under the SEM.

The specimens were re-polished a third time. After this, they were subjected to 10% phosphoric acid treatment for 3-5 seconds (Gwinnett and Kanca, 1992), followed by 5% sodium hypochlorite immersion for 5 min (Wang and Nakabayashi, 1991), so as to dissolve mineralized tissue and remove any collagen fibers which had not been enveloped by the adhesive resin (Gwinnett and Kanca, 1992). After thoroughly rinsing in water, the treated specimens were air-dried, gold-sputter-coated, and observed with the SEM.

Results

The mean shear bond strengths and standard deviations for the four groups are presented in Table 5.2. No significant interaction was found between the adhesive systems and the RmGIC when the data was analyzed by two-way Anova.

The shear bond strength of Fuji II LC was significantly higher than that of Vitremer ($p<0.001$) in the control group. When the adhesive systems were applied, a significant increase of the shear bond strengths of Vitremer to dentine occurred, and no significant difference between the two RmGICs could be detected. Although bond strengths increased significantly when Clearfil Liner Bond II was used with Vitremer, this difference was less so for Fuji II LC. Bond strengths for Vitremer seemed to be more affected by the adhesive systems than those for Fuji II LC. The bond strength of Vitremer was low (7.4 MPa) when bonded directly to the dentine, but significantly increased when used in conjunction with the bonding systems (2 of which included dentine etch and one-self-etching)(Photo Bond = 12.0 MPa, $p<0.005$; Liner Bond = 12.6 MPa, $p<0.01$; and Liner Bond II = 17.3 MPa, $p<0.001$). On the other hand, the bond strength of Fuji II LC with Photo Bond (14.5 MPa) or Liner Bond (15.0 MPa) did not differ significantly from the control (13.4 MPa, $p>0.05$). The bond strengths of Vitremer or Fuji II LC applied in conjunction with Liner Bond II were identical (17.3 MPa, $p>0.05$). The failure modes for all materials were classified as mixed with cohesive material failure and adhesive failure between the bonding resin and dentine; however, three LB II specimens showed cohesive failure in dentine.

The RmGIC/dentine control interfaces are shown in Figs. 5.1 and 5.2. SEM photographs of the RmGIC/adhesive/dentine interfaces are shown in Figures 5.3 – 5.8. Even after desiccation and exposure to the high vacuum for SEM observation, no separation between the RmGIC/adhesive interface, and adhesive/dentine interface was observed. The micromorphology of the interfaces exhibited interaction between the adhesive systems and the underlying dentin, demonstrating the formation of hybrid layers. Photo Bond strongly demineralized the underlying dentine, producing a 4 - 5 μm thick

hybrid layer. For Photo Bond specimens only, the layer of bonding resin was not observed between the RmGICs and underlying hybrid layer. Ca-agent created less demineralization of the surface, with a hybrid layer approximately 2 – 3 μm thick being observed. Compared with the other adhesive systems, Liner Bond II produced the thinnest hybrid layer, about 1 – 2 μm thick. However, the bond strength for this system was the greatest and identical for both Vitremer and Fuji II LC. All adhesive systems demonstrated a continuous link between the bonding resins and RmGICs yielding a continuous interface.

Figures 5.7 and 5.8 show Fuji II LC/Liner Bond II/ dentine and Vitremer/Liner Bond II/ dentine interfaces, respectively. Long resin tags penetrating dentinal tubules and a thin hybrid layer were clearly observed. Little effect of the RmGIC on dentin bonding over the adhesive bonding resins was recognized, and particles of the RmGIC were not observed within the resin tags.

Discussion

Because of the large number of teeth required for these types of study, and a lack of human teeth, bovine teeth were selected as a substitute for human teeth in the bond strength testing. The size of bovine teeth may eliminate some factors that can influence bond strength testing, as well as simplify the experimental procedure (Suzuki and Finger, 1988; Tagami *et al.*, 1993). Furthermore, similarities concerning bond strength tests have been reported when comparing human and bovine teeth (Nakamichi *et al.*, 1983; Fowler *et al.*, 1992). Therefore, bovine incisors are believed to be suitable substrates for evaluating bonding systems. On the other hand, because it is also important to evaluate and predict how dental restorative systems affect human dentine, human teeth were used for the SEM observation part of the study.

To date, various attempts have been made to improve mechanical and adhesive properties of RmGICs to enamel and dentine. In order to improve bond strengths of RmGICs to dentine, various acidic conditioners and primers have been used to pre-treat the dentine (Powis *et al.*, 1982; Berry and Powers, 1994). Pre-treatment of the dentine with acidic conditioners removes the smear layer and demineralizes the superficial dentine layer, allowing the HEMA (2-hydroxy ethyl methacrylate) incorporated in the RmGICs to penetrate the exposed collagen fiber network (Titley *et al.*, 1996; Friedl *et al.*, 1995).

The shear bond strengths for Vitremer obtained in this study are comparable to previously reported results ranging between 5.9 to 9.7 MPa (Triana *et al.*, 1994; Bell and Barkmeier, 1994; Berry *et al.*, 1994; Erickson and Glasspoole 1994; Pawlas *et al.*, 1994). The bond strength of Vitremer was low (7.4 MPa) when bonded directly to the dentine, but increased significantly when used in conjunction with the bonding systems. The results for Fuji II LC were similar to results previously published, which ranged from 11.0 to 15.4

MPa (Triana *et al.*, 1994; Friedl *et al.*, 1995; Berry *et al.*, 1994; Charlton. and Havemann 1994; Kato *et al.*, 1995; Fritz and Finger, 1996; Garcia-Godoy; 1992). Interestingly, when the adhesive system Liner Bond II was used, bond strengths for both Vitremer (17.3 MPa) and Fuji II LC (17.3 MPa) increased, being significantly greater than the other groups tested. In previous work, Liner Bond II has been demonstrated to produce greater bond strengths to bovine dentine compared with the other two bonding systems (Burrow *et al.*, 1994) when using a resin composite. This same situation also occurred in this study, therefore, the results in this study demonstrate that RmGICs can bond very well to bonding resins, producing similar results to bonding of resin composites.

The SEM observations disclosed distinct interactions between the bonding resins and the underlying dentine. As expected, the different conditioners and adhesive resins produced different degrees of demineralization and thickness of hybrid layers. It was also noted that the RmGICs exerted little effect on the bonding capacity of the different adhesive systems, and consequently in bond strengths. Since the RmGICs contain HEMA and other resinous components, they were able to interact with the bonding resin forming a chemical union (Figs. 5.3 – 5.8). Interestingly, the Photo Bond group specimens did not display a layer of bonding resin (Figs. 5.3 and 5.4). This may be probably a result of an incompletely polymerized bonding resin and a very thick hybrid layer that may have acted as a sponge absorbing the uncured resin.

Resin-modified glass-ionomer materials have been widely used and are recommended for restoring cervical lesions that include enamel and dentine margins, and for the caries-prone patient because of the possible cariostatic effect of fluoride. Adhesion of recently developed RmGICs to dentine has been improved; however, comparable bond strengths to dentine bonding adhesives have not yet been achieved. Therefore, we improved bond strengths by applying adhesive resins prior to placement of a RmGIC. Our results demonstrated that the shear bond strengths of Vitremer was significantly less when applied directly to the dentine. Since Fuji II LC requires conditioning with 10% polyacrylic acid, and Vitremer requires a primer, the difference in dentine conditioners and material compositions may be the reason for the different bond strength values (Mitra and Kedrowski, 1994; Triana *et al.*, 1994). Current studies are being carried out to analyze what factor was responsible for the increased bond strength.

It has also been previously reported that fluoride ions could diffuse through adhesive resins when RmGICs were coated, although in smaller quantities (Castro *et al.*, 1994). Thus, should it be that fluoride ions could diffuse through adhesive resins, the benefit of using RmGICs over dentine bonding materials may not be lost. Further studies are necessary in order to determine this possibility.

Conclusions

Shear bond strength of Vitremer to dentine was significantly increased when recent adhesive resin systems were used. Shear bond strength of Fuji II LC to dentine significantly increased when the self-etching primer system was used. A technique of bonding dentine with a resin and then applying RmGIC may be a good solution for those materials that produce weak bonding to dentine.

Table 5.1. Restorative materials employed

Material	Brand name	Components	Batch	Manufacturer
Resin-modified glass-ionomer cement	Fuji Ionomer Type II LC	Conditioner: Powder: Liquid:	071141 071241 291141	GC Corp., Tokyo, Japan
Resin-modified glass-ionomer cement	Vitremer	Primer: Powder: Liquid:	3303P 3303A3 3303L	3M Dental Products, MN, USA
Adhesive Resins				
	Clearfil Photo Bond	K-etchant: Photo Bond:	K: 145 Catalyst: 236 Universal: 339	Kuraray Co., Osaka, Japan
	Clearfil Liner Bond	CA-agent: SA Primer: Photo Bond:	1134 021 Catalyst: 191 Universal: 296	Kuraray Co., Osaka, Japan
	Clearfil Liner Bond II	Primer LB Bond	001 0002	Kuraray Co., Osaka, Japan

K-etchant: 37% H_3PO_4; CA-agent: 10% citric acid in 20% calcium chloride; SA Primer: 3% n-methacryloyl 5 aminosalicylic acid in ethanol

Table 5.2. Shear bond strength of Fuji II LC and Vitremer using different adhesive systems and statistical analysis

	Control	Photo Bond	Liner Bond	Liner Bond II
Fuji II LC	13.4 (1.6)	14.5 (2.6)	15.0 (2.9)	17.3 (6.3)
Vitremer	7.4 (2.6)	12.0 (2.0)	12.6 (1.8)	17.3 (4.9)

Bars indicate no statistically difference among figures by the Fisher's PLSD Test ($p>0.05$).

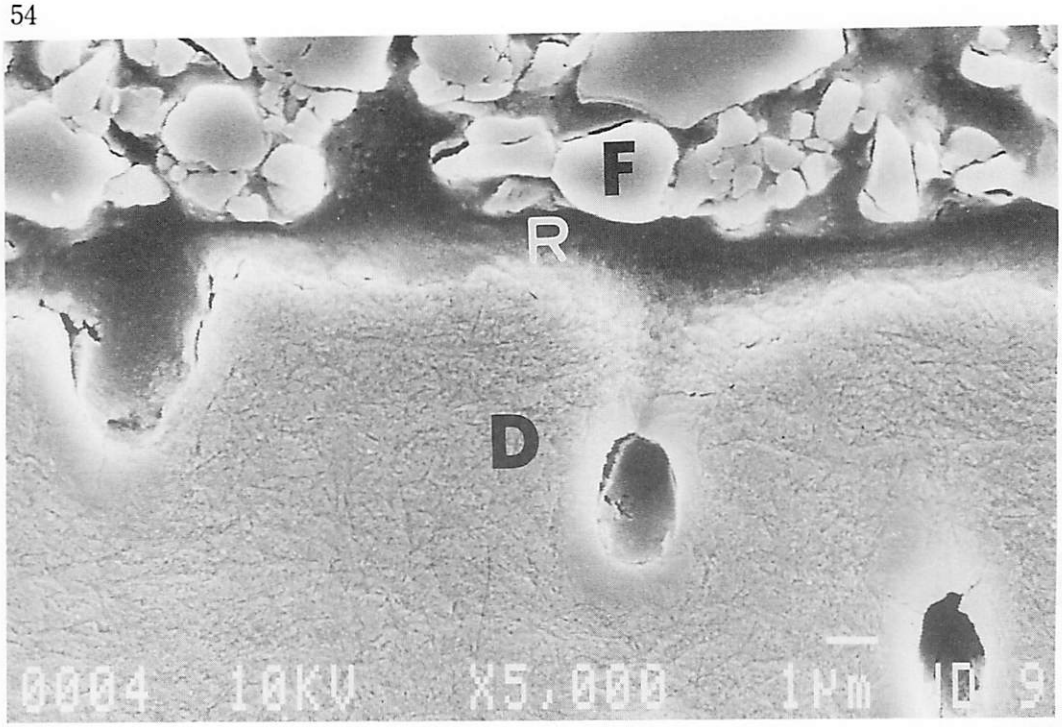


Figure 5.1. Polished Fuji II LC (F) / dentine (D) interface after surface treatment with 10% polyacrylic acid. Note a resin-rich layer (R) that was slightly worn off during polishing procedures.

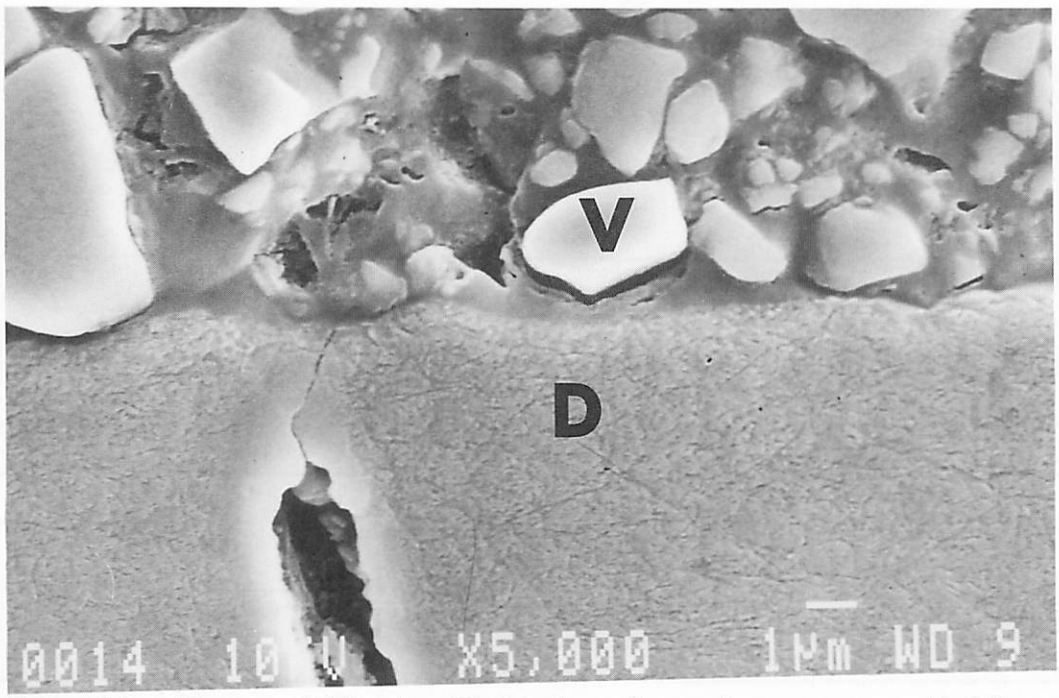


Figure 5.2. Vitremer (V)/dentine (D) interface after surface treatment with Vitremer primer. A resin rich layer can not be clearly observed.

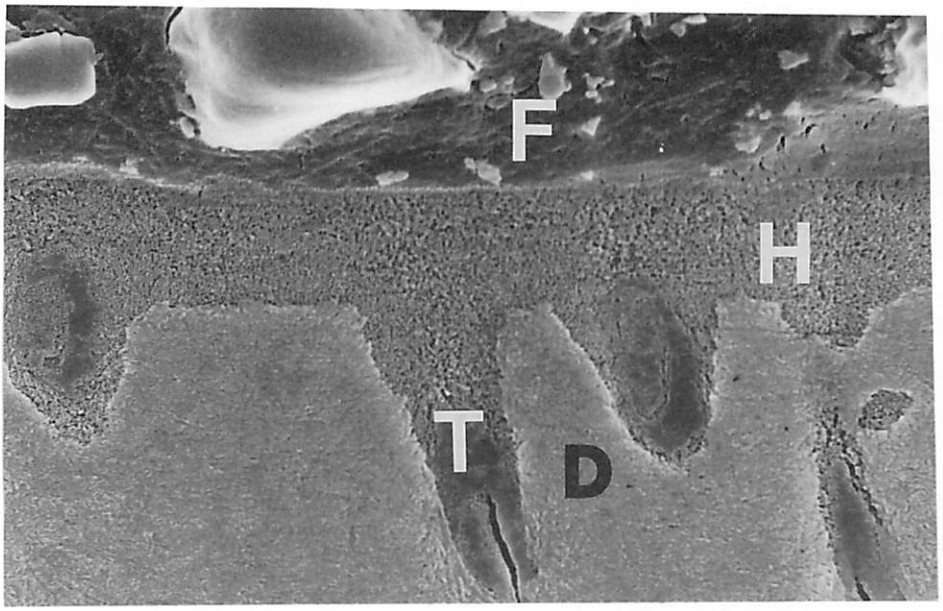


Figure 5.3. Fuji II LC/Clearfil Photo Bond/dentine (D) interface after argon ion beam etching. The specimen displays a 4 – 5 μm thick hybrid layer (H). The bonding resin is imperceptible between the RmGIC (F) and hybrid layer. Note the good interaction between the RmGIC and hybrid layer, and formation of resin tags (T) although bonding resin is imperceptible.

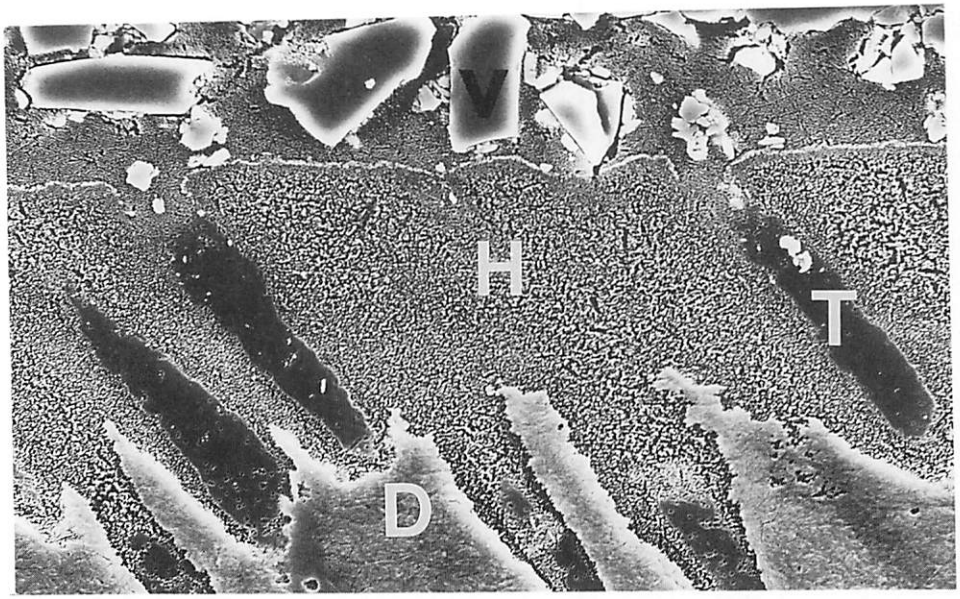


Figure 5.4. Vitremer (V)/Clearfil Photo Bond/dentine (D) interface after argon ion beam etching. This specimen displays a hybrid layer (H) of approximately 8 μm thick, and the layer of bonding resin is imperceptible. Resin tags are apparent (T).

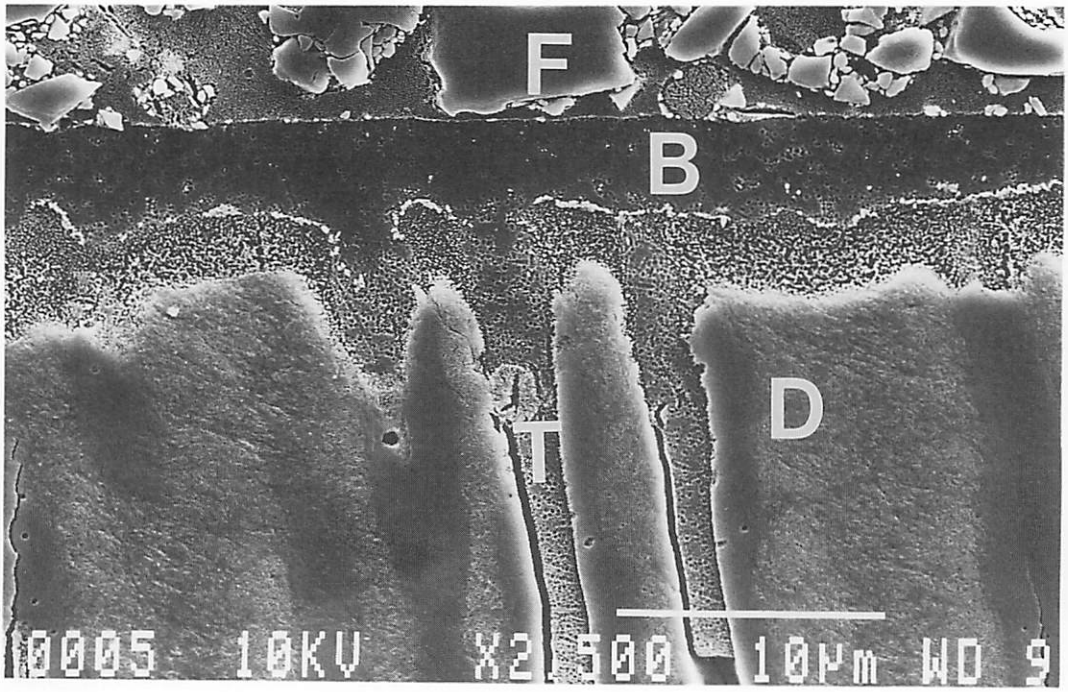


Figure 5.5. Fuji II LC/Clearfil Liner Bond/dentine (D) interface after argon ion beam etching. A 2 – 3 µm thick hybrid layer, resin tags (T), and a distinct border between the bonding resin (B) and the RmGIC (F) are observed.

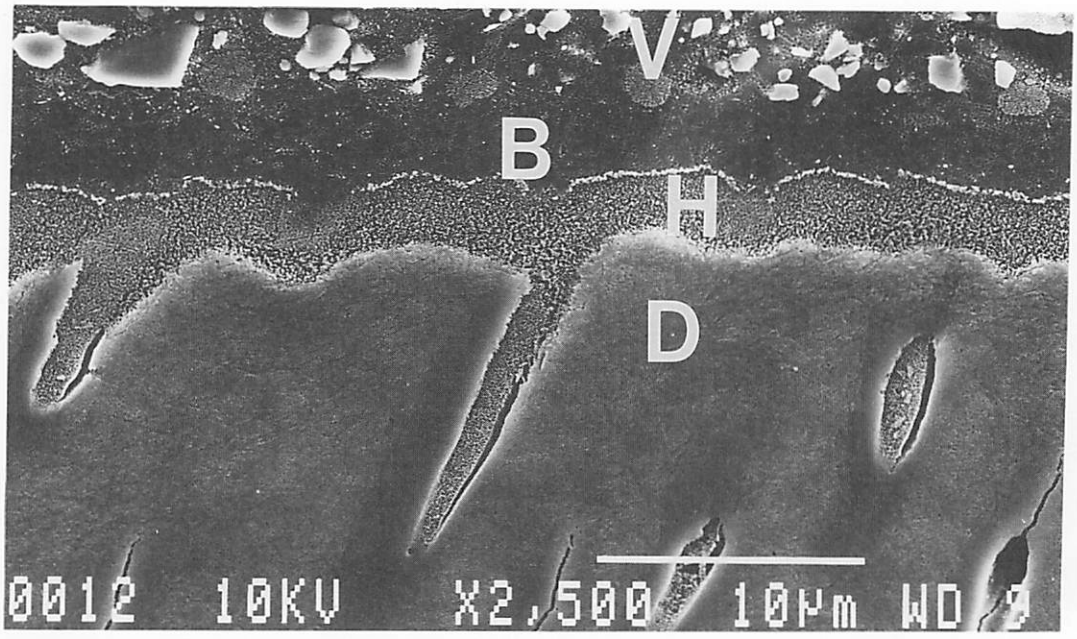


Figure 5. 6. Vitremer (V)/Clearfil Liner Bond/dentine (D) interface after argon ion beam etching. Note a 2 – 3 µm thick hybrid layer (H) and a non-distinct border between the bonding resin (B) and the RmGIC.

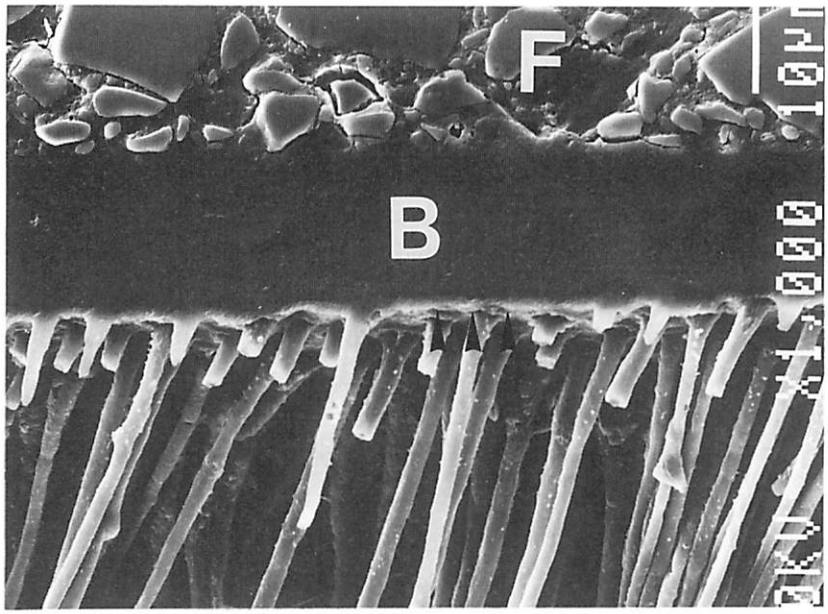


Figure 5.7. Fuji II LC/Clearfil Liner Bond II interface after exposure to 10% phosphoric acid for 3 – 5 seconds followed by immersion in 5% sodium hypochlorite for 5 minutes. A hybrid layer of 1 – 2 μm thick (black arrowheads), and long resin tags can be observed. A good interaction between the bonding resin (B) and the RmGIC (F) can be seen.

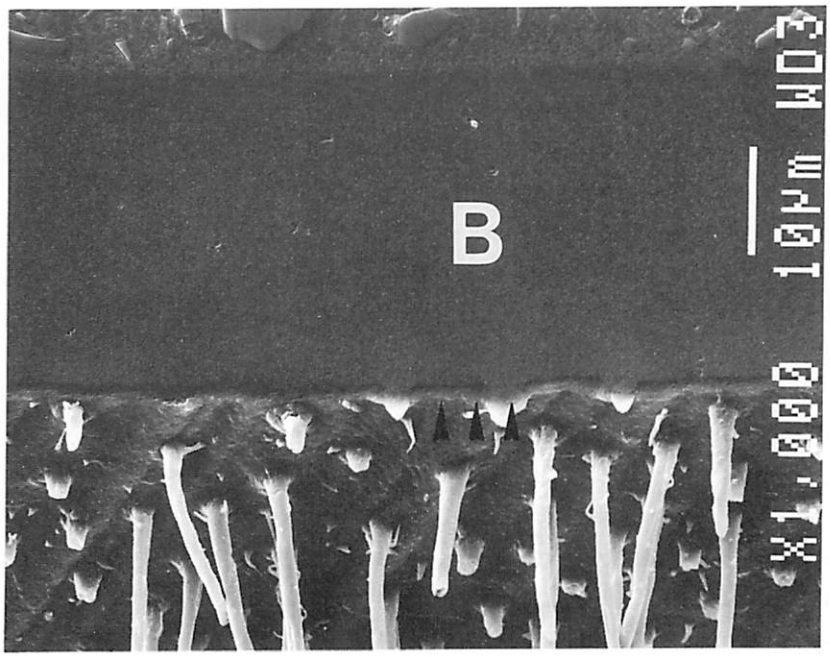


Figure 5.8. Vitremer – Clearfil Liner Bond II/dentine interface after exposure to 10% phosphoric acid for 3 – 5 seconds followed by immersion in 5% sodium hypochlorite for 5 minutes. A hybrid layer of 1 –2 μm thick (black arrowheads), and long resin tags can be observed. Note a very good interaction between the bonding resin (B) and the RmGIC (V).

Chapter 6

General conclusions and future perspectives

Secondary caries and dentin bonding has become two of the most challenging and studied topics in the restorative dentistry. As patients live longer the probability of cervical lesions and/or secondary caries around cervical restorations to occur increases, lead by periodontal problems, decrease in coordination movements and consequent plaque accumulation. However, root surface caries present specific operative difficulties, which are reduced retention capacity and difficult access of the restoration. Adhesive restorative materials, especially the resin-modified glass ionomer cements (RmGICs) have been indicated as first choice restoratives for root surface caries because of their potential anticariogenic effect. Fluoride has been also incorporated to recent adhesive restoratives and bonding systems as to protect the restoration against secondary caries. These recent bonding systems produce high bond strengths however, fail to inhibit secondary caries. In order to increase the adhesive capacity of the resin-modified glass ionomer cements, preserving the potential anticariogenic effect of these materials, improved conditioners and use of adhesive resins should be considered.

Chapter 2

The aims of this investigation were to determine through polarized light microscopy the capacity of three glass ionomeric restoratives and a fluoride releasing resin composite system to inhibit *in vitro* secondary caries in root dentin, as well as to measure the width and height of the inhibition zones, and the depth of the outer lesions. The results indicated that although the three glass ionomeric materials used in this study produced an inhibition zone adjacent to the restorations, the height and width of this zone was material dependent. The conventional glass ionomer cement (Fuji Ionomer Type II; GIC) produced an inhibition zone with greatest height and thickness compared to the resin-modified glass ionomers (Fuji II LC and Vitremer; RmGIC). The outer lesions that were produced were similar in depth for all materials, indicating that they were little influenced by the restorative materials. Since previous studies have suggested that fluoride released by GIC and RmGIC are similar or greater for the latter, it can be speculated that other factors, e.g. other ions that are released together with fluoride, may be responsible for a *in vitro* caries inhibition. The resin composite containing fluoride failed to produce an inhibition zone, even though visual separation of the restoration at the cavity margin was not evident. Presumably, the demineralizing solution may have leaked through the base of the hybrid layer creating a wall lesion. Therefore, even recent adhesive resin composite systems that produce high bond strengths to dentin do not protect the restoration against secondary caries when the environment is prone to caries formation.

Chapter 3

In chapter 2, the presence of inhibition zones was determined and the dimensions of these zones measured. However, polarized microscopy by itself does not reveal the quality of the inhibition zone, for example, the degree of mineralization. Therefore, the purpose of the investigation of this chapter was to measure the microhardness of the inhibition zones and outer lesions produced adjacent to the glass ionomeric restorations when subjected to *in vitro* acid challenge. Knoop hardness number (KHN) of all outer lesions were initially measured along the lesion and parallel to the cavity wall. However, because the size of the knoop indenter is greater than the width of the inhibition zone, an alternative microhardness tester that measures the triangular hardness (HT) was used to determine the hardness of the inhibition zone. The HT of the outer lesions and inhibition zones were determined and correlated with KHN values, and a linear correlation obtained. Therefore, the triangular hardness test showed to be efficient to measure the hardness of narrow areas. When comparing hardness of the different inhibition zones, the zone created adjacent to the GIC was significantly greater than those created by the RmGICs. These results confirmed the previous polarized microscopy data (chapter 2), and demonstrated that the mineral content of the inhibition zone around the GIC was greatest. Similar to chapter 2, it can be speculated that other factors, rather than fluoride alone, may influence the formation of mineralized inhibition zones.

Chapter 4

This study focused in testing the effect of an improved resin-modified glass ionomer cement (Fuji II LC-I) on shear bond strengths to enamel and dentin that were conditioned with an agent composed of 20% polyacrylic acid and 3%$AlCl_3$ (cavity conditioner), and analyzing the micromorphology appearance of the cement/tooth interface. Since bonding of glass ionomeric restoratives can be inconsistent (Helwett et al., 1991; Ngo et al., 1986) without removal of the smear layer, and clinical retention and bond strengths to dental substance are improved when the smear layer is removed (Powis et al., 1982), pre-treating the cavity walls with 10% polyacrylic acid solution (dentin conditioner) became routine prior to restoring with ionomeric materials. The Fuji II LC-I posses superior polishability to the former version, and the cavity conditioner requires a 10 second application rather than a 20 second application for the dentin conditioner.

The results indicated that bond strengths of both versions to enamel were similar at 5 minutes after light-curing, and that they significantly increased at 1 day remaining stable to 1 week, being the improved version significantly higher than the former version. Regarding bond strengths to dentin, the improved version showed significant higher bond strengths than the former version at 5 minutes after light-curing, remaining stable to a period of 1 week. The increase of early bond strengths after light-curing of Fuji II LC-

I/cavity conditioner system is possibly the greatest clinically-related advantage over the former system, because it permits the clinical operator to finish and polish the restoration safely after light-curing.

Chapter 5

Bonds of the restorative materials to dentin or enamel may fail losing its retention to the cavity, promoting leakage of fluids from the oral environment into the cavity, being detrimental to the longevity of the restoration. Since bond strengths of resin-modified glass ionomer cements are low and volumetric shrinkage during light-curing is similar to the resin composite systems, the study in chapter 5 aimed to increase shear bond strengths of RmGICs to dentin by first bonding the dentin with an adhesive resin. Three adhesive resin systems (Clearfil Photo Bond, PB; Clearfil Liner Bond, LB; and Clearfil Liner Bond II, LBII) and two resin-modified glass ionomer cements (Fuji II LC and Vitremer,) were used in the present investigation. The results indicated that shear bond strengths of both Fuji II LC and Vitremer were quite low when applied directly to the dentin surface, however significantly increased when a recent self etching primer system (LBII) was used. Among the three systems used in this study, bond strengths of resin composites have been reported to be highest for the LBII (Burrow et al., 1994). These previous results demonstrate that RmGICs can bond very well to bonding resins, producing relatively high bond strengths to dentin. The micromorphology appearances disclosed a good interaction between the bonding systems and the RmGICs, as well as good interaction between the bonding systems and the underlying dentin. Moreover, because fluoride ions have been reported to diffuse through adhesive resins (Castro et al., 1994), the anticariogenic benefit of the ionomeric materials may not be lost. Therefore, the technique of bonding dentin with an adhesive resin and then applying a RmGIC may be a good restorative solution for those materials that produce weak bonding to dentin.

Unfortunately, to date no restorative material posses all the properties desired in a perfect filling material (Watts, 1996). Successful and appropriate application of glass ionomeric restoratives will depend upon conscientious indication and careful handling skills, as much as the inherent restorative formulation. The ideal restorative material should be able to provide short- and long-term sealing of the cavity by bonding to the tooth tissue, undergo minimal contraction during light-curing, and possess anticariogenic properties by releasing and uptaking fluoride into and from the adjacent restoration or teeth, and oral environment.

References

Akinmade AO (1994). Adhesion of glass polyalkenoate cements to collagen. *J Dent Res* Special issue 73:181 abst. 633.

Akinmade AO and Nicholson JW (1993). Glass-ionomer cements as adhesives. Part I Fundamental aspects and their clinical relevance. *J Mater Sci.* Materials in Medicine 4:95-101.

Almqvist H, Lagerlof F (1993). Influence of constant fluoride levels in solution on root hard tissue de- and remineralization measured by ^{125}I absorptiometry. *Caries Res* 27:100-105.

Antonucci JM, McKinney JE, Stansbury JW (1988). Resin-modified glass-ionomer dental cements field of invention. US patent application 7160856. Springfield, VA: National Technical Information Service.

Arends J and Schuthof J (1975). Fluoride content in human enamel after fluoride application and washing. An in vitro study. *Caries Res* 9:363-372.

Arends J, Schuthof J, Jongebloed WG (1979). Microhardness indentations on artificial white spot lesions. *Caries Res* 13:290-297.

Arends J, Schuthof J, Jongebloed WG (1980). Lesion depth and microhardness indentations on artificial white spot lesions. *Caries Res* 14:190-195.

Asmussen E, Hansen EK, Peutzfeldt A (1991). Influence of the solubility parameter of intermediary resin on the effectiveness of the Gluma bonding system. *J Dent Res* 70:1290-1293.

Bell RB, Barkmeier WW (1994). Glass-ionomer restoratives and liners: Shear bond strength to dentin. *J Esthet Dent* 6:129-134.

Berry EA III, Powers JM (1994). Bond strength of glass ionomers to coronal and radicular dentin. *Oper Dent* 19:122-126.

Bhaskar SN (1976). Orban's Oral histology and embryology, eigth edition, Mosby Co., saint Loius.

Blackwell G and Käse R (1996). Technical Characteristics of light curing glass-ionomers and compomers. Proceedings of conference on clincally appropriate alternatives to amalgam: Biophysical Factors in restorative Decision-making. Transactions 9:77-87.

Bowen RL, Cobb EN, Rapson JE (1982). Adhesive bonding of various materials to hard tooth tissues: improvement in bond strength to dentin. *J Dent Res* 61:1070-1076.

Buonocore MG (1995). A Simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. *J Dent Res* 34:849-853.

Burgess JO, Burkett L (1993). Shear bond strength of four glass ionomers to enamel and dentin. *J Dent Res* 72:388, Abstr. No. 2276.

Burguess J, Norling B, Summitt J (1994). Resin ionomer restorative materials: The new Generation. *J Estht Dent* 6:207.

Burrow MF, Tagami J, Hosoda H. (1993). The long term durability of bond strengths of dentin. *Bull Tokyo Med Dent Univ* 40:173-191.

Burrow MF, Tagami J, Negishi T, Nikaido T, Hosoda H (1994). Early tensile bond strengths of several enamel and dentin bonding systems. *J Dent Res* 73:522-528.

Busscher HJ, Retief DH, and Arends J (1987). Relationship between surface-free energies of dental resins and bond strengths to etched enamel. *Dent Mater* 3:60-63.

Caldwell RC, Gilmore RW, Timberlake P, Pigman J, Pigman W (1958). Semiquantitative studies of *in vitro* caries by microhardness tests. *J Dent Res* 37: 301-305.

Carvalho RM, Yoshiyama M, Horner JA, Pashley DH (1995). Bonding mechanism of Variglass to dentin. *Am J Dent* 8:253-258.

Caughman WF, Caughmen GB, Dominy WT, Schuster GS (1990). Glass ionomer and composite resin cements: Effects on oral cells. *J Prosthet Dent* 63: 513-521.

Charlton DG, Havemann CW (1994). Dentin surface treatment and bond strength of glass ionomers. *Am J Dent* 7:47-49.

Ciucchi B, Bouillaguet S., Delaloye M, Holz J (1997). Volume of the internal gap formed under composite restorations *in vitro. J Dent* 25: 305-312.

Craig RG, and Peyton FA (1958). The microhardness of enamel and dentin. *J Dent Res* 37:661-668.

Cranfield M, Kuhn AT, Winter Gb. (1982). Factors relating to the rate of fluoride ion release from glass ionomer cement. *J Dent* 10: 333-341.

Crisp S, Lewis BG, Wilson AD (1976). Glass ionomer cements: chemistry of erosion. *J Dent Res* 55:1032-1041.

Darling AI (1956). Studies of the early lesion of enamel caries with transmitted light, polarized light and microradiography. *Br Dent J* 101:289-197, 329-341.

Darling AI (1958). Studies of the early lesion of enamel caries. Its nature, mode of spread, and points of entry. *Br Dent J* 105: 119-35.

Darling AI, mortimer KV, Poole DFG, Ollis WD (1961). Molecular sieve behavior of normal and carious human dental enamel. *Arch Oral Biol* 5:251-273.

Davidson CL, Hoekstra IS, Arends J (1974). Microhardness of sound, decalcified and etched tooth enamel related to the calcium content. *Caries Res* 8:135-144.

Diaz Arnold AM, Holmes DC, Wistrom DW, Swift Jr EJ. (1995). Short-term fluoride release/uptale of glass ionomer restoratives. *Dent Mater* 11: 96-101.

Dionysopoulus P, Kotsanos N, Kolinitou E, Papagodiannis Y (1994). Secondary caries formation in vitro around fluoride-releasing restorations. *Oper Dent* 19:183-188.

Dionysopoulus P, Kotsanos N, Papadigiannis Y (1990). Lesions in vitro associated with a Fl-containing amalgam and a stannous fluoride solution. *Oper Dent* 15: 178-185.

Donly KJ (1994). Enamel and dentin demineralization inhibition of fluoride-releasing

materials. *J Dent* 7:275-278.
Douglas WH (1989). Clinical status of dentin bonding agents. *J Dent* 17:209-215.
Dunne SM, Goolnik JS, Millar BJ, and Seddon RP (1996). Caries inhibition by a resin-modified and a conventional glass ionomer cement in vitro. *J Dent* 24:91-94.
Eick JD, Robinson SJ, Chappell RP, et al. (1993). The dentinal surface: its influence on dentinal adhesion. Part III. *Quint Int* 24;571-582.
Eick JD, Wilko RA, Anderson CH, Sorensen SE (1970). Scanning electron microscopy of cut tooth surfaces and identification of debris by use of the electron microprobe. *J Dent Res* 49:1359-1368.
Erickson RL (1989). Mechanism and clinical implications of bond formation for two dentin bonding agents. *Am J Dent* 2:117-123;
Erickson RL (1992). Surface interactions of dentin adhesive materials. *Oper Dent* Suppl. 5:81-94.
Erickson RL, Glasspoole EA (1994). Bonding to tooth structure: a comparison of glass-ionomer and composite-rein systems. *J Esth Dent* 6:227-244.
Featherstone JBD, Glena R, Shariati M, Shields CP (1990). Dependance on in vitro demineralization of apatite and remineralization of dental enamel on fluoride concentration. *J Dent Res* 69 special issue:620-625.
Featherstone JDB, McIntyre JM, Fu J (1987). Physicochemical aspects of root caries progression. In: thylstrup A, Leach SA, Quist A. Dentine and dentine reactions in the oral cavity. Oxford: IRL Press, 127-139.
Feilzer AJ, De Gee AJ, Davidson CL (1988). Curing contraction of composites and glass-ionomer cements. *J Prosth Dent* 59: 297-300.
Forss H (1993). Release of fluoride and other elements from light-cured glass ionomers in neutral and acidic conditions. *J Dent Res* 72:1257-1262.
Forsten L (1977). Fluoride release from a glass ionomer cement. *Scand J Dent Res* 85: 503-504.
Forsten L (1990). Short- and long-term fluoride release from release from glass ionomers and other fluoride-containing filling materials *in vitro*. *Scand J Dent Res* 98: 179-185.
Forsten L (1991). Fluoride release and uptake by glass ionomers. *Scand J Dent Res* 99:241-244.
Forsten L (1995). Resin-modified glass ionomer cements: fluoride release and uptake. *Acta Odontol Scand* 53: 222-225.
Fowler CS, Swartz ML, Moore BK, Rhodes BF (1992). Influence of selected variables on adhesion testing. *Dent Mater* 8:265-269.
Friedl KH, Powers JM, Hiller KA (1995). Influence of different factors on bond strength of hybrid ionomers. *Oper Dent* 20:74-80.
Fritz UB, Finger WJ, Uno S (1996). resin-modified glass ionomer cements: Bonding to enamel and dentin. *Dent Mater* 12:161-166.

Fukusawa M, Matsuya S, Yamane M (1987). Mechanism for erosion of glass-ionomer cements in an acidic buffer solution. *J Dent Res* 66:1770-1774.

Fusayama T (1979). Two layers of carious dentin: diagnosis and treatment. *Oper Dent* 4: 63-70.

Fusayama T(1988). The problems preventing progress in adhesive restorative dentistry *Adv Dent Res* 2: 158-161;

Fusayama T, Okuse K, Hosoda H (1966). Relationship between hardness, discoloration, and microbial invasion in carious dentin. *J Den Res* 45: 1033-1046.

Gaberoglio R and Brännström M (1976). Scanning electron microscopic investigation of human dentinal tubules. *Arch Oral Biol* 21:355-362.

Gladys S, Van Meerbeek B, Braem MJA, Lambrechts P, Vanherle G (1977). Comparative Physico-mechanical characterization of New hybrid restorative materials with conventional glass-ionomer and resin composite restorative materials. *J Dent Res* 76: 883-894.

Glasspoole EA and Erickson RL (1993). In vitro investigation of the caries inhibition effects of fluoride releasing materials. *J Dent Res* 72 spec issue: abstract 1448.

Griffin F, Donly KJ, Erickson R (1992). Caries inhibition by fluoride-releasing liners. *Am J Dent* 5:293-295.

Gustafson G (1957). The histopathology of caries of human dental enamel, with special reference to the division of the lesion into zones. *Acta Odontol Scand* 15: 13-55

Gwinett AJ, Kanca J (1992). Interfacial morphology of resin composite and shiny erosion lesions. *Am J Dent* 5:315-317.

Gwinnett AJ, Kanca JA (1992). Micromorphology of the bonded dentin interface and its relationship to bond strength. *Am J Dent* 5:73-77;

Gwinnett AJ, Matsui A. (1967). A Study of enamel adhesives. The physical relationship between enamel and adhesive. *Archs Oral Biol* 12:1615-1620.

Hammesfahr PD (1994). Developments in resionomer systems. Glass ionomers: the next generation. Proceedings of the 2nd international symposium on glass ionomers. Pennsylvania, 47-55.

Harniratissai C, Inokoshi S, Shimada Y, Hosoda H (1991). Penetration pattern of resin into caries affected and acid conditioned dentin. *Adhes Dent* 8:147-148.

Hatibovic-Kofman Sahza, Koch G (1991). Fluoride release from glass ionomer cement in vivo and in vitro. *Scand Dent J* 15:253-258.

Hatibovic-Kofman Sahza, Suljak JP, Koch G (1997). Remineralization of natural carious lesion with a glass ionomer cement. *Swed Dent* J 21:11-17.

Hegdahl T and Hagebö T (1972). The load dependence in micro indentation hardness testing of enamel and dentin. *Scand J Dent Res* 80:449-452

Herkströter FM, Witjes M, Ruben J, Arends (1989). Time dependency of microhardness indentations in human and bovine dentine compared with human enamel. (Short

Communication) *Caries Res* 23:342-344.
Hewlett ER, Caputo AA, Wrobel DC (1991). Glass ionomer bond strength and treatment of dentin with polyacrylic acid. *J Prosthet Dent* 66:767-72.
Hicks MJ and Silverstone LM (1984). Acid-etching of caries-like lesions of enamel: a polarized light microscope study. *Caries Res*18:315-326.
Hicks MJ, Flaitz CM, Silverstone LM (1986). Secondary caries formation in vitro around glass ionomer restorations. *Quint Int* 17: 527-532.
Hinoura k, Miyazaki m, Onose H (1991). Dentin bond strength of light-cured glass-ionomer cements. *J Dent Res* 70:1542-1544.
Hodge HC (1936). Hardness tests on teeth. *J Dent Res* 15:271-279.
Holtan JR, Nystrom GP, Olin PS (1990). Bond strength of a light-cured and two auto-cured glass liners. *J Dent* 18:271-275.
Hörsted-Binslev P and Mjör IA (1988). Modern Concepts in Operative Dentistry. 1st edition, Munksgaard, Copenhagen.
Iijima Y and Koulourides T (1988). Mineral density and fluoride content of in vitro remineralized lesions. *J Dent Res* 67:577-581.
Inokoshi S, Hosoda H, Harniratissai C, Shimada Y, Tatsumi T (1990). A Study on the resin-impregnated layer of dentin: Part I. A comparative study on the decalcified and undecalcified sections and the application of argon ion beam etching to disclose the resin-impregnated layer of dentin. *Jpn J Conserv Dent* 33:427-442;
International Organization for Standardization. ISO TR 110405 dental materials-guidance on testing of adhesion to tooth structure. Geneva, Switzerland: WHO.
Kato S, Tosaki S, Hirota K (1995). Effect of surface conditioning materials on glass ionomer bonding. *J Dent Res* 74:106, Abstr. No. 759.
Kemp-Scholte CM and Davidson CL (1990). Complete marginal seal of class V composite restorations effected by increased flexibility. *J Dent Res* 69:1240-1243.
Koulourides T, Cueto H, Pigman W (1961). Rehardening of softened enamel surfaces of human teeth by solutions of calcium phosphates. *Nature* 189: 226-227.
Koulourides T, Phantumvanit P, Housch T (1975). Effect of a single 2% NaF Topical vs. frequent exposures to 1ppm fluoride (F-) on experimental cariogenesis. *J Dent Res* (Spec issue A) no.L307
Kouluorides T and Cameron B (1980). Enamel Remineralization as a factor in the pathogenesis of Dental caries. *J Oral Pathol* 9:255-269.
Limeback H (1996). Treating dental caries as an infectious disease. *Ont dent* 73: 23-25.
Linde A (1985). The extracellular matrix of the dental pulp and dentin. *J Dent Res* 64 (Special issue):523-529.
McCaghren RA, Retief DH, Bradley EL, Denys FR (1990). Shear bond strength of light-cured glass ionomer to enamel and dentin. *J Dent Res* 69:40-45.
McLean JW (1992). Clinical applications of glass-ionomer cements. *Oper Dent* suppl

5:184
McLean JW, Nicholson JW, Wilson AD (1994). Proposed nomenclature for glass-ionomer dental cements and related materials. *Quint Int* 25:587-589.
McLean JW, Wilson AD (1977) The clinical development of the glass-ionomer cement. III. The erosion lesion Australian Dental journal 22 190-195.
McLean JW. Glass-ionomer cements (1988). *Br Dent J*; 164:293-300.
Mellberg JR and Singer L (1977): Discussion of Weatherell, Deutsch, Robinsn and Hallsworth. *Caries Res* 11 (supl 1): 101-115.
Meyron SD, Smith AJ, (1984). A comparison of fluoride release from three glass ionomer cements and a polycarboxylate cement. *Int Endod J* 17:16-24, 1984.
Mitra SB (1991). Adhesion to dentin and physical properties of a light-cured glass-ionomer liner/base. *J Dent Res* 70:72-74.
Mitra SB (1991). In vitro fluoride release from a light-cured glass-ionomer liner/base. *J Dent Res* 70: 75-78.
Mitra SB (1994), Curing reactions of glass ionomer materials, Proceedings of the 2nd international symposium on glass ionomers. International Symposia in Dentistry, PC, Philadelphia, PA; 1:13-33.
Mitra SB, Kedrowski BL (1994). Long-term mechanical properties of glass ionomers. *Dent Mater* 10:72-82.
Mjör IA and Fejerskov O (1986). Human oral embryology and hystology. Copenhagen:Munksgaard.
Momoi Y, McCabe JF (1993). Fluoride release from light-activated glass ionomer restorative cements. *Dent Mater* 9:151.
Mount GJ (1993). Clinical placement of modern glass-ionomer cements. *Quint Int* 24: 99-107.
Mount GJ (1994). Glass-ionomer cements: Past, Present and Future. *Oper Dent* 19: 82-90.
Nakabayashi N (1982). Resin reinforced dentin due to infiltration of monomers into dentin at the adhesive interface. *J Jpn Dent Mater* 1:78-81;
Nakabayashi N (1984). Biocompatibility and promotion of adhesion to tooth substrates. *CRC Critical Review Biocompatibility* 1:25-52.
Nakabayashi N, Kojima K, Masuhara E (1982). The promotion of adhesion by infiltration of monomers into tooth substrates. *J Biomed Mater Res* 16:265-273;
Nakamichi I, Iwaku M, Fusayama T (1983). Bovine teeth as possible substitutes in adhesion test. *J Dent Res* 62:1076-1081.
Ngo H, Earl A, Mount GJ (1986). Glass-ionomer cements: a 12-month evaluation. *J Prosthet Dent* 55:203-5.
Nikaido T, Nakajima M, Higashi T, Kanemura N, Pereira PNR, Tagami J (1997). Shear bond strengths of a single-step bonding system to enamel and dentin. *Dent Mater J* 1:40-

47.
Nyvad B and Fejerskov O (1986). Active root surface caries converted into inactive caries as a response to oral hygiene. *Scan J Dent Res* 94:281-284.
Nyvad B and Fejerskov O (1987). Active and inactive root surface caries: structural entities? In: Thylstrup A, Leach SA, Quist A. Dentine and dentine reactions in the oral cavity. Oxford: IRL Press 165-179.
Ohgushi K and Fusayama T (1975). Electron microscopic structure of the two layers of carious dentin. *J Dent Res* 54:1019-1026.
Pashley DH (1984). Smear layer: physiological considerations. *Oper Dent* 3:13-29.
Pashley DH (1990). Interactions of dental materials with dentin. *Trans Acad Dent mater* 3:55-73.
Pashley DH, Horner JA, Brewer PD (1992). Interactions of conditioners on the dentin surface. *Oper Dent* suppl 5:137-150.
Pashley DH. The smear layer: physiological considerations. *Oper Dent* 1984; 3:13-29.
Pawlus MA, Swift, Jr. EJ, Vargas MA (1994). Shear bond strengths of resin ionomer restorative materials. *J Dent Res* Abstr 1812.
Pereira PNR, Okuda M, Yoshikawa T, Sano H *et al.,* (1997). Effect of water and regional difference on dentin bond strength. *J Dent Res* 76 spec issue: abstr 56.
Pereira PNR, Yamada T, Tei R, Tagami J (1997). Bond strength and interface micromorphology of an improved resin-modified glass ionomer cement. *Am J Dent* 10: 128-132.
Powis DR, Folleras T, Merson SA, Wilson AD (1982). Improved adhesion of a glass-ionomer cement to dentin and enamel. *J Dent Res* 61:1416-1422.
Prado C, Triana R, Llena C, Forner L, Garro J, Garcia-Godoy F (1994). Influence of acid-etching on modified ionomer dentin bonding. *J Dent Res* Spec issue 73: abstr 1807.
Rezk-Lega F, Ogaard B, Rolla G (1991). Availability of fluoride from glass-ionomer luting cements in human saliva. *Scand J Dent Res* 99:60-63.
Ryge G, Foley DE, Fairhurst CW (1961). Micro-indentation hardness. *J Dent Res* 40: 1116-1126.
Rølla G and Saxegaard E (1990). Critical evaluation of the composition and use of topical fluorides with emphasis on the role of calcium fluoride in caries inhibition. *J Dent Res* 69:780-785.
Sano H, Pereira PNR, Kanemura N, Morigami M, Yoshikawa T, Tagami J, Pashley DH (1998). Long-term Durability of Dentin Bonding in vivo. *J Dent Res* abstr.1535.
Sano H, Takatsu T, Ciucchi B, Horner J A, Matthews WG, Pashley DH (1995). Nano-leakage: Leakage within the hybrid layer. *Oper Dent* 20:18-25.
Schüpback P, Lutz F, Guggenheim B (1992). Human root caries: histopathology of arrested lesions. *Caries Res* 26:153-164.
Scott JH, Symons NBB (1982), Introduction to dental anatomy. Ninth edition. Churchill

livingstone, New York.
Seppä L, Forss H, Ogaard B (1993). The effect of fluoride application on fluoride release and the antibacterial action of glass ionomers. *J Dent Res* 72:1310-1314.
Seppä L, Torppa-Saarinen E, Luoma H (1992). The effect of different glass ionomers on the acid production and electrolyte metabolism of Streptococcus mutans Ingbritt. *Caries Res* 26:434-438.
Shono T (1995). Pulpal responses to light-cured restorative glass polyalkenoate cements and ultrastructure of cement-dentin interface. *Jap J Conserv Dent* 38: 514-548.
Sidhu SK, Watson TF (1995). Resin-modified glass ionomer materials. A status report for the American journal of dentistry. *Am J Dent* 8:59-67.
Silverstone LM (1967). Observations on the dark zone in early enamel caries and artificial caries-like lesions. *Caries Res* 1:260-174.
Silverstone LM (1968). The surface zone in caries and in caries-like lesions produced in vitro. *Br Dent J* 20: 145-157.
Silverstone LM (1970). The histopathology of early approximal caries in the enamel of primary teeth. *ASDC J Dent Child* 39: 201-210.
Silverstone LM (1973). The structure of carious enamel, including the early lesion. In: oral Sciences Reviews, No. 3, Dental Enamel. Melcher AH, Zarb GA, Eds. Copenhagen, Munksgaard 100-160.
Silverstone LM (1983). Remineralization and enamel caries: Significance of fluoride and effect on crystal diameters. In: demineralization of the teeth, Leach Sa, Edgar WM, Eds. Oxford. IRL Press Ltd., 185-205.
Skarveit L, Wefel JS, Ekstrand J (1991). Effect of fluoride amalgams on artificial recurrent enamel and root caries. *Scand J Dent Res* 99: 287-294.
Smith DC (1992). Polyacrylic acid-based cements: Adhesion to enamel and dentin. *Oper Dent* suppl 5:177.
Souder W, Schoonover IC (1944). Experimental Remineralization of Dentin. *J Am Dent Assoc* 31:1579-1596
Souto M, Donly KJ (1994). Caries inhibition of glass ionomers. *Am J Dent* 7: 122-124.
Stanford JW (1985). Bonding of restorative materials to dentin. *Int Dent J* 35: 133-138.
Sugizaki J (1991). The effect of various primers on the dentin adhesion of resin composites. SEM and TEM observations of the resin-impregnated layer and adhesion promoting effect of the primers. *Jpn J Conserv* Dent 34:228-265.
Suzuki T, Finger WJ (1988). Dentin adhesives; Site of dentin vs. bonding of composite. *Dent Mater* 4:379-383.
Swartz ML, Phillips RW (1952). Solubility of enamel on areas of known hardness. *J Dent Res* 31:293-300.
Swartz ML, Phillips RW, Clark HE (1984). Long-term F release from glass ionomer cements. *J Dent Res* 63:158-160.

Swift Jr EJ (1988). Effect of mixing time on fluoride release from a glass ionomer cement. *Am J Dent* 1:132-134.

Swift Jr EJ (1989). *In vitro* caries-inhibitory properties of a silver cermet. *J Dent Res* 68:1088-1093.

Swift Jr EJ, Bailey SJ, Hansen SE (1990). Fluoride release from fast setting glass ionomer restorative materials. *Am J Dent* 3:101-103.

Swift Jr EJ, Hammel SA, Perdigao J, Wefel JS (1995). Prevention of root surface caries using a dental adhesive. *J. Am. Dent. Assoc.* 125:571-576.

Swift Jr EJ, Pawlus MA, Vargas MA (1995). Shear Bond Strengths of Resin-modified Glass-Ionomer Restorative Materials. *Oper Dent* 20: 138-143.

Swift Jr EJ, Perdigao J, Heymann HO (1995). Bonding to enamel and dentin: a brief history and state of the art, 1995. *Quintessence Int* 26:95;

Tagami J, Nakajima M, Shono T, Takatsu T, Hosoda H (1993). Effect of aging on dentin bonding. *Am J Dent* 6:145-147.

Takahashi K, Emilson CG, Birkhed D (1993). Fluoride release *in vitro* from various glass ionomer cements and resin composites after exposure to NaF solutions. *Dent Mater* 9:350-354.

Tao L and Pashley DH (1988). Shear bond strengths to dentin: effects of surface treatment, depth and position. *Dent Mater* 4:371-371.

Tay FR, Gwinnett AJ, Wei SHY, P (1996). The overwet phenomenon: A transmission electron microscopic study of surface moisture in the acid-conditioned, resin-dentin interface. *Am J Dent* 9:161-166.

Ten Cate JM, Duijsters PPE (1983a). The influence of fluoride solution on tooth demineralization. I. Chemical data. Caries Res 17:193-199.

Ten Cate JM, Duijsters PPE (1983b). The influence of fluoride solution on tooth demineralization. II. Microradiographic data. *Caries Res* 17: 513-519.

Ten Cate JM, van Duinen RNB (1995). Hypermineralization of dentinal lesions adjacent to glass-ionomer cement restorations. *J Dent Res* 74:1266-1271.

Thorton JB, Reteif DH, Bradley EL (1986). Fluoride release from and tensile bond strength of Ketac-fil and Ketac-Silver to enamel and dentin. *Dent Mater* 2: 241-246.

Titley KC, Smith DC, Chernecky R (1996). SEM observations of the reactions of components of a light-activated glass polyalkenoate (ionomer) cement on bovine dentine. *J Dent* 24:411-416.

Tosaki S, Hirota K (1994). Current and future trends for light-cured systems. Proceedings of the 2nd international symposium on glass ionomers, International Symposia in Dentistry, PC, Philadelphia, PA; 1: 35-47.

Triana R, Prado C, Garro J, Garcia-Godoy F (1994). Dentin bond strength of fluoride-releasing materials. *Am J Dent* 7:252-254.

Tveit AB, Gjerdet NR, (1981). Fluoride release from a fluoride containing amalgam, a

glass ionomer cement and a silicate cement in artificial saliva. *J Oral Rehab* 8:237-241.
Tveit AB, Hals E, Isrenn R, Totdal B (1983). Highly acid Sn_2 and TiF_4 solutions. Effect on chemical reaction with root dentin in vitro. *Caries Res* 17: 412-418.
Van Dijken JW (1992). Three year evaluation of effect of surface conditioning on bonding of glass ionomer cement in cervical abrasion lesions. *Scan J Den Res* 100:133-135.
Van Meerbeek B, Dhem A, Gorat-Nicaise M, Braem M, Lambrechts P, Vanherle G (1993). Comparative SEM and TEM examination of the ultrastructure of the resin-dentin interdiffusion zone. *J Dent Res* 72:495-501.
Van Meerbeek B, Inokoshi S, Braem M, et al., (1992). The morphological aspects of the resin-dentin interdiffusion zone with different dentin adhesive systems. *J Dent Res* 71:1530-1540.
Van Meerbeek B, Perdigao J, Gladys S, Lambrechts P, Vanherle G (1996). Operative Dentistry: A contemporary approach. Quintessence books.
Walls AWG (1986). Glass polyalkenoate (glass-ionomer) cements: a review. *J Dent* 14:231-246.
Wang T, Nakabayashi N (1991). Effect of 2-(mehtacryloxy)-ethyl phenyl hydrogen phosphate on adhesion to dentin. *J Dent Res* 70:59-66.
Watson TF (1990). A confocal microscopic study of some factors affecting the adaptation of a light-cured glass ionomer to tooth tissue. *J Dent Res* 69:152-538.
Watson TF, Billington RW, and Williams JA (1991). The interfacial region of the tooth/glass ionomer restoration: a confocal optical microscope study. *Am J Dent* 4: 303-310.
Wefel JS, Heilman JR, Jordan TH (1995). Comparisons of *in vitro* root caries models. *Caries Res* 29:204-209.
Wei SHY and Forbes WC (1968). X-ray defraction analysis of carious dentin treated with stannous fluoride. *Arch Oral Biol* 13:407-409.
Wilson AD and Kent BE (1972). A new translucent cement for dentistry. The glass ionomer cement. *British Dent J* 132:133-135.
Wilson AD and McLean JW (1988). Glass-ionomer Cement. Quintessence publishing Co Inc., Chicago.
Wilson AD, Groffman DM, Kuhn AT (1985). The release of fluoride and other chemical species from a glass-ionomer cement. *Biomater* 6: 431-433.
Wilson AD, Prosser HJ, Powis DM (1983). Mechanism of adhesion of poly-electrolyte cements to hydroxyapatite. *J Dent Res* 62:590-592.
Yoshikawa T, Hirasawa M, Tosaki S, Hirota K (1994). Concentration of HEMA eluted from light-cured glass ionomers. *J Dent Res* 72 abstr no. 254.