A New In Vitro Method for the Study of Micro-leakage of Dental Restorative Materials

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TABLE OF CONTENTS

ABSTRACT

Microleakage is an important topic in restorative dentistry. A large number of different techniques have been developed for the investigation of microleakage. However, these methodologies have been considered less reliable due to the nature of specimen preparation.

The major objective of this investigation was to introduce a non-destructive technique for the study of microleakage. This objective has been partly met with the use of micro-computed tomography. By scanning the whole restoration with high spatial resolution, microleakage could be detected non-destructively and three-dimensionally.

In order to detect microleakage by micro-computed tomography, an X-ray contrast dye solution was developed to reveal microleakage at the tooth/restoration interface. In addition, a suitable model of tooth/cavity complex was designed in order to gain the best resolution from micro-computed tomography. Finally, with the application of advanced image analysis software, three-dimensional analysis of microleakage was achieved quantitatively and qualitatively.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and to the best of my knowledge and belief, contains no material previously published or written by another person except where due reference has been made in the text.

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CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Microleakage has been defined as the passage of bacteria, fluids, molecules or ions along the tooth-restoration interface (Kidd 1976). This leakage may be clinically undetectable, but is a major factor influencing the longevity of dental restorations as it causes many severe biological effects on the restored tooth including the recurrence of caries, pulp pathology, hypersensitivity and marginal breakdown (Hersek 2002). The investigation of microleakage is, therefore, important in the assessment of restorative materials.

A variety of in vitro methods have been introduced into the study of microleakage including compressed air, neutron activation, electrochemical, fluid filtration, bacteria and the use of dyes (Kidd 1976; Taylor and Lynch 1992; Karagenç and Cansever 2006). In addition, various techniques such as scanning electron microscopy, transmission electron microscopy and electron probe microscopic analysis have been used to image and measure leakage. However, the above specimen preparation techniques are two dimensional in nature and do not take the whole tooth-restoration interface into account, as some sections obtained randomly are taken to measure microleakage. Over the past few years there have been efforts to investigate microleakage of restorative materials three-dimensionally (Youngson 1992; Gale 1994; Lyroudia 2000; Iwami 2005). However, this methodology was also destructive as images were reconstructed from serial cross sections of continuously ground surfaces.

One of the most advanced techniques in medicine in recent years has been the advent of micro-computed tomography (MCT) that can achieve a spatial resolution at the micron level. Recently, the MCT Skyscan 1072 (MCT 1072) has been introduced into dentistry for the study of many relevant subjects including dental materials, dental morphology and dental implants (Bergmans 2001; Park 2005; Santis 2005). Santis et al (2005) first used MCT to study microleakage and although these workers introduced a non-destructive method, the model they chose had some weaknesses. From the clinical perspective only flat dentine surfaces were studied rather than definite cavities, as in the clinical situation. Furthermore, no three dimensional quantitative and qualitative analysis was undertaken.

In considering various methods that have been used to study microleakage, the technology of MCT would appear to offer significant advantages over two dimensional methods involving sectioning of specimens.

However, in order to image microleakage using MCT, an X-ray contrast dye solution is needed. Although a solution of 50% silver nitrate has been commonly used in microleakage studies (Taylor and Lynch 1992; Taylor and Lynch 1993; Besnault and Attal 2003; Tsatsas, Meliou et al. 2005), it has been suggested that the nitrate solution has affinity with tooth structures, binding to tooth substances and restorative materials, leading to false results. As a consequence, one of the aims of the present study was to identify a better contrast dye solution for microleakage studies.

The restorative material to be chosen to validate the methodology was a Glass Ionomer Cement (GIC), which is considered one of the most challenging classes of materials in microleakage studies (Ngo, personal communication). This is because the GIC is a water-based cement, which significantly absorbs dye agents, thereby making it difficult to accurately interpret microleakage.

It is from this fundamental basis that the project evolved, with firstly, the need to explore the potential of using MCT scanning on dental restorations and secondly, to develop a suitable X-ray contrast dye medium to expose regions of microleakage. Thirdly, there was the requirement to quantify the amount of microleakage in three dimensions.

1.2 Literature review

As an introduction to this investigation it is necessary to review the adverse effects of microleakage on the restored teeth. In addition, etiologies and dynamic processes of microleakage will be considered. Furthermore, the current methodologies with their main advantages and disadvantages are discussed.

Having discussed the current thinking on microleakage, there will be a further discussion of X-ray contrast dye solutions and MCT. There is also an overview of GICs which is used for the validation of a new methodology. Finally, the factors that can influence the results of microleakage studies are reviewed.

1.2.1 Microleakage definition

As mentioned previously, microleakage can be the passage of bacteria, fluids, molecules or ions into the tooth/restoration interface. Trowbridge (1987) has also stated that microleakage can be considered as the ingress of oral fluids into the space between tooth structure and a restoration. These descriptions have been widely used by the researchers studying microleakage (Youngson 1990; Taylor and Lynch 1992; Youngson, Jones et al. 1999; Matharu 2001). From these studies it is evident that microleakage can be at micron level or at nanometer level.

1.2.1.1 Leakage at micron level (bacterial microleakage)

It can be inferred from the above microleakage definition that marginal gaps around a restoration permit bacteria to pass into the tooth/restoration interface. This is considered to be bacterial microleakage, which is at the micron level. Numerous studies have shown that once cariogenic bacteria gain an entrance to the tooth-restoration interface they are able to successfully proliferate along this area with the potential to cause an adverse response from the pulp and recurrent caries (Browne and Tobias 1986; Brannstrom 1987; Bishop and Briggs 1995; Mount and Hume 2005)

However, it is still questionable about the marginal gap size around restorations and the occurrence of recurrent caries. It was reported that even though the size of bacteria is as small as 2-4 µm, no secondary caries was found in amalgam restorations where the marginal gaps are less than 50 µm (Jorgensen and Wakumoto 1968). It was also reported that recurrent caries rates significantly increased with the extent of a wide marginal gap. In fact a crevice at the tooth/restoration interface between 250-400 µm was considered a major problem in terms of recurrent caries (Kidd, Joyston-Bechal et al. 1995). Currently, there was a statement that there seems to be no clear correlation between the dimension of marginal gaps around restorations and the development of recurrent caries (Mjör 2005).

The origin of bacteria which are found at the tooth/restoration interface is still uncertain and their relation to the development of recurrent caries remains to be established. While it is believed that bacteria trapped within the smear layer are able to multiply (Brannstrom 1984), on the other hand, it was stated that micro-organism contamination occurring during cavity preparation had little opportunity to survive in the absence of microleakage, and that bacteria found at the tooth/restoration interface were mainly derived from the oral environment through microleakage.

It is also noted that most of bacteria in the oral environment are non-pathogenic and bacteria that are found at the tooth/restoration interface may not be cariogenic. Therefore, in evaluating the role of microleakage, more investigation is needed in terms of the nature, the constitution, concentration and the biological activity of all microleakage factors (Trowbridge 1987; Mjör 2005).

In order to gain information regarding the clinical significance of bacterial leakage many attempts have been made to mimic the oral environment by introducing bacteria. However, the technique itself is considered complicated and somewhat unreliable (Taylor and Lynch 1992).

1.2.1.2 Leakage at submicron level (nanoleakage)

It can also be interpreted from the above definition that restorations with marginal gaps that permit ions and molecules to gain access can have microleakage at the nano level. Apparently, leakage can occur at the tooth/restoration interface while bacteria may not be able to enter.

It is agreed that fluid flow containing ions and molecules access dentinal tubules with ease, particularly when the dentin surface is treated with acid-etch or other conditioning agents. It is also reported that the passage of fluid through dentin is affected by dentin permeability which is markedly influenced by a number of factors including the volume of dentinal tubules, the characteristics of dentine (such as density), dentin smear, dentin calcification and topical applications (Mount and Hume 2005).

Recently, nanoleakage research has focused more in composite resins, particularly at the hybrid layer (Heping Li 2003). However, it is still controversial, as to the clinical significance, in relation to recurrent caries (Taylor and Lynch 1992; Mjör 2005).

1.3 Development of Microleakage

There are many factors that can cause microleakage. Polymerization shrinkage of adhesive restorations has been commonly documented, where the hardening phase causes a considerable contraction in volume, creating stresses and forming gaps between cavity walls and a restoration (Rees and Jacobsen 1989). Secondly, some restorative materials such as GICs have the property of thermal expansion and water absorption, which is susceptible to leakage formation (Retief 1994). Thirdly, long-term effect of mechanical loading and thermal changes can cause elastic deformation and physical alterations of both tooth substances and restoration, resulting in microleakage (Trowbridge 1987; Hilton 2002).

Marginal gaps of a restoration can also be created by improper manipulation of materials by operators. For example, material such as composite resin is highly technique sensitive. Its sealing ability is markedly influenced by the presence of moisture, cavity surface treatment, incremental placement and adequate light-curing time. In addition, factors such as cavity shape, cavity location and cavity depth are of great importance in microleakage creation. For example, composite resins bond well to acid-etched enamel however their bonding to cementum or dentin is still modest (Causton, Braden et al. 1984). As a result, microleakage can be much more easily initiated at composite/cementum and composite/dentin margins. Finally, the level of compatibility of restorative materials to tooth substances is also considered as an important factor in microleakage generation.

1.4 Microleakage modification

It has been believed that microleakage is an active process and thus varies with time. The progression of microleakage is due to long-term bio-chemical reaction within the material itself and between the material and surrounding environment, where the distance between tooth/restoration interfaces may worsen or improve over time (Trowbridge 1987). This can be partially seen in the case of glass ionomer cements where chemical adhesion to tooth surfaces via ionic exchanges can result in enrichment of the ion-bonded hybrid layer at the tooth/restoration interface, which may improve marginal gaps over time reducing microleakage (Mount and Hume 2005). In other circumstances, long-term dimensional changes of restorations caused by environmental and functional factors such as masticatory forces (Qvist 1983) may also alter marginal adaptability, and hence the microleakage of a restoration.

It has been also documented that gradual accumulation of mineral substances at the marginal gaps due to long-term contact with the surrounding saliva environment can stimulate the progress of blocking out the marginal gaps around restorations (Brannstrom 1984). Moreover, microleakage caused by initial contraction may be compensated by the expansion due to water absorption and thermal alterations as seen in amalgam restorations (Trowbridge 1987).

1.5 Adverse effects of microleakage

Restorative marginal gaps that permit the ingress of oral fluid are considered a major reason for pulpal reaction and in time pulpal injuries (Brannstrom 1984; Mount and Hume 2005). Moreover, it is reported that the most substantial biological effect of microleakage on a restored tooth may be the development of recurrent caries, which accounts for approximately 50% of causes of clinical failure for restorations (Trowbridge 1987; Mjör 2005).

Recurrent caries is sometimes named as secondary caries and can be clinically and radiographically identified at the restoration margins, most frequently on the gingival margins of class II and class V restorations. Recurrent caries may develop from a primary lesion or may initiate at the restoration margins, where dental plaque accumulation is accelerated by the presence of microleakage (Trowbridge 1987).

Fluid leakage may cause an acute reaction of the pulp following the placement of a restoration, leading to post-operative hyper-sensitivity or even acute pain (Youngson, Jones et al. 1999; Mount and Hume 2005). It is believed that the symptoms are due to the fluid flow within dentin tubules, which is favored by the presence of microleakage. The problems may become more severe during function as the restoration can act as a plunger during mastication, causing increased fluid motion in the dentin tubule.

These pulpal symptoms may gradually disappear if there is no cariogenic factors involved. There appears to be the process of gradual recovery of pulpal tissue, probably resulting from the self-correction and replacement of dentinal fluid within the pulp chamber. In addition, there is also the involvement of dentinal sclerosis and dentinal calcification during the recovery process (Mount and Hume 2005).

Other adverse effects of microleakage may include marginal defects which favor dental plaque accumulation, leading to periodontal problems. Microleakage may also cause esthetical consequences such as marginal discoloration, with associated aesthetic consequences.

1.6 Microleakage studies

The most effective method for evaluating the sealing of restorative materials to cavity walls is by microleakage studies (Hilton 2002) that use colored dye agents or chemical tracers which are able to penetrate into and stain the tooth/restoration interface. The specimens are then sectioned longitudinally through the restorations and assessed with stereo optical-microscopy or scanning electron microscopy (SEM). Bacteria and radioactive isotopes have also been widely used as markers. Techniques employing ions as markers which can then be detected by neutron activation has also been used. Some other methodologies include electrical conductivity measurement, direct observation with microscopy or SEM and compressed air and compressed fluid.

An understanding of leakage patterns of restorative materials can lead to an increased awareness of the mechanism and etiology of microleakage, resulting in the establishment of the microleakage pattern. Subsequently this will have relevance for restorative material selection in dental practice. (Taylor and Lynch 1992;Hilton 2002).

The following is an overview of current microleakage methodologies and factors that may influence microleakage results. The methodologies which are related to MCT methodology are also emphasized.

1.6.1 Air pressure method

This technique is based on the introduction of compressed air into the pulp space of a restored tooth while investigating the delivery of air bubbles at restoration margins which are placed in fluid. The method was first introduced to test the margins of amalgam restorations (Harper 1912) and then applied to acrylic restorations (Fiasconaro and Shernam 1952). The introduction of microscopic observation (Pickard and Gaynford 1965) to examine the release of air bubbles at restoration margins was a major improvement in establishing a standard method to monitor leakage in the long-term.

One advantage of the method is that leakage can be investigated without sectioning a sample. Thus the restorations can be monitored non-destructively and longitudinally. In addition, the technique can give a quantitative analysis by measuring the loss of pressured air. However, it has many drawbacks. Firstly, the microleakage cannot be photographed as the specimens are immersed in the fluid. Therefore, apart from the drop in pressurized air it cannot give a qualitative analysis of microleakage (Derkson and Pashley 1986). Secondly, it is difficult to interpret microleakage results because the observation of air release is purely to inform air leakage through dentin and then a restoration. Because air flow may pass through the restoration and tooth cracks it is difficult to determine whether air leakage is due to marginal gaps or cracks among tooth structures and restorations. Finally, the method may not provide clinical relevance because it is merely a reflection of air leakage, which is not representative of bacteria or other microorganisms (Kidd 1976; Taylor and Lynch 1992).

1.6.2 Fluid filtration method

The fluid filtration method was developed on the principle of an air-pressurization technique where instead of using pressurized air, a pressurized-liquid was applied to the pulp chamber of a restored tooth with a constant pressure generated by a gas system (Derkson and Pashley 1986).

The sealing ability of a restoration is indicated by the resistance to the dentine permeability. Dentin permeability is defined as the rate of the fluid filtration in the dentin tubules. The dentine permeability rate is measured once a cavity has been prepared and ready for the insertion of a restoration. This rate is assigned as a value of 100%. The rates of the fluid filtration that are measured after the insertion of the restoration are expressed relative to dentine permeability. The changes in the permeability of dentin of the restored tooth, for example values below 100%, indicate that the restoration has affected the dentine permeability by sealing the dentinal tubules (Derkson and Pashley 1986).

A main advantage of the methodology is that it is a non-destructive test. It, therefore, allows samples to be reinvestigated over a period of time. Another advantage is that it provides some level of quantitative and qualitative analysis as fluid flow can be measured as well as photographed.

The sealing ability of the restoration, according to the methodology, was indicated by the rate of the fluid filtration which is applied to the restored tooth through dentin tubules. This quantitative analysis is subject to the variations of the research design and varies significantly from experiment to experiment because of the remarkable changes in dentine permeability caused by dentin conditioning techniques and the thickness of the remaining dentin beneath the cavity. This makes it difficult to compare the results between studies (Youngson, Jones et al. 1999; Karagenç and

Cansever 2006). In addition, the actual amount of microleakage and exact location of leakage cannot be directly determined.

1.6.3 Electrochemical method

In an attempt to develop a technique that can assess restorative microleakage longitudinally, the electrochemical methodology was introduced using a "conductimetric technique" in which the cavity wall/restoration interface (using a glass tube filled with silicate) was incorporated into an electrochemical unit (using lactic acid). The measurement of current changes flowing through this unit demonstrated changes in the dimensions of the interface and thus the tooth/restoration interface can be interpreted (Jacobsen and Von 1975). The technique is apparently not used for conductive materials.

NOTE: This figure is included on page 11 of the print copy of the thesis held in the University of Adelaide Library.

Figure 1: A model developed by Jacobsen

The technique was then applied to extracted tooth models involving the insertion of an electrode into the root of an extracted tooth in such a way that it makes contact with the base of the restoration. Once filled, the cavity is theoretically sealed preventing electrical leakage through the tooth/restoration interface while immersed in an electrolytic bath (Momoi and Iwase 1990).

NOTE: This figure is included on page 12 of the print copy of the thesis held in the University of Adelaide Library.

Figure 2: A model developed by Momoi

The technique was also used to compare its reliability with other techniques such as autoradiography and dye penetration (Delivanis and Chapman 1982). However, it is hard to make any conclusion from this comparison because there seemed to be no reliable correlation among these techniques. Lim (1987) used the same methodology to compare the microleakage of two treatments of glass ionomer cements. There were again broad variations in the values.

The electrochemical methodology of investigating leakage is destructive of tooth structure. The technique is extremely sensitive because it is highly related to the property of electric transmission of restorative materials. In addition, this dielectric property is changed with time due to the continuous setting reaction of the materials.

Similar to air pressure studies, qualitative and quantitative analysis are hard to be drawn as the measurements purely described whether there is a current flow via the restoration.

1.6.4 Neutron activation method

The technique basically used non-radioactive manganese (Mn) salt as a chemical marker which was allowed to leak around the margins of restorations. The specimens were then placed in the core of a nuclear reactor and exposed to a pulsed neutrons/sp cm/sec, where the ^{55}Mn is activated to ^{56}Mn. The gamma-ray emission of ^{56}Mn formed during irradiation was measured with the solid-state scintillation detector. The number of radioactive counts is considered to be proportional to the uptake of Mn per specimen (Going, Mayers et al. 1968).

While there is the advantage of quantifying the results, the method has many disadvantages. Firstly, the technique is complicated, requiring nuclear engineers and involving radioactive isotopes. Secondly, the path and depth of tracer cannot be identified. In addition, the origin of leakage was not well defined as the method failed to identify whether the leakage is at the tooth/restoration interface or due to the uptake of the restoration. Finally, the presence of manganese, either in the tooth or in the restoration can lead to variability of the results.

1.6.5 Bacterial method

Using bacteria to investigate microleakage was first introduced by Fraser (1929), who examined the presence of bacteria after the immersion of glass tubing packed with amalgam into the cultured broth. The technique was then modified by the use of filled teeth in stead of the glass tubing (Kraus 1951; Seltzer 1955).

Bacterial techniques continue to be used and upgraded as they have some clinical relevance (Matharu 2001; Britto 2003; Holke and Drake 2003; Balto and Mansour 2005; Deus and Murad 2006; Karagenc and Gencoglu 2006). Advanced technology such as the SEM has been used to investigate the presence of bacteria at the tooth/restoration interface. Recently, Matharu (2001) introduced the use of the “constant depth film fermentor”, which helped generate a selected bacterial flora and was able to generate large number of biofilms that simulated an oral environment. The authors also suggested that the methodology could be improved by the investigation of positive pulpal pressure on bacterial leakage and the identification of bacteria at the tooth/restoration interface.

The most apparent advantage of the bacterial method is its capacity of replicating and simulating the clinical problems of bacterial leakage, which is considered the main origin of recurrent caries of restored teeth. However, there are many disadvantages arising from such techniques and associated research results. Firstly, the technique is complicated and difficult in terms of cultivating and controlling the bacterial population. Secondly, the methodology lacks standardized models and lacks reproducibility. Therefore, the results amongst various studies are hard to compare. Thirdly, the results are purely qualitative on the basis of whether or not there is presence of bacteria at the tooth-restoration interface. In addition, the results can only display the gaps at which bacteria can pass through. This does not reflect the smaller gaps which can be accessed by fluid flow such as ions, toxins and bacterial by-products (Taylor and Lynch 1992). The results of bacterial studies are therefore not entirely representative of microleakage images of the restoration.

1.6.6 Radioisotope method

Radioactive isotopes have been widely applied in microleakage studies with a broad range of substances including ^{45}Ca, ^{131}I, ^{35}S, ^{22}Na, ^{32}P, ^{86}Rb and ^{14}C which have been introduced as markers. Basically, microleakage is expressed with isotopes by immersing specimens in the isotope solution. The isotope leakage at the tooth/restoration interface is detected by the autoradiography of a sectioned specimen (Hembree 1989; Fitchie and Reeves 1990; Saunders and Grieve 1990). Recently, Hersek (2002) simplified the technique of isotope identification with the use of a Kodak film model that is used in nuclear medicine.

The radioisotope method, on the one hand, may bring with it some important advantages. It is convincing that isotopes are able to penetrate through gaps as small as 40 nm, which investigates the under-sized gaps that bacterial studies cannot reveal. In addition, it is believed that isotopes are more capable at demonstrating microleakage than that of dye (Taylor and Lynch 1992). It has also been demonstrated that radioisotopes such as ^{14}C can be used for long-term monitoring of microleakage (Alani and Toh 1997).

On the other hand, there are many disadvantages arising from the radioisotope study. Firstly, the method is again destructive of specimens and still qualitative in the analyses of results. Secondly, a two-dimensional autoradiograph image is not representative of the three-dimensional image of microleakage. Thirdly, an isotope such as ^{45}Ca has an affinity with tooth structure or restorative materials, leading to increased measurement errors. In addition, isotopes are able to pass through tooth structure or restoration flaws because of their tiny size, resulting in misinterpretation of leakage (Taylor and Lynch 1992). Moreover, because of the complicated procedure of radioisotope leakage recording, the results can be affected by other factors such as isotope selection, source and emulsion distance, exposure length and rinsing. Finally, the technique has potential to produce hazardous radiation.

1.6.7 Dye penetration method

Staining microleakage by using colored agents has been the most popular technique (Taylor and Lynch 1992). The method allows microleakage to be demonstrated in contrasting colors to both tooth and restoration.

Basically, the methodology involves the immersion of a specimen into a dye solution for the pre-set time, after which the tooth-restoration interface is examined for stain. This staining layer is contrasted in color to both tooth structure and the restoration.

A diverse range of dye agents with different concentrations has been introduced into the technique, in which 0.5% Basic Fuchsin, 2% Methylene Blue and 50% silver nitrate solution have been most frequently used (Taylor and Lynch 1992; Hilton 2002).

The methodology has many advantages over the other techniques. First of all, the microleakage is demonstrated by single colored agent without the need for any further introduction of chemical reaction or hazardous radiation. In addition, the researchers can have a range of choices of available dye agents, which allows the method to be easily conformed to the instruments and methods available at the center in which the research is to be carried out. The technique is, therefore, highly feasible in any circumstances and can be easily repeated. They can, to some extent, have clinical significance since the particles size of dye agents can be pre-measured.

One again the method is destructive because the specimen is required to be sectioned so that the staining dye layer is measured and recorded under light microscopy or scanning electron microscopy. This neither allows the method to be reproduced nor is the specimen capable of being long-term assessed. In addition, the results are recorded only from one or two slices obtained from sectioning, which does not represent the whole image of microleakage which is three-dimensional. The results are therefore unreliable. Finally, it is highly technique sensitive and is not able to exclude the diffusion of the dye substance into tooth structures and the restoration from the measurement. The results again do not demonstrate the nature and the patterns of the leakage (Taylor and Lynch 1992; Hilton 2002).

The current studies have failed to make it clear whether a dye solution selected is suitable to use with tooth structure and restorative materials tested. For example, one particular frequent dye solution used, basic fuchsin and its solvent, propyl glycol, has been well documented to react with dentine. This process can cause the leakage image collected to be greater than the true image (Kidd 1976; Taylor and Lynch 1992). The other consideration is the particle size of the dye solution used. The final results can be extremely less reliable if the particle dimension of dye solution used is too small or too big in comparison with bacteria and dentinal tubule diameter (Taylor and Lynch 1992).

1.6.8 Metal solution tracers

Metal solutions have been commonly used as tracers to express the tooth/restoration gaps. It was frequently seen that the technique involves the use of at least two colorless chemicals to produce a colored precipitate at the tooth/restoration interface (Taylor and Lynch 1992; Li, Burrow et al. 2003). The microleakage deposition is, therefore, dependent on the penetration of both chemicals, since a precipitate may not occur if only one chemical, or the smaller of the two chemicals, exist (Taylor and Lynch 1992).

Early chemical tracing of leakage was introduced by Kornfield (1953), in which barium sulphide solution was used to investigate microleakage of acrylic resin containing lead glass. The reaction between barium and lead glass results in the formation of the lead sulphide which is in black and precipitates at the tooth/restoration interface, allowing microleakage to be determined (Kornfield 1953).

Manganese salts were also introduced as a non-radioactive marker for a microleakage study (Going, Mayers et al. 1968). However, it was commented that the presence of manganese, either in the restoration or in tooth structure, can result in the variability of the result. As a result, dysprosium was recommended as an alternative (Meyer, Dennison et al. 1974).

Recently, a solution of 50% silver nitrate has been most frequently used in conjunction with photo-developing solution (hydroquinone) to produce a precipitate at the restoration-tooth interface. This combination has been widely used as dental leakage dying technique (Taylor and Lynch 1992; Youngson, Jones et al. 1999; Mathew 2001; Hilton 2002).

The problem of the chemical tracer technique is that it involves the use of many chemicals and the microleakage result is dependent on chemical reactions.

A criticism arising from using silver nitrate is its clinical relevance, because of its molecular size (Douglas 1989; Matharu 2001). The particle dimension of silver ions (0.059 nm) is absolutely small in comparison with bacteria (2-4 µm) and the dentinal tubule diameter (1.0-4.0 µm), so the result of silver nitrate leakage is markedly sensitive because of the easy penetration of silver through the restoration-tooth interface and dentine tubules. It is believed that with the introduction of photo-developing solution whose molecular size is significantly larger than that of silver nitrate, the precipitate is, therefore, representative of bacterial size (Taylor and Lynch 1992).

The mechanism of silver staining at the tooth/restoration interface is still uncertain (Li, Burrow et al. 2003). On one hand, it was found that silver deposition was essentially affiliated with collagen fibrils (Adam and Whittaker 1972); in another hand, it was assumed that minute silver is precipitated freely at the tooth/restoration interface (Tay and Pang 1995). It seems that silver ions are highly active and therefore they are easily converted into silver metal, which can act as a stable dying agent.

1.6.9 Three-dimensional (3D) methods

Most microleakage researchers have recognized the disadvantages of two - dimensional analysis because of its simple sectioning into the specimen. A few studies (Youngson 1992; Gale 1994; Iwami and Hayashi 2007) have been developed to analyze microleakage three dimensionally.

The three-dimensional analysis was pioneered by Youngson (1992) who introduced the technique of producing serial sections using a water-cooled wire saw. Each section was approximately 200µm thick and separated by 280 µm. Three-dimensional models were then created by hand tracing projected transparencies and reconstructed by computer aided tools. Computer image analyzer was then applied to count the surface areas of dye leakage but the volume of leakage was calculated manually (Gale 1994). It was reported that microleakage in this three-dimensional analysis was significantly greater than that of the two-dimensional analysis. This is probably due to the more thorough examination of the object compared to the single sectioning technique.

However, the methodology is again destructive of specimens and thus all the disadvantages of sectioning technique were unavoidable. In addition, the technique is highly cumbersome and the restoration structure itself can be altered due to the comprehensive sectioning preparation. Apparently, the distance between the slices is still significant. The microleakage images were therefore comparatively low in pixel resolution and the loss of three-dimensional information is inherent to the methodology. Finally, manual tracing of the dye leakage is inherently subjective.

The methodology was then applied and upgraded by Gale (1994), who developed a reconstructed model with higher resolution, in which the surface separation was approximately 100-200 µm compared to 280 µm in the previous study. The images of consecutive surfaces, which were created by sequential grinding, were photographed by a computer with image resolution about 9.3 µm per pixel. For the microleakage staining procedure, instead of using water-soluble eosin, which was consider to be significantly leached during sectioning, the author used a high contrast, water-fast tracer, which was 50% silver nitrate solution. This solution was not leached through the grinding process. Recently, Iwami (2007) introduced an improved method based on the technique of continuous surface reductions similar to the above technique in conjunction with an electrical method. In this study, the sequence of surface reduction was more consistent by 100 µm and image taking was made with an operation (i.e. surgical operating) microscope. Three dimensional images were also created by computer software.

Although there were some improvements due to better control of surface reduction compared to serial sectioning, the methodology was again destructive of specimens.

Grinding processes of specimens may generate some mechanical deformation and unparallel surfaces, leading to increased measurement errors. In addition, the image resolution in depth direction was still low.

The micro-computed tomography 1072 was introduced in a microleakage study by De Santis (2005), who stated that the MCT-1072 is able to determine the silver deposition at the tooth-restoration interface non-invasively. However, there were many drawbacks relating to the study. Firstly, the experimental method did not reflect a true clinical situation as prepared cavities were not used. Secondly, the 50% silver nitrate solution that was used in the study was not buffered and can cause marginal erosion, leading to confounding results. Finally, no qualitative and quantitative analysis was presented.

1.7 Factors influencing microleakage studies

1.7.1 Substrate for microleakage studies

It is well documented that a myriad amount of microleakage research has been done on extracted human teeth although bovine teeth have been sometimes used (Hilton 2002). It was also cited that living human teeth are the best substrate for bonding tests and also to conduct microleakage tests. However, it is extremely hard to have these studies done in vivo, leading to exclusive use of extracted human teeth for in vitro study (Rueggeberg 1991).

The limited availability of human teeth and the concern about infection control have made bovine teeth a useful substitute. However, little research has been done to compare the microleakage results between the use of bovine and human teeth.

1.7.2 Storage factors

The factors such as time, media and temperature for the storage of extracted teeth and specimens can play a role on microleakage studies. These factors could be related to the period of time after extraction, before specimen fabrication, after specimen fabrication. In addition, due to the concern about infective diseases, most extracted teeth were placed in sterilizing/disinfecting solutions for a period of time before changing to another media for storage.

Research comparing the effects of autoclave and ethylene oxide sterilization procedures on bonding strength with those of non-sterilized specimens, found that there was no difference in shear bond strength and dentin permeability, and that either method of storage could be applied (Pashley, Tao et al. 1993).

The time vector after extraction has not been specified by most studies. The most common words “freshly extracted” were used to describe sample collection but it seems hard to extrapolate the exact time period from the “freshly extracted”. Generally, it ranged from minutes to years (Hilton 2002). A thorough review done by Rueggeberg (1991) concluded that time after extraction has no impact on bonding result. He also concluded that storage time after cavity preparation but before material placement could be more important, and that restorations should be completed immediately after cavity preparation to better simulate clinical procedures (Rueggeberg 1991).

Another time vector is storage duration after specimen fabrication. It was reported that there was a remarkable reduction in shear bond strength and increased gap at the cavity floor between 24 hours and six months, but no marginal gaps were found in the study done with Class V microleakage for two bonding agents with composite resin (Gwinnett and Ju 1994). There were also a number of studies investigating and comparing microleakage over time (Meiers and Turner 1988; Crim 1993; Gwinnett and Ju 1994), that reported little change in marginal gaps over time with adhesive materials or amalgam lined with adhesive agents.

A broad range of medium solutions have been used for the storage of extracted teeth, including formalin, thymol, chloramines, sodium azide, saline and water. These media may have different effects on enamel and dentin. It was found that physiological saline can make enamel softer while distilled water less so and sodium chloride had no effect on enamel surface hardness (Muhlemann 1964). It was also cited that formaldehyde is not an appropriate medium for storing extracted teeth as an oxidation process can form formic acid, which causes changes in pH of the medium solution (Rueggeberg 1991).

It seems that dentin was more affected by storage solution than enamel. Teeth stored in saline demonstrated the greatest changes in dentin permeability over time. It was found that the shear bond strengths of composite and dentin fluctuate with storage media and time after extraction. It was also reported that ethanol and formalin provided stable results, while the saline results were dramatically variable. The authors also found that microleakage markedly rose in teeth stored in chloramines solution after 48 days, but no further surge up to 135 days. These changes could be caused by the modification in dentin due to ion exchanges, changes in dentin collagen framework and dentin tubules (Goodis and Allart 1993).

1.7.3 Cavity design

Cavity design including size, shape and location can be important in a microleakage study because these variables closely relate to bonding efficiency of adhesive materials and thus microleakage results (Gale and Darvell 1999; Hilton and Ferracane 1999; Hilton 2002). It has been suggested that it is necessary for cavities to be as standardized as possible so as to eliminate variation among specimens.

Cavity size is an important variable for the microleakage testing of adhesive materials as polymerization shrinkage can be significantly altered by volume of the restoration. It was reported that the volumetric contraction during the setting phase of composite resins and GICs ranged from 1.0-3.6% by volume after 30 seconds and these shrinkages can reach a range of 2.8-7.1% after 24 hours (Feilzer, De Gree et al. 1988). The authors also stated that chemically setting GICs contracted less than that of light-cured resins. Despite the apparent significance of volumetric shrinkage associated with cavity size, a review done by Taylor and Lynch (1993), reported that very few studies gave details about cavity design and the cavity dimensions were rarely investigated.

Cavity properties such as depth can also be related to the extent of microleakage. This is likely due to the differences in the dentinal tubule diameter and dentinal tubule density, leading to differences in bonding effectiveness of the material (Trowbridge 1987).

Cavity shape is considered to be the factor that relates closely to the restoration stresses and so to microleakage formation. These stresses were shown to be proportional to the contact surface area which bonds to the restoration (Davidson and De Gree 1984). It was stated that the increase in the ratio of bonded surface to free surface can increase the internal stress within the restoration. The degree of internal stresses, therefore, varies among different class cavities and the highest values can be with Class I and Class V cavities.

It can be seen in the literature that cavity design varies amongst studies with respect to the dental material being analyzed. (Taylor and Lynch 1993; Hilton 2002). For example, some authors introduced the beveling of enamel margins to compare the microleakage of composite resins with non-beveled cavities or butt margins and found that beveling enamel margins reduced leakage (Holtan, Nystrom et al. 1990). Another cavity modification was introduced with one and two notches placed at the axial-gingival line angle in Class II cavities and found that the notches improved marginal sealing (Coli, Blixt et al. 1993). Moreover, a variety of cavity shapes have been also introduced such as saucer-shaped preparations (Krejci and Lutz 1990), wedge-shaped Class V cavities (Prati and Nucci 1991), and Cylindrical Class V cavities (Kamel and Retief 1990).

Location of cavities can be also an important factor closely relevant to microleakage results. This is because adhesive materials may behave differently among enamel, dentin and cementum, resulting in internal stresses and marginal adaptation differences. It was also noted that the majority of microleakage studies preferred having margins involved in both enamel and dentin and that cementum has been importantly ignored (Hargreaves, Grossman et al. 1989; Taylor and Lynch 1993).

It has been suggested that in-vitro cavity designs for microleakage studies should involve cementum, as clinically cervical lesions are increasingly prevalent, due to the fact that, these lesions may be proportional to the increasingly aging population. Such lesions may have special treatment requirements in bonding ability and cavity preparations (Hargreaves, Grossman et al. 1989). There have been some studies comparing the sealing ability of composite resins in cavity preparations with margins involving cementum (Phair and Fuller 1985; Staninec and Mochizuki 1985). They concluded that the etching-bonding condition on cementum showed little effect on composite resin sealing. However, a suitable method of preservation of cementum surface and cementum condition was not mentioned in these studies. The conclusion about composite bonded to cementum was therefore less reliable.

1.7.4 Microleakage expression and analysis

As discussed previously, the most popular technique for the investigation of restoration sealing is through a microleakage study (Taylor and Lynch 1992; Gale and Darvell 1999; Hilton 2002), in which the use of dyes for in-vitro experiments has been dominant. As a result of this work a number of issues concerning methodology reliability and technique identity have arisen. Of particular concern are the issues of microleakage expression and analysis, both of which can affect microleakage results.

First of all, a variety of techniques have been used for the immersion of specimens in the dye solution. Many studies have compared microleakage using different methodologies and found that dye immersion time and different thermocycling techniques did not affect microleakage (Hilton 2002). In this literature review, Hilton also stated that the time used for specimen immersion commonly ranged from one hour to two weeks but most commonly 24 hours. In addition, it was found that dye temperature during staining was not specified, commonly mentioning room temperature or 37ºC.

Another concern is the use of different types of dyes in microleakage studies. Dying agents may behave differently due to different molecular size and different level of affinity to tooth structures and restorative materials. A large range of dyes have been used in microleakage studies and thus it seems very hard to locate identical study protocols. As a result, it is difficult to interpret the differences in microleakage results collected from different types of dyes.

Microleakage assessment is considered as a factor influencing study results. As can be seen, most of studies by far have sectioned the specimens to be assessed and most of these had a single section through the center of the restoration (Taylor and Lynch 1992; Hilton 2002). As discussed previously, this evaluation technique does not reflect the whole image of microleakage.

Recently, attempts have been made to assess microleakage three-dimensionally by serial sectioning of the specimen into very thin two-dimensional slices which can then be reconstructed and interpolated into a three-dimensional image (Youngson 1992). Another technique was introduced by Gale (1999), who presented a sequential grinding of the specimen and image reconstruction by computer. The authors stated that three dimensional techniques revealed markedly greater microleakage than the two dimensional assessment.

Microleakage recording and statistical analysis are also crucial. Almost all of the current methods of recording data simply code the two dimensional extent of dye leakage with cardinal or ordinal scale for the statistical calculation, which is subjective.

1.8 Glass Ionomer Cements (GICs) and microleakage studies

There are two main reasons for selecting GICs to validate the proposed methodology. Firstly, there has been an increasing interest in using GICs in practice but little research on microleakage has been done. Secondly, questions raised from previous microleakage studies were that the cements tend to take up dye agents, making it difficult to accurately measure microleakage. The previous techniques have been unable to distinguish leakage staining from that occurring via marginal gaps or from restoration absorption. The proposed methodology is hoped to overcome these problems.

It has been believed that GICs would be able to prevent microleakage as a result of its chemical bonding to tooth substances (Mount and Hume 2005). In spite of that, results from current in-vitro microleakage studies have been equivocal. Many studies (Prati 1989; Sparks and Hilton 1992; Hallett and Garcia 1993; Quo and Drummond 2002) have consistently shown that marginal sealing of GICs to tooth structures was not sufficient enough to prevent dye leakage into the tooth-restoration interface. A study comparing microleakage of one chemically-cured and two resin-modified GICs on Class V cavities reported that all three cements behaved similarly in marginal leakage, showing slight leakage (Brackett, Gunnin et al. 1995). Another study also comparing two resin-modified with one chemically-cured glass ionomer cement on Class V cavities reported that no leakage was found for these three cements (Crim

1993). Other studies (Davidson and Abdalla 1994; Sidhu 1994) reported that microleakage of resin-modified GICs was slightly less than that of chemically-cured GICs. Similarly, another study comparing microleakage of self-cured GICs with third generation dentin bonding agents/resin-based composite, reported no differences in microleakage among groups at one week, 6 months, and one year and that microleakage at one year was more severe than at one week (Reeves, Fitchie et al. 1990). Another comparable study using the same dentin bonding agents on Class V cavities concluded that GICs demonstrated less leakage than that of dentin bonding agents (Sidhu and Henderson 1992).

In terms of secondary caries, a long term clinical study (Mjör 2005) about the causes of restoration replacement reported that recurrent caries rates of glass ionomer restorations are as high as that of composite, which accounts for approximately 50% of the whole reasons for restoration failure.

It is believed that the study of microleakage with GICs involves many difficulties. It is well agreed that GICs tend to take up moisture and ions as they are basically acid-based cements. As a result, it is suggested that GIC restorations be immediately covered with a varnish or unfilled resin in order to isolate the material. This procedure, if applied in the microleakage study, can bring about other confounding factors. It is hard to be sure that the microleakage results are not influenced by surface coating. In addition, if this surface is removed by a cleaning finishing technique the researcher can not be certain whether or not the coating substances penetrate into marginal gaps, interfering with the dye leakage. Finally, as a consequence of moisture and ion uptake by GICS, the exact amount of microleakage is hard to identify (Sidhu and Henderson 1992).

1.9 Conclusion

Microleakage is definitely an important issue in modern dentistry, particularly when new versions of adhesive materials are constantly introduced. Various methodologies have been developed but none are reliable. It is reasonable to conclude that research should focus on microleakage methodology in order to develop a reliable technique before applying it to the study of microleakage on materials.

CHAPTER 2: HYPOTHESIS AND OVERVIEW OF APPROACHES

2.1 Hypothesis of the study

As pointed out in the literature review, the ideal method would be non-destructive and the results should be analyzed three-dimensionally. Based on this suggestion, a hypothesis was constructed.

This study was designed to test the following hypothesis:

Microleakage of restorative materials can be detected non-destructively and microleakage results can be analyzed three-dimensionally.

2.2 Overall approach to achieving the objectives

As introduced previously, a major emphasis of this study was to investigate a non-destructive three-dimensional method for studying microleakage of restorative materials. Having reviewed the current methodologies and the advanced medical technology, it is likely, at this time, that a way forward would be to explore the potential of MCT in not only identifying microleakage non-destructively but also the possibility arises to be able to analyze the result in three dimensions. One of the most important parts of the project was to obtain a study protocol that can be effectively and efficiently used with the MCT/Skyscan-1072 (MCT-1072), which is available at the University of Adelaide.

The first step was to investigate the x-ray contrast dye solution that can reveal microleakage of restorative materials. Then, it was to assess if this microleakage expression can be photographed by the MCT-1072 and then quantified.

It was also necessary to conduct an investigation involving the specimen-restorative dimensions in order to determine the best spatial resolution that could be obtained in the scanning process. In addition, the programs used for image analysis and 3D microleakage analysis were evaluated. Finally, the main experiment was carried out to finally validate this methodology.

The material chosen for the study was a conventional glass ionomer restorative material, Fuji IX. This material is considered to have adhesive properties to enamel and dentine but has not been widely investigated with respect to its microleakage potential.

CHAPTER 3: MATERIALS AND METHODS

3.1 Introduction in the MCT-1072

3.1.1 Introduction

Generally, an X-ray system generates two-dimensional shadow images from three-dimensional structures. In pure two-dimensional radiography the depth information is completely missed. Only an X-ray tomography system allows three-dimensional object structures to be viewed and analyzed in an absence of sample preparation or chemical fixation. Basically, the spatial resolution of traditional medical computer tomography scanners falls between 1-2 mm. The MCT-1072 allows a spatial resolution in order of microns. Similar to conventional computer tomography scanners, the fully three-dimensional structures of the specimen can be reconstructed non-destructively.

3.1.2 Skyscan 1072 system overview

The Skyscan 1072 is an advanced digitalized system for x-ray microscopy and micro-tomography, which is the combination of an x-ray shadow microscopic unit and a computer installed with tomographic reconstruction software. The system allows the production of a non-destructive three-dimensional reconstruction of the highly detailed internal structure of objects from two-dimensional x-ray shadow projections.

Figure 3: The Skyscan 1072 system

The equipment contains an x-ray microfocus tube with high-voltage power supply, a specimen stage with precision manipulator, two-dimensional x-ray CCD-camera connected to the frame-grabber and a Dual Pentium (IBM) computer with color monitor. The system can be summarized as follows (Skyscan).

The x-ray microfocus tube with several microns focal spot size operates at 20-80kV/100 µm or 20-100kV/0-250 µA. The special x-ray CCD-camera is based on high-resolution (1024x1024) cooled CCD-sensor with fibre optic coupling (3.7:1 image reduction) to x-ray scintillator or 768x560 pixels CCD-sensor with lens coupling to x-ray scintillator (Skyscan).

NOTE: This figure is included on page 33 of the print copy of the thesis held in the University of Adelaide Library.

Figure 4: Schematic working chart of the Skyscan 1072 (Skyscan)

The x-ray shadow projections digitized as 1024 x 1024 pixels with 4096 brightness gradation (12 bit) for cooled camera or 256 gradations (8 bit) for analog camera. The reconstructed cross-sections have a 1024x1024 (or 2048x2048, 512x512…) pixels (float point) format. Typical circle of data collection for reconstruction contains of shadow image acquisition from 200 to 400 views over 180 or 360 degrees of object rotation.

For the reconstruction of 3D objects a serial reconstruction of cross sections is operated with reconstruction programs. It starts with one acquisition cycle followed by an “off-line” reconstruction of the complete three-dimensional object in a resolution of 1024x1024xmax1024 layers. After the serial reconstruction, the cross-sections of the object can be displayed on the screen and a realistic view of the three-dimensional object is obtained.

There are two main programs for image analysis and visualization of the results from the MCT-1072: CT-an (Skyscan) for 2D visualization and 2D and 3D analysis, and

ANT (Skyscan) for realistic 3D visualization. In addition, T-view and Data-viewer (Skyscan) can be used to primarily visualize images. With these programs, qualitative analysis and quantitative analysis of microleakage results can be comprehensively analyzed. More details about these programs are presented in the next part of this paper.

3.2 Selection of dye solution

3.2.1 Introduction

As discussed previously, dye techniques have been a popular method in microleakage studies. The dye agent that that can be identified by the MCT-1072 has to be an x-ray contrast solution. As a result, the search for a suitable agent was focused on solutions containing metal ions.

It is well agreed that a dye solution for microleakage studies should meet some fundamental requirements. Firstly, it must be able to penetrate into the tooth/restoration interface. Secondly, it must satisfactorily stain the tooth/restoration interface. Thirdly, it should not react with tooth structures and restorative material.

As stated in the introduction to Chapter 1, the metal solution, containing 50% silver nitrate has been broadly used in the field. However, it is highly invasive, interacting with the restorative material and tooth substances.

It was decided to test other metal solutions. Barium nitrate and lead nitrate solutions were chosen for the test as it is thought that they may be chemically less invasive than that of silver nitrate. However, the possibility of staining could occur due to metal precipitation formed as a result of a chemical reaction with metal salts existing in the Fuji IX.

3.2.2 Preparing x-ray contrast dye solutions

A 50% silver nitrate solution (Chem-Supply, Batch Ref 10 23015, code SA 087), a saturated concentration of barium nitrate (10%) and lead nitrate solution (15%) were prepared.

It was noticed that that the non-buffered silver nitrate solution with pH 3.5 is highly acidic and thus corrosive to tooth structures and Fuji IX. The solution therefore can produce its own path into tooth/restoration interface (Li, Burrow et al. 2003). It was decided to buffer the pH of the 50% silver nitrate solution in order to diminish the corrosive potential.

However, the pH of barium and lead nitrate was not determined at this stage, as this was only a preliminary experiment to identify whether or not these solutions can be used as dye solutions for microleakage studies.

It was found that the 50% silver nitrate solution was extremely chemically active. Many common buffering systems were tried but not successful due to their chemical reaction to nitrate solution, causing precipitate. The successful buffer formula for the 50% silver nitrate solution found was therefore presented as a useful system for future use.

The buffer system is based on acid boric/ sodium tetra-borate and the formula as follows:

Solution A: 0.2 M boric acid (mw: 62.5), which was made from 1.24 g boric acid in 100 cm^3 DDW.

Solution B: 0.5 M sodium tetra-borate (mw: 381.4), which was generated from 1.9 g sodium tetra-borate in 100 cm^3 DDW.

90 cm^3 solution A was mixed with 10 cm^3 solution B to make a buffering solution having pH 7.4. This solution was used to bring pH of the 50% silver nitrate solution to 6.5-7

3.2.3 Testing the interactions between the dye solution and Fuji IX

This experiment was designed to examine the surface interaction between the above metal solutions with Fuji IX, which will be used in the main experiment for the validation of the proposed methodology.

Sixty cylindrical specimens approximately 4 mm in diameter 6 mm in height of Fuji IX (GC Corp., Tokyo, Japan, lot no. 05033081) were produced using a split mould, stored in 100% humidity for 48 hours, allowing the material to mature. The specimens were then divided into 3 groups of 20 each, immersed in the above three solutions for 24 hours at room temperature (silver nitrate was placed in darkness), rinsed in running water for 5 minutes.

In order to determine surface reaction between the material and the solutions, specimens were embedded in epoxy resin at a ratio of 100:25 for epoxy resin LC 191 (Adelaide Epoxy Supplies, Adelaide, Australia), using plastic circular moulds. The cylindrical specimens were then mounted both horizontally and longitudinally so that every surface can be examined via cross sectioning. The specimens were then sectioned in halves using a water cooled low speed diamond saw (Buehler, USA), with Diamond Wafering Blade, No. 11-4244 (Buehler, USA). Since a smooth surface is required for analysis, the samples were then polished manually using medium grit with an aluminum oxide micro-abrasive system (Struers, Copenhagen, Denmark). The specimens were assessed using optical microscopy.

Figure 5: Isomet Slow –speed saw

3.2.4 Testing staining properties

As discussed previously, the ability to penetrate and stain the tooth-restoration interface is an essential prerequisite for the metal solutions. Except for silver nitrate which has been used as a dye solution in earlier microleakage studies, the barium and lead nitrate solution have not been confirmed as suitable dye solutions in the field. The ability to demonstrate microleakage using these solutions was therefore investigated.

In order to test marginal staining properties of the above two metal solutions, microleakage of a tested restoration was promoted so that the dye solutions were free to enter the tooth/restoration interface and so express this space.

The material of choice for this experiment was also Fuji IX. The reason was that it is necessary to examine the same material of choice so that the results have consistency

For the detection of microleakage, specimens were prepared to be examined with the MCT-1072. By using the MCT-1072, at this stage, the suitability of the machine could be assessed for its ability to display microleakage.

Ten non-carious premolars, extracted for orthodontic treatment, were collected, stored in de-ionized distilled water (DDW) containing 1% thymol at room temperature. After surface debridement with a hand scaling instrument and cleaning with a rubber cup and pumice, cavity preparations were placed in the buccal and lingual root surface 1 mm apical to the cementum-enamel junction. 20 preparations were produced 1 mm deep and 2 mm wide using round diamond burs (Komet Brasseler, USA) and high-speed hand-piece (Sirona, Siemens, Germany).

The reason for choosing the root was that its size is relatively smaller than that of the coronal part. This smaller size part can increase the spatial pixel resolution of the

MCT-1072 as the resolution of the MCT-1072 is dependent on the dimension of the object scanned. In addition, it has been suggested that studying microleakage of GICs should be done on dentin because of it clinical relevance.

The teeth were filled with Fuji IX (GC Corp., Tokyo, Japan, lot no. 05033081) and stored in 100 % humidity for 48 hours for maturing the restorative material. As mentioned above, the cavity surfaces were not treated with conditioning in order to promote microleakage of the Fuji IX. The metal solution was therefore encouraged to enter the tooth/restoration interface.

Groups of three teeth were made and immersed in each solution for 24 hours, encouraging the penetration of the metal solution into the tooth-restoration interface. The specimens were then rinsed under running water and dried for mounting in the MCT-1072. The extra tooth was treated the same but with the non-buffered silver nitrate solution. This was to examine the surface erosion of the restoration caused by the non-buffered solution.

Each specimen was securely placed and fixed into the specimen holder of the MCT1072. In order to achieve the best spatial resolution, the image magnification was adjusted to as high as possible while allowing for full rotation of the specimen within the CT machine. The program was commenced with source voltage set at 100 kV and source current at 120 µAmps, beam hardening set to 10.

The time required for each specimen to be completely scanned was roughly 2 hours, producing approximately 1000 projections in TIFF. These images were then converted to tomograms (cross-sections) saved in BMP, using NRecon (version 1.4.3; Skyscan). The images were then examined for microleakage using image analysis programs provided by Skyscan such as T-view and CT-an.

3.2.5 Development of study model

3.2.5.1 Introduction

It was felt that an appropriate model designed to realize the advantages of the MCT-1072 was essential for the proposed methodology. By the time this project started, there was no previous microleakage studies using MCT published. It was decided to develop an in vitro model of microleakage that could be best used with the available technology-the MCT1072.

It should be emphasized that the restoration must be non-destructively examined and its size must be harmonious to the whole tooth model. In addition, the spatial resolution of the MCT-1072 is dependent on the whole tooth dimension, particularly the diameter of the specimen. As a result, cavity size and cavity location should be located close to where the 4 mm in diameter of the specimen can be achieved. In addition, the cavity is closely related to the material of choice for the clinical relevance. Finally, it is necessary that the programs that are able to quantitatively and qualitatively analyze the images should be incorporated within the model.

3.2.5.2 Specimen preparation

As discussed previously, human teeth were chosen for this experiment as it has been shown that microleakage patterns of restorative materials in human teeth may have more clinical relevance than that on animal teeth (Taylor and Lynch 1992; Hilton 2002).

There were other factors contributing to the development of the proposed model. Firstly, as Fuji IX was the material of choice to validate the methodology it was decided to prepare cavities on root surfaces. Secondly, since the tooth containing a cavity to be scanned must be within the limit of 4 mm in diameter, the premolars were chosen. Moreover, the longitudinal sectioning in halves mesio-distally of the premolars produced two specimens of 4 mm in diameter (Figure 17). Finally, the cavity sizes were decided after considering these concerns by such conditions.

To validate the model it was realized that this part of the research should be considered as a pilot experiment so that a small study sample was used. Four non-carious premolars, extracted for orthodontic reasons, stored in DDW containing 1% thymol were selected. After surface debridement with a hand scaling instrument and cleaning with a rubber cup and pumice, a standardized cavity preparation was placed in the buccal and lingual root surface, 1 mm apical to the cementum-enamel junction. Uniform round preparations were made 1 mm deep and 2 mm wide using round diamond burs (Komet Brasseler, USA) and high-speed hand-piece (Sirona, Siemens, Germany). These cavities were compatible to the specimen size as pointed out above. The cavities then were measured to be 2 mm ± 0.3 in diameter and 1 mm ± 0.3 in depth with the whole margin in the root surface.

The cavities were then checked for cracks at the margins using a light stereomicroscope. This step was necessary to eliminate those cavities with defects that may allow dye solution to ingress through microscopic spaces of the tooth-restoration interface, thereby giving false positive results.

Only the buccal cavities (marked by a notch at the buccal enamel edge) were treated with dentin conditioner (3M ESPE, USA) according to the manufacturer's instruction and all cavities were filled with Fuji IX (GC Corp., Tokyo, Japan, lot no. 05033081). The restorations were then stored in 100% humidity at room temperature for 48 hours.

To prevent dye penetration in areas other than the exposed margins, the apices were covered with Fuji 9 and the teeth were sealed with two layers of nail varnish to within 1 mm away from restoration margins.

The 50% silver nitrate solution (buffered to pH 6.5) was an x-ray contrast solution of choice based on the above testing. The silver nitrate solution proved to stain well the tooth-restoration interface and this layer of silver deposition was highly distinct from dentin and materials. Although previous studies have suggested that photo-developing solution helps precipitate silver ions to metal silver to stain the tooth-restoration interface (Taylor and Lynch 1992; Heping Li 2003), the above part of this study showed no difference in silver deposition when images were compared to non-developing solution. Therefore, this study demonstrated that photo-developing solutions were not required.

The specimens were immersed in 50% silver nitrate solution in darkness for 24 hours, rinsed with running water for 5 minutes and exposed to light for 8 hours allowing conversion of silver ions to silver metal that stayed stable at the tooth-restoration interface. The specimens were then cleaned slightly with Soflex disks (3M ESPE, USA) to partly eliminate silver staining at the restorative surface.

Each tooth was then sectioned longitudinally via a mesial-distal direction (using Isomet low-speed saw, Buehler), giving two specimens within 4 mm in diameter (Figure 6). The specimens can be adjusted at the sectioning surface so as to ensure that every specimen has the diameter at about 4 mm. It should be noted that the longitudinal sectioning must be carried out carefully so that the whole restoration can be completely preserved non-destructively. These models were then ready for scanning with the MCT-1072.

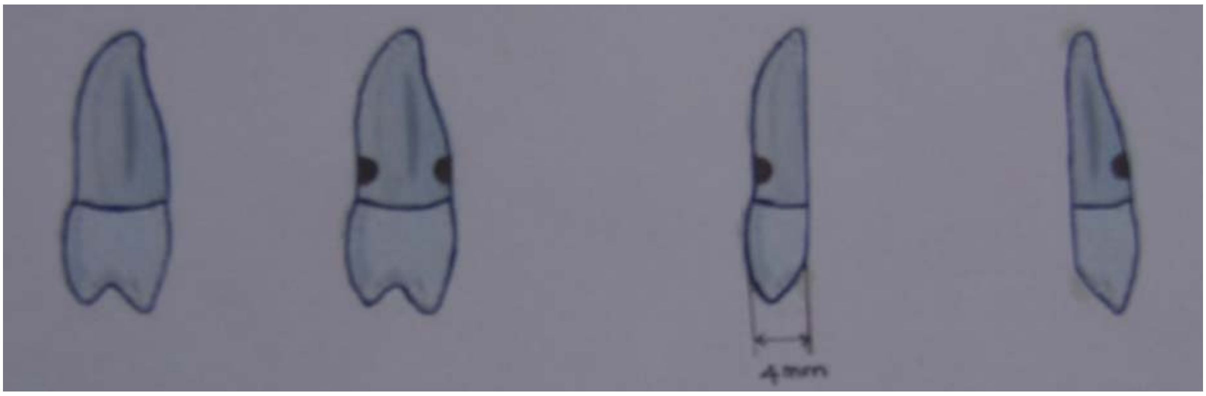


Figure 6: the proposed model (4 mm in diameter)

The specimen was securely placed and fixed into the specimen holder of the MCT1072. The specimen holder 4 mm in diameter was chosen so that the maximum magnification for small object sizes was reached (Figure 7-8). As a result, the best spatial resolution of the MCT 1072 was obtained. During operation with this specimen holder, the specimen position and its rotation were carefully checked by the visual camera to avoid the object touching the x-ray tube or other parts inside the system. The program was commenced with the source voltage set at 100 kV and source current at 120 µAmps, beam hardening set to 10. The magnification was 75X and thus approximately 4.166 µm of image pixel was obtained.

Figure 7: Specimen holder

Figure 8: Special specimen holder 4 mm in diameter

The time required for each specimen to be completely scanned was roughly 2 hours. Each specimen was entirely scanned, producing approximately 1000 projections in TIFF. The projections were constructed to tomograms (cross sections) saved in BMP using NRecon (Skyscan). All information about the scanning procedures and their results for every specimen, particularly the spatial resolution, were automatically reported and saved. The report of every specimen was checked for resolution consistency.

3.2.5.3 Qualitative and quantitative analysis

3.2.5.3.1 Selection of programs for image analysis

The image analysis programs used in this work were T-view, Data-viewer, CT-an and ANT (Skyscan, Aartselaar, Belgium). The tomograms (cross-sectioning images) saved in BMP were compatible to all these programs so the images can be viewed and analyzed easily.

T-view is able to convert between TIFF and BMP files with adjustment of color palette, inversion, renaming, resizing and combining of datasets. Data-viewer assists in visualizing 2D images in three intersecting orthogonal sections, which can be turned and each intersecting slice independently moved by simple control. As a result, horizontal images (or cross-sections), coronal images and sagittal images can be viewed simultaneously. CT-an is the most important program that is able to calculate the volume of microleakage. ANT is the program that reconstructs 3D images of microleakage, giving a realistic view on microleakage structures.

3.2.5.3.2 Image analyses

Approximately 1000 cross-sectioning images or tomograms were produced from every single specimen. However, in order to limit the numbers of images that have validity, only images that contained the restoration were selected. Even so, there were about 550 -600 images that needed to be analyzed in each specimen.

It is apparent that it is impossible to analyze every image because of the huge numbers of images. In other words, 2D quantitative analysis is impractical. It was decided to focus on analyzing microleakage quantitatively three-dimensionally using computer software programs.

Initially, T-view was used for two-dimensional visualization. It was convenient to display images as a slice-by-slice by scrolling the mouse so as to visualize every single image. Also, it was possible to scroll forwards and backwards for any further examination. This enables the examiner to record the severity of microleakage in 2D images and thus this can be used as a reference in 3D analysis. In addition, T-view is useful in terms of identifying silver deposition at dentinal tubules and in the restoration.

Further image analyses were done with Data-viewer, where three intersecting orthogonal sections can be visualized, allowing the extent of silver spreading to the surrounding structures to be confirmed.

3.2.5.3.3 Image analysis with CT-an

One of the aims of the proposed methodology was to identify and exclude any dye agent that had diffused into the dentinal tubules and the restoration from the microleakage result. It was found that the CT-an allowed the identification of areas to be measured (i.e. region of interest).

CT-an is able to quantitatively analyze microleakage both two-dimensionally and three-dimensionally. Originally, the program was designed to measure bone volume and as a consequence, in microleakage images, silver deposition at the tooth/restoration interface was considered as bone density.

Initially, the MCT results were loaded in CT-an. The shadow images and list of files are displayed in the top part of the program window and one of the cross sections in the lower part. This image can be then magnified for closer investigation.

Microleakage can be measured in 2D images. However, as discussed previously, microleakage calculated from 2D images cannot be representative of the 3D structure of the microleakage. In addition, approximately 1000 cross-section images were produced from any single specimen, in which 500-600 images needed to be analyzed. The 2D quantitative analysis was therefore impractical. It was decided not to measure the dimension of the microleakage in the cross sectional images.

The CT-an provides many useful functions for volumetric calculation. These functions are activated once a dataset is loaded, including original image view, selection of region or volume of interest, conversion of images to binary for quantitative image analysis.

As also discussed above, in general, silver deposition at the tooth-restoration interface is highly distinct from surrounding structures because of difference in the level of x-ray contrast density. This silver deposition was therefore outlined from the surrounding structures and calculated in volume.

It was realized that the accuracy of the microleakage result could be improved if the technique could identify the amount of dye agent that infused into the surrounding structures. This meant that only the microleakage at the tooth/restoration interface was measured and the amount of leakage that extended into the restoration and dentinal tubules could be identified and excluded from the microleakage result.

CT-an provided the tool that allowed selecting only the region that needed to be measured. This meant that it was possible to outline the silver deposition at the tooth/restoration interface only.

Microleakage was located by activating the “volume of interest” menu and drawing with the left mouse button, following the microleakage shape as close as possible to the image of silver deposition at the tooth/restoration interface in the cross-sectional image. This shape could be copied to all the cross sections in the dataset. It was realized that the microleakage of each restoration was complex in morphology and significantly varied from tomogram to tomogram. It was therefore important to accurately draw the microleakage individually. The software was able to interpolate them automatically through all intermediate cross sections.

Of course, only the images that contained the restoration needed to be analyzed. These images were more than 550-600 for a single specimen. Therefore, the volume-of-interest could be limited within these images and this could be done by moving two sliders in the vertical direction bar to the right of the files list. The blue bar appeared between the top slider and the bottom slider in the vertical bar, indicating the number of files selected. Double-clicking on the blue bar displayed the conversation boxes in which upper and lower limits could be entered numerically. The region-of-interest containing the microleakage limit was then saved and reset.

The next step was to prepare all parameters for quantitative analysis. It was initiated with the binary images and by opening the “histogram” dialog window. It was important to accurately select the upper and lower global threshold levels for silver deposition by using the sliders above and under the histogram. The white part represented silver deposition at the tooth/restoration interface. The histogram could also be saved.

The histogram could be used for the 2D and 3D quantitative analysis. However, as discussed above, 2D analysis was not a focus of this work. The whole volume of microleakage was calculated by starting the 3D analysis button. By selecting all the required parameters, a data text report could be saved and printed out

3.2.5.3.4 Creation of 3D image with ANT

One of the interesting parts of the work was to reconstruct 3D images of the whole tooth-restoration-microleakage complex. It was hoped that creation of 3D images would provide a realistic qualitative analysis of microleakage.

Some earlier studies had tried to reconstruct the 3D image of microleakage from serial sectioning of the specimens. However, these images lacked reality because of the low spatial resolution (more than 100 microns between two sections). With the spatial resolution of 4 microns, it was hoped that the 3D images of microleakage were more representative. It was decided to use ANT for the 3D reconstruction as it had all necessary functions for creating the 3D model: movement, rotation, surface and illumination adjustments, re-cutting and many other possibilities.

It involved many steps to create and manipulate a full 3D model of microleakage. This included, in the order of time, getting started, model creation, model visualization, plane and shadow, visualization of internal structure, scene controls and flight, and creating cross sections with arbitrary orientation. In addition, there were other commands that could rotate the models.

Of special mention was the significant time required to three-dimensionally reconstruct one model. Time taken was dependent on the parameters of object densities that were set in the “density window”. These parameters include the “Step”, “Locality” and “Tolerance”. The “Step” indicated the step size of the original dataset. The “Locality” helped adjust the size of neighboring area pixels, which could be scanned to find the object connectivity. The “Tolerance” helped select the accuracy in the surface reconstruction. In addition, these parameters could influence the levels of smoothness and sharpness of the models.

It was suggested that the “footstep” be set at 1 for silver, 15 for restorations and to 30 for teeth. The “Locality” was set at 1 for silver, 15 for restoration and 100 for teeth and the “Tolerance” was set at 1 for all components. These settings ensured the model would be created efficiently and effectively so that the microleakage of the restoration could be visualized in relation to its surrounding structures.

In the step of "Model Visualization", when 3D images appeared on the screen, all the buttons of movements and rotations could be accessed, allowing movement and rotation of the object model around corresponding axes.

There are two main ways for visualizing the object's internal structures. The first one was to make the model body semi-transparent, which can be done in "model properties". Another way was to cut a corner of the model and show the internal structure.

It was decided to set the silver, the restoration and the tooth at different levels of translucency and then color code, allowing each structure to be visualized clearly.

3.2.6 Main experiment-studying microleakage of Fuji IX

3.2.6.1 Introduction

The above pilot study showed that the above model of the tooth-cavity complex together with the MCT-1072 and its software programs can be a useful model in 3D microleakage studies. Furthermore, it is a truly non-destructive methodology with comprehensive 3D analysis.

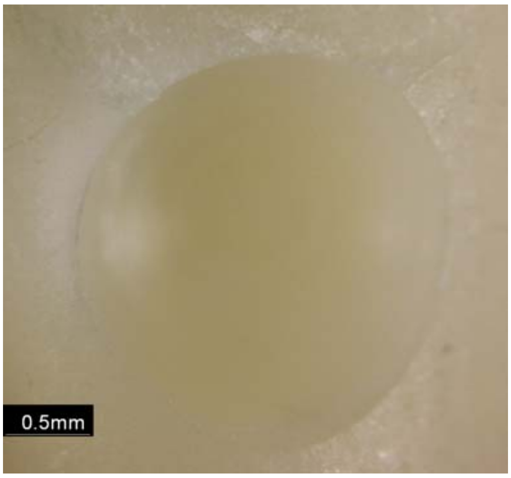
However, it was realized that a number of aspects of the technological mechanisms and their sensitivity for assessing microleakage needed validation and the consistent results achieved from the MCT-1072 should be confirmed. It was decided to develop an experiment for the assessment of the consistency and sensitivity of the model. In addition, it was conducted to study microleakage of the restorative material.

The material selection for the validation of the model has already been discussed. Fuji IX was a challenging material for the microleakage study because of its water-based property. It was hoped that with the use of appropriate computer software, microleakage of this material could be analyzed more accurately, so that the problems raised by previous studies were no longer an issue.

3.2.6.2 Experiment preparation

Twenty non-carious premolars stored in 1% thymol in DDW were selected. After surface debridement with a hand scaling instrument and cleaning with a rubber cup and pumice, a standardized cavity preparation was placed in the buccal and lingual root surface 1 mm apical to the cementum-enamel junction. Uniform round preparations were made 1 mm deep and 2 mm wide using round diamond burs (Komet, Brasseler, UK) and high-speed handpiece (Sirona, Siemens, USA). The cavities then were measured 2 mm ± 0.3 in diameter and 1 mm ± 0.3 in depth with the whole margin in the root surface.

The cavities were then checked for cracks at the margins using light stereomicroscopy (figures 9-10) to eliminate those cavities with defects that may allow dye solution to ingress through microscopic spaces of the tooth-restoration interface, giving false positive results.



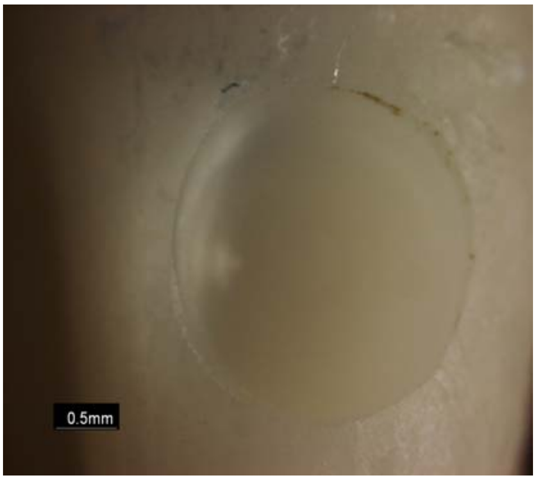


Figure 9 Figure 10

The teeth were then divided randomly into two groups of ten each. In the group 1, only the buccal cavities (marked by a notch at the buccal enamel edge) were treated with dentin conditioner (3M ESPE, USA) according to the manufacturer's instruction and all cavities were filled with Fuji IX (GC Corp., Tokyo, Japan, lot no. 05033081). Vaseline was used for coating the restoration surface immediately after restoration placement to limit water absorption and dehydration. Group 2 were treated exactly as group 1 except that the restorations were not protected by Vaseline. The restorations were then stored in 100% humidity at room temperature for 48 hours.

To prevent dye penetration in areas other than the exposed margins, the apices were covered with Fuji IX and the teeth were sealed with two layers of nail varnish to within one mm away from restoration margins.

The 50% silver nitrate solution (pH buffered to 6.5 using boric/sodium tetra-borate buffer system) was the X-ray contrast solution of choice based on a pilot study which was part of this research. In addition, it was decided not to use photo-developing solution in conjunction with silver nitrate solution as suggested by previous studies (Taylor and Lynch 1992; Heping Li 2003).

The specimens were immersed in 50% silver nitrate solution in darkness for 24 hours, rinsed with running water for 5 minutes and exposed to light for 8 hours, allowing conversion of silver ions to silver metal that stays stable at the tooth-restoration interface. The specimens were then cleaned slightly with Soflex disks (3M ESPE, USA) to eliminate partly silver staining at the restorative surface.

The tooth models for scanning with the MCT-1072 were prepared according to the above methodology and 20 specimens 4 mm in diameter were produced.

The procedures of placing the specimen in the MCT-1072 were described in the above experiment.

The time required for each specimen to be completely scanned was roughly 2 hours. Each specimen was entirely scanned, producing approximately 1000 slices saved as TIFF files which were then converted into BMP files using the NRecon software version 1.4.3 (Skyscan, Belgium).

These slices can be visualized using T-view and Data-view. With the T-view, all 2D images were screened allowing silver leakage to be identified. DATA-viewer can separate these slices into three spatial directions.

CT-an was used to quantify the volume of silver leakage while assessing the restoration three dimensionally. In order to eliminate any silver diffusing into dentin tubules and the restoration, the program allowed the identification of areas of interest to be measured.

Silver deposition at the tooth-restoration interface was distinguished from the tooth structure and the restoration as its X-ray density was different with these structures. In addition, this silver deposition was seen easily since the whole restoration was depicted.

Finally, three dimensional images were constructed using 3D-ANT in which Step is set at 1 for silver, 15 for restorations and to 30 for teeth. The Locality was set at 1 for silver, 15 for restoration and 100 for teeth and Tolerance was set at 1 for all components. In addition, the silver, the restoration and the tooth were set at different level of translucency and color, allowing each structure to be visualized.

CHAPTER 4 RESULTS

4.1 Surface reactions

The experiment was designed to observe whether or not the metal solutions chosen reacted with or were absorbed by Fuji IX. Qualitative analysis was therefore an emphasis of this experiment.

The surface staining was observed and photographed using an optical microscope (figure 11-13). It was obvious that silver reacted strongly with Fuji IX and that silver was absorbed into the material (figure 11), making a thick black smear around the whole surface of the Fuji IX specimens. The patterns of surface staining of barium nitrate were similar to that of lead nitrate solution and were less severe than that of silver nitrate. It was noted that about one to two surfaces were stained by these two metal solutions (Figures 12-13).

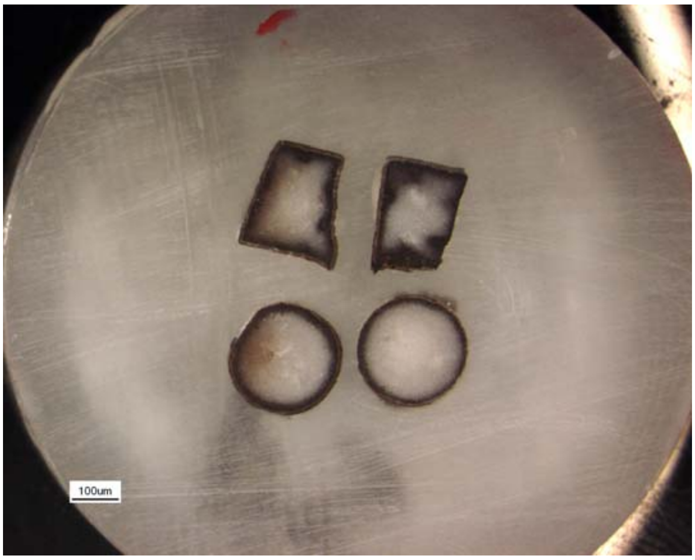


Figure 11: silver nitrate

Figure 12: lead nitrate

Figure 13: barium nitrate

In order to compare the surface reactions among the solutions, marginal staining of rectangular shaped specimens was chosen and recorded. This was because the marginal staining at rectangular pieces could be scored with the cardinal numbers for student test. It was scored as follows:

0: No evidence of marginal staining

0.5: Half a single margin was stained

1: One single margin was stained

1.5: One and a half margins were stained

2: Two margins were stained

2.5: Two and a half margins were stained

3: Three margins were stained

3.5: Three and a half margins were stained

4: Four margins were stained

The incidence of surface interaction between the three solutions and Fuji IX were presented in table 1. The t test results showed that surface reactions were significantly different between silver nitrate and barium and lead nitrate ($p<0.01$). There was no significant difference between barium and lead nitrate ($p>0.05$).

Table 1: Degree of surface reactions on a scale of 0-4

Sample no.	Silver nitrate	Barium nitrate	Lead nitrate
1	4	1.5	1.5
2	4	1.5	1.5
3	4	3	1
4	4	2	1.5
5	4	1	1.5
6	4	2	1.5
7	4	1	2
8	4	2	2
9	4	2.5	1.5
10	4	2	1
Mean	4	1.85	1.5

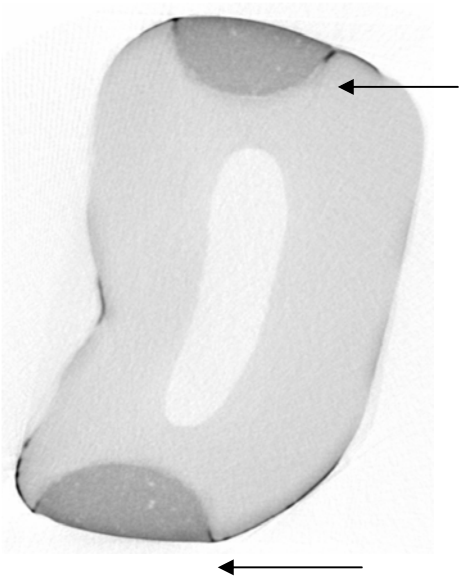
4.2 Staining properties

Each specimen was scanned with the MCT-1072, producing approximately 1000 projections which were converted into tomograms as cross-sectional images.

Similar to the previous analysis, it was mainly focused on analyzing results quantitatively because the aim was to identify if the above solutions are able to stain the tooth/restoration interface.

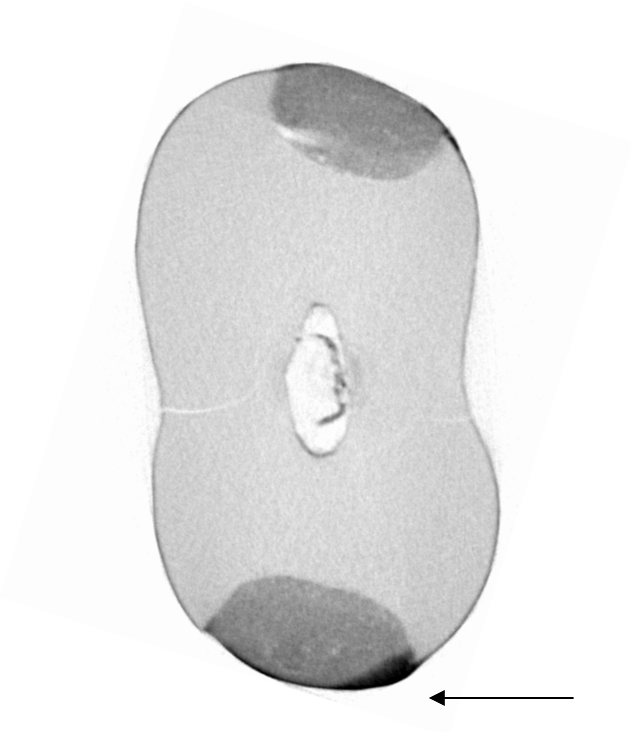
All cross-section images containing the restoration were examined using T-view and Data-viewer (Skyscan). Results showed that while silver nitrate solution consistently stained the tooth/restoration interface and this silver deposition was sharply displayed in the MCT images (Figures 14), barium nitrate and lead nitrate solution did not present at the tooth/restoration interface (Figures 15 and 16). However, lead nitrate again proved to significantly stain the surface of Fuji IX (Figure 15).

It was also reported that spatial resolution achieved from the above model was 10.2 µm. Non-buffered silver nitrate solution was highly corrosive to the Fuji IX surface (Figure 17), whereas the buffered solution was not corrosive to the material (Figure 18).



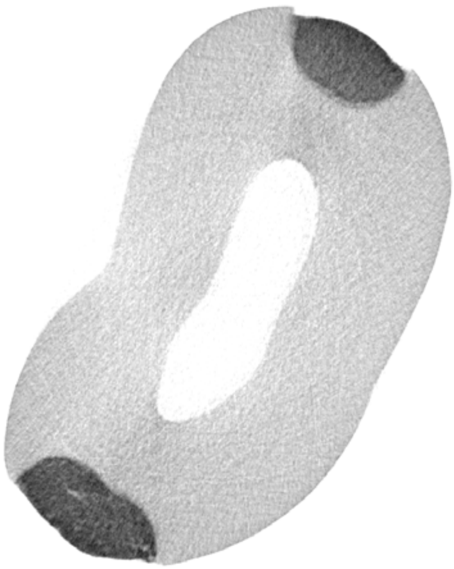
Silver deposition at tooth/restoration interface (arrows) was photographed by the MCT-1072

Figure 14: 2D image-silver nitrate solution



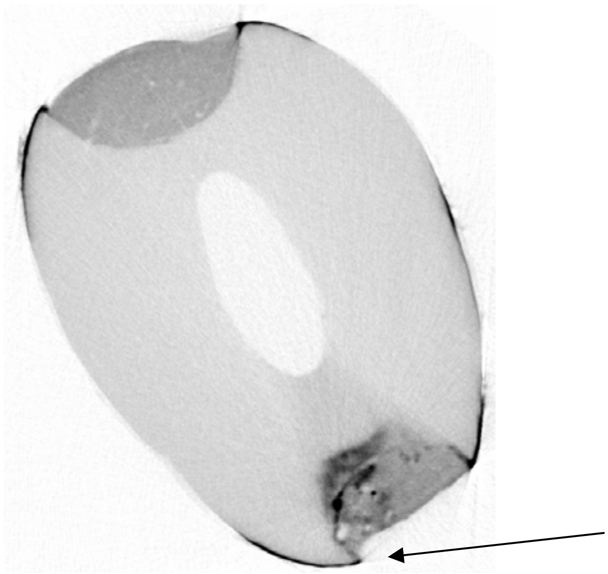
Lead stained restoration surfaces but not in the tooth/restoration interface

Figure 15: 2D image-lead nitrate solution



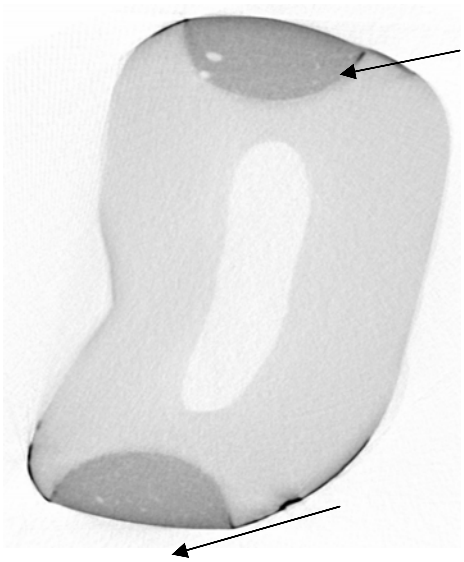
Showed no staining at tooth/restoration interface

Figure 16: 2D image-barium nitrate solution



Non-buffered silver nitrate was corrosive to Fuji IX.

Figure 17: 2D image-the non-buffered silver nitrate solution



The Fuji IX surface (arrow) was intact with the buffered silver nitrate solution.

Figure 18: 2D image-buffered silver nitrate (pH 6.5)

4.3 Development of study model for the MCT-1072

Similar to the above experiment, this experiment was conducted as a small pilot investigation for the main experiment which was carried out in the following part of the project. The primary results were mainly descriptive and statistical analysis was not a focus as the study sample was small.

It was consistently reported that the spatial resolution achieved from the above models was approximately 4.16 µm. The following is an example of the individual report for each specimen (e.g. tooth 1_a: conditioning group) scanned.

[System]

Scanner=SKYSCAN1072_3

[Acquisition]

Converted by = NRecon (Version: 1.4)

Conversion time=Mar 15, 2006 21:03:10

Original configuration file=mls_1_1_a__par.txt

Conversion description=Retrieved info. No conversion.

Acquisition time=Wednesday, March 15, 2006, 9:32(file creation time)

Source Voltage (kV) = 80.000000

Source Current (uA) = 120.000000

Use 360 Rotation=NO

Rotation Step (deg) = 0.675000

Object to Source (mm) = 204.000000

Optical Axis (line) = 513

Image Pixel Size (um) = 4.166

Rotation Direction=CC

Image Format=TIFF

Depth (bits) = 16

[Reconstruction]

Reconstruction Program = NRecon

Program Version=Version: 1.4.3

The following is an individual report of 3D image analysis of the above tooth:

CT Analyser, Version: 1.3.3.11

Date and time: 23.03.2006 15:17

Operator identity analysis

Computer name: ANALYSIS2

Computation time: 00:02:18

Dataset: mls_1_1_a__rec

Lower grey threshold: 215

Upper grey threshold: 255

Number of layers: 577 (cross-section images)

Lower vertical position: 0.91667 mm

Upper vertical position: 3.31668 mm

Pixel size: 4.166 um (spatial resolution)

Tissue volume: 2.63840 mm^3 (volume of restoration)

Bone volume: 0.00080 mm^3 (volume of silver nitrate)

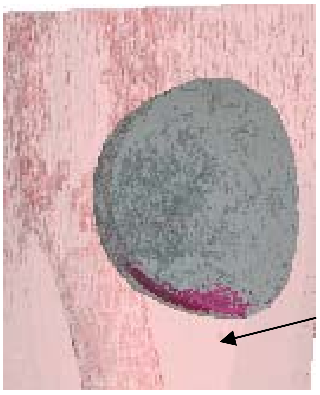
Percent bone volume BV/TV: 0.03043 % (silver/restoration ratio)

Tissue surface TS: 30.82264 mm^2 (surface of the restoration)

Bone surface BS: 0.26400 mm^2 (surface of silver nitrate)

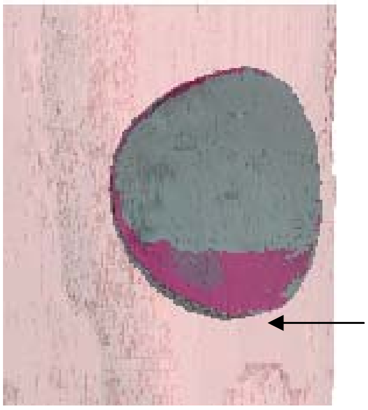
It was noted that approximately 1000 cross-sectioning images were produced from each specimen. But about 550-600 images contained the restoration and these images were located by setting the lower grey and upper grey threshold during image analysis with CT-an. Every single image could be identified, analyzed and relocated whenever required, as this cross-section image was a single file.

Qualitative and quantitative were carried out with the programs chosen and the technical procedures were also discussed. More details in quantitative analysis will be discussed in the result part of the main experiment of this research. The following are examples of 2D and 3D images of this pilot study.



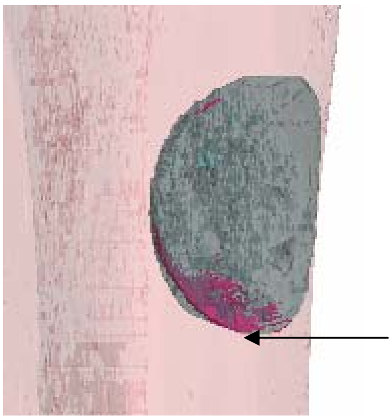
Microleakage was viewed in 3D image (arrow). The volume could be calculated, being representative of the whole leakage of the restoration.

Figure 19: 3D image of microleakage



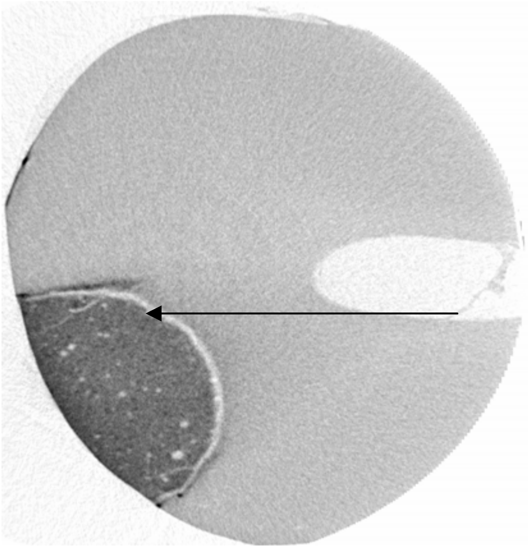
Microleakage (red) was viewed in the 3D reconstruction. This 3D image could be viewed through every single angle in the spatial rotation.

Figure 20: 3D image of microleakage



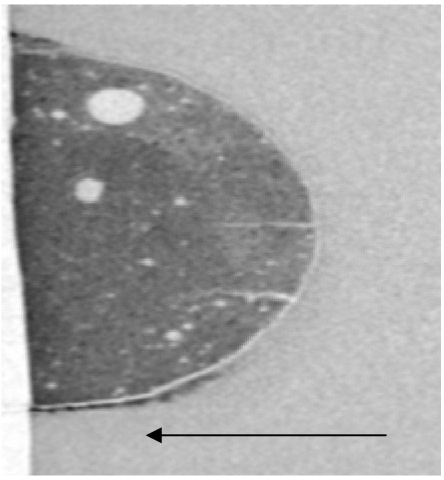
Similarly, microleakage was reconstructed three-dimensionally.

Figure 21: 3D image of microleakage



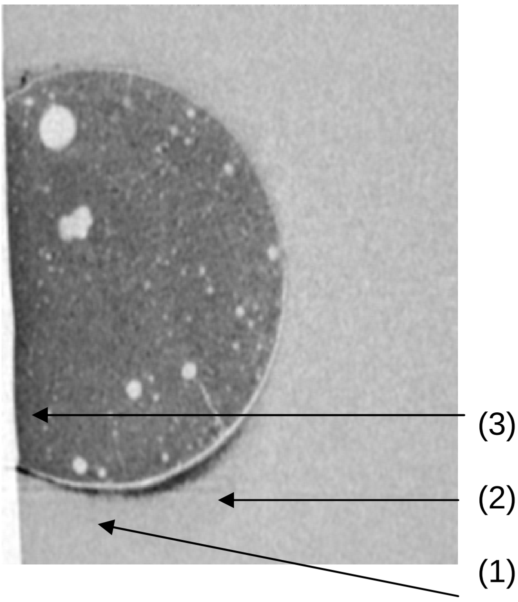
Silver leakage (strong black) was viewed in 2D image in T-view. Silver leakage diffusing into dentinal tubules was noted (arrow).

Figure 22: 2D image



Silver leakage (arrow) at the marginal area viewed in the Data-viewer program. This is a single image scanned in the frontal plane

Figure 23: Coronal single image of the same specimen in Data-viewer dataset



(1)Microleakage was viewed in the sagittal plan. (2) Silver diffused into dentinal tubules. (3) Silver was absorbed into the restoration.

Figure 24: Sagittal single image of the same specimen in Data-viewer

4.4 Main experiment-microleakage of Fuji IX

Quantitatively, the whole amount of silver deposition at the tooth-restoration interface was calculated in volume. Table 2 and table 3 show silver leakage of Fuji IX restorations for the two groups. In order to compare the mean values between groups the t test was applied. The results of the t test showed that no significant differences in silver leakage between conditioning and non-conditioning groups ($p>0.05$). However, there were significant differences in volume silver deposition between the Vaseline and the non-Vaseline groups ($p<0.001$).

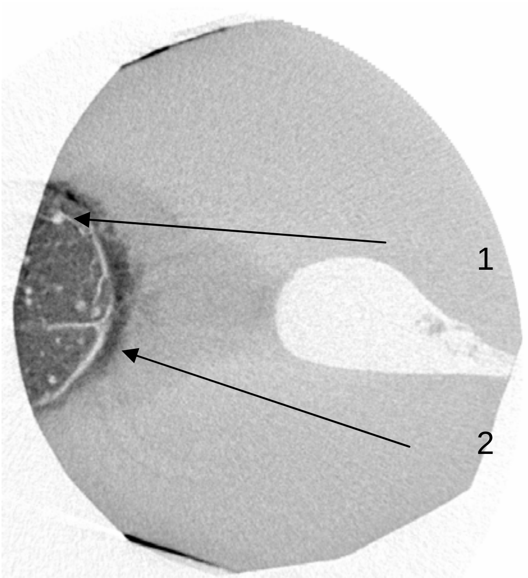
However, it should be noted that when the observed data were plotted it was observed that there seemed to be not a normal distribution within the groups. Analysis of the data to provide means and standard deviation for each group also showed that each group was associated with a significant large standard deviation. A non-parametric statistical analysis was considered appropriate to analyze this parameter and therefore the Mann-Whitney test was applied. It was seen that the statistical results analyzed by the Mann-Whitney were the same as what was reported above with the t test. This is to say a statistically significant difference in volume silver leakage between the Vaseline and the non-Vaseline groups was reported ($p<0.001$). The results of the Mann-Whitney U test also showed that no differences between conditioning and non-conditioning groups ($p>0.05$).

Table 2: silver deposition volume (μ^3) of group 1(Vaseline)		
Sample no.	Conditioning	Non-conditioning
Tooth 1	800	1040
Tooth 2	5520	5890
Tooth 3	2650	34380
Tooth 4	1620	350
Tooth 5	710	11350
Tooth 6	230	1080
Tooth 7	29660	230
Tooth 8	51850	1060
Tooth 9	2460	330
Tooth 10	990	14360
Mean	10611	6190
Median	2460	1060
Standard deviation	18035	11204

Table 3: silver deposition volume (μ^3) of group 2 (Non-Vaseline)		
Sample no.	Conditioning	Non-conditioning
Tooth 11	94610	111920
Tooth 12	7340	117910
Tooth 13	5440	11220
Tooth 14	25020	10070
Tooth 15	5420	20010
Tooth 16	2020	2680
Tooth 17	22540	58620
Tooth 18	22540	9880
Tooth 19	10570	78350
Tooth 20	168600	17720
Mean	36410	43838
Median	16555	18865
Standard deviation	53738.96	44513

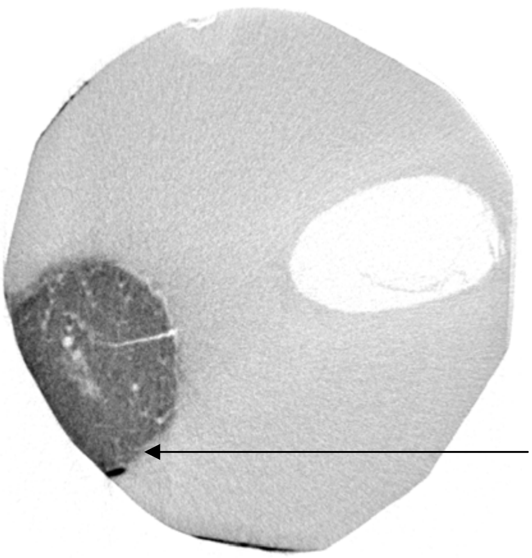
Qualitatively, 2D images and 3D models were established for each specimen. Figures 26-29 are the examples of 2D images extracted from T-view and Data-viewer, showing that silver deposition was found only at the tooth/restoration interface. This is possibly because of the absence of dentinal tubules at these areas. For these specimens, microleakage results can be measured accurately. However, in some other specimens silver leakage diffused into dentinal tubules, making microleakage result less accurate (Figures 25).

For the 3D qualitative analyses the pattern of microleakage of each restoration was reconstructed three-dimensionally using ANT. It seems that the images transferred to this thesis have lost resolution compared with the original images. It was understood that the degree of image sharpness depends on the levels of “Step”, “Locality” and “Tolerance” set. However, it was extremely time-consuming to have one 3D model finished with a high level of sharpness. It was therefore decided to reduce the amount of time creating a 3D model by setting as presented previously. It is to be noted that pink, grey and red were chosen for teeth, restorations and silver depositions respectively and these structures were set at different levels of translucency so that the structures could be seen internally (Figures 30-31) .



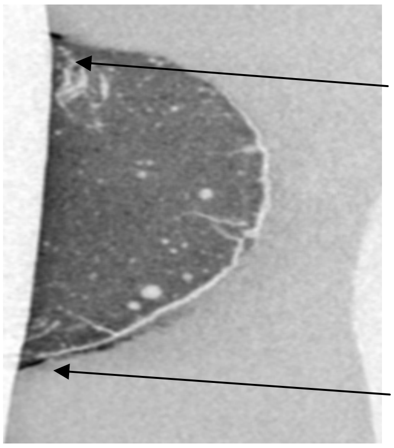
Microleakage at the tooth/restoration interface (1) and its diffusion into dentinal tubules (2) are observed in 2D image. The silver leakage was not very sharp.

Figure 25: 2D image of tooth 8, slice 306, a non-conditioned cavity



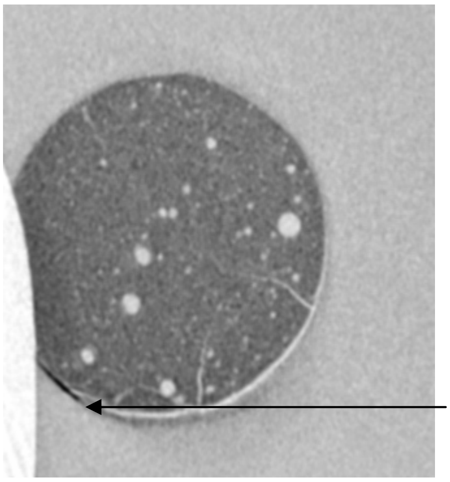
Microleakage was viewed on T-view. The image of silver leakage at the tooth/restoration interface was highly sharp and well defined

Figure 26: Single 2D image of tooth 1, slice 721, non-conditioned cavity



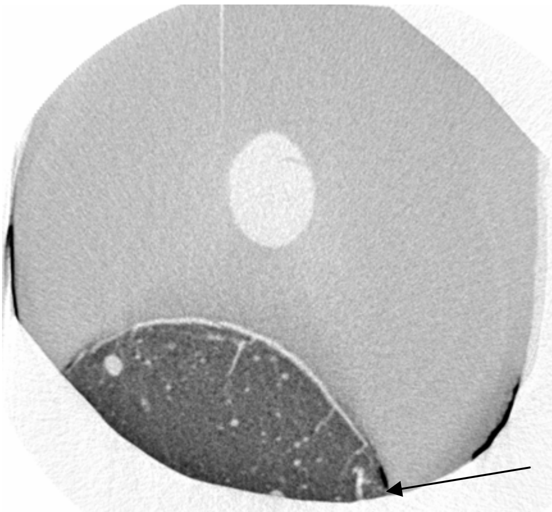
Microleakage (arrow) was viewed on Data-viewer in sagittal plane.

Figure 27: Sagittal single image of the tooth 2 viewed in Data-viewer



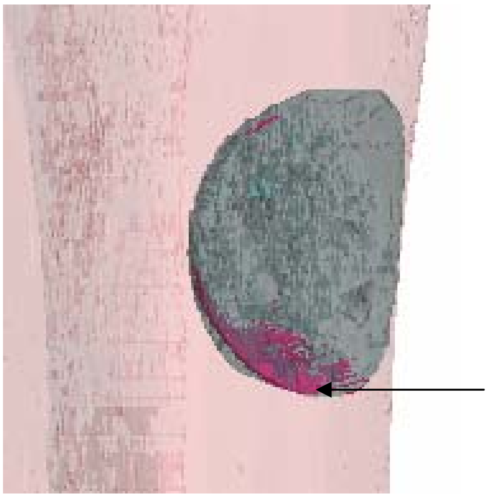
Silver leakage (arrow) at the marginal area viewed in the Data-viewer program. This is a single image scanned in the frontal plane

Figure 28: Coronal single image of the same specimen in Data-viewer



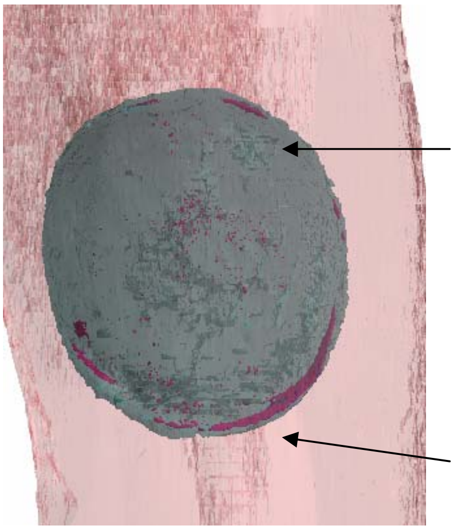
Silver leakage (arrow) was highly sharp and defined

Figure 29: Horizontal single image of the same specimen in Data-viewer



Microleakage (arrow) was viewed on 3D image. The microleakage volume was calculated 51805 μ^3.

Figure 30: Silver deposit in tooth 8, a non-conditioned cavity



Microleakage (arrows) was viewed in 3D image. The microleakage volume was calculated 5890 μ^3.

Figure 31: microleakage on tooth 2, a non-conditioned cavity

CHAPTER 5 DISCUSSION

5.1 Surface reaction test

It was shown by simple experimentation that the interaction between the proposed dye solutions and the test material could be investigated with no special advanced technology; the testing of suitable agents undertaken in the basic laboratory. It was seen that silver nitrate is highly active and invasive to the Fuji IX, which is in agreement with previous studies (Taylor and Lynch 1992; Santis 2005).

Lead and barium were investigated and proved to be the promising dye agents as they were chemically less active compared to silver nitrate in terms of interaction with Fuji IX. The reasons for this could be related to chemical behavior.

The reactivity between the test material e.g. Fuji IX and the dye agent is an important consideration to bear in mind when calculating microleakage. Ideally, such measurements would be made somewhat easier if the microleakage revealing dye solution was inert with respect to the reactivity of the test restorative material. However, it seems impossible to find any metal dye solutions that are totally inert to glass ionomer cements as these cements tend to absorb ions and water because of their water-based properties. Newer technology involving better computer programs could be employed for the image analysis to identify material reactivity that amplifies the microleakage results. Most microleakage studies have ignored the ionic uptake of the test material and the invasion of the dye solution into the surrounding structures. This is probably because the issues were too complicated for the current methodologies to identify the quantity of dye agent taken by the material. And also it is because of two-dimensional sectioning of the specimens that the severity of the dye agent invasion was underestimated.

The experiment was considered as a preliminary pilot study that could provide an overall view on the degree of severity of material reaction occurring between the test material and the proposed dye solution. This simple design could be used for future studies on determining suitable dye solutions.

5.2 Microleakage staining of the metal solutions

5.2.1 Barium and lead nitrate solution

Although barium and lead nitrate were significantly less invasive than that of silver as mentioned above, the results from the MCT-1072 showed that these solutions were not able to stain the tooth/restoration interface. It is understood that the size of the metal atom used as the radiographic contrast medium may have some influence on the penetration of the metal solution into the tooth/restoration interface.

Whatever atom is selected it must be capable of diffusing between the cavity margin and the restorative material. The above parts of the study conducted in preparation for this investigation observed larger metallic atoms, such as barium and lead could produce good x-ray contrast, but it was likely that the prepared solutions were highly viscous and therefore unlikely to penetrate the margins. In some cases the material was insoluble and therefore entirely unsuitable.

There would be other reasons for not finding barium and lead nitrate at the tooth/restoration interface. It may be due to the fact that x-ray density of the amount of barium and lead at the marginal areas was at the same level as that of the surrounding structure, possibly because of the low saturated concentration of the solutions. Barium and lead deposition at the tooth/restoration could not be, therefore, picked up by the MCT-1072. In addition, it could be that barium and lead ions are not able to stably stain the tooth/restoration interface as they were not easily converted into metal precipitate as the silver ions do, and hence, may be washed away during specimen cleaning.

It is possible that the introduction of another chemical that can react with barium or lead may help produce metal precipitate at the tooth/restoration interface. However, because of the fact that barium and lead are not chemically highly active and their concentration at the tooth/restoration interface low, the chemical reaction at the interface was hard to efficiently happen. Also these issues were beyond the outline of the project.

The study failed to find an alternative for silver nitrate solution. This is to say, the invasive and highly chemically active nature of this solution was still used as a dye agent for this research.

It was hoped that with the use of the MCT and its advanced software in image analysis, the problems of invasive dye solution could be overcome. It could be a significant advantage if the image analysis software could automatically distinguish the invasive dye areas and exclude them from the results, thereby increasing the accuracy of the microleakage results.

It is also believed that from this experiment, further investigation can be done with manganese and dysprosium salts, which were discussed as chemical tracers used in the past in the chapter 1.

5.2.2 Silver nitrate solution

The results showed that although silver nitrate is highly active and invasive, it can be used to express microleakage and this leakage can be detected by the MCT. In addition, the silver nitrate solution itself is able to stably stain microleakage without the need to introduce photo-developing solutions as stated in previous research. This finding helps minimize the involvement of chemicals being introduced into the tooth/restoration interface, which by their nature might also be reactive and invasive.

Silver nitrate solution proved to stain the tooth/restoration interface consistently. This layer was displayed reasonably sharply in 2D images (figure 22, 23, 24, 26, 27, 28, 29) and constructed into 3D images (figures 30, 31).

The MCT images also demonstrated that the non-buffered 50% silver nitrate solution was significantly corrosive to both tooth structures and restorative surfaces, particularly at marginal areas, making the microleakage pattern more severe (Figure 17). However, when buffered to pH 6.5, the solution showed no corrosion to the tooth structures and the restoration (Figure 14, 18). This result was in agreement with earlier work(Li, Burrow et al. 2003).

Previous studies did not buffer the silver nitrate solution. This was probably due to the difficulties faced in searching an appropriate buffering system for silver nitrate as this solution is highly chemically active.

It was significant that the present study introduced a buffering system, namely boric acid/ sodium tetra-borate. With the formula presented in detail, it is hoped that silver nitrate solution can be buffered for future microleakage studies.

5.3 Development of study model

The experiment outlined the overall procedure that can produce a model that the best resolution of the current MCT can be achieved. The result showed that the model produced a resolution of approximately 4.16 microns while the model from the above experiment can only achieve a resolution of more than 10 microns. This is a significant improvement because the sharpness and details of the microleakage images were markedly improved. This is to say the accuracy regarding qualitative and quantitative of microleakage improved with the current model.

It was realized that the development of a suitable model to be used effectively with the MCT-1072 was an important step of this work. This model can be used for the future study of microleakage using the same technique. It was also realized that some aspects relevant to the suitability of the new model needed validation, in which identifying and applying the appropriate software programs for the analysis of microleakage results can be particularly important.

In terms of software programs used in this experiment, it was shown that T-view and Data-view are effective programs for 2D analysis and CTan and ANT are sufficient for 3D analyses.

Although the sample size of the experiment was not sufficient for statistical analysis, the results from the experiment have partially met the major objectives. This is to say, the highest spatial resolution of the MCT-1072 was consistently achieved with the model introduced.

As discussed above, although 2D analysis of microleakage is not representative of the 3D structure of microleakage, there were some useful findings when the whole 2D images were scrolled. 2D analysis programs used in this research were T-view and Data-viewer. 2D analysis done by these programs may preliminarily determine the general severity of leakage. Also, it provided information about the levels of dye agent infusing into surrounding structures. As a result, the fundamental pattern of leakage of the restoration could be imaged and so be more easily outlined in the 3D analysis program.

For 3D analysis, it was found that the whole amount of silver deposition at the tooth-restoration interface could be calculated in volume (μm^3) and the 3D image of the microleakage in relation to the restoration reconstructed. In this way, a realistic pattern of the microleakage for each restoration could be displayed three-dimensionally. These findings are particularly important for this methodology

The volumetric analysis of microleakage has been considered the most relevant parameter, because the result reflects the whole leakage at the tooth/restoration interface rather than the leakage value calculated from some sections (Lyroudia 2000).

It is also noted that one of the conveniences of the technique is that the data of whole images can be stored digitally and the study models kept intact, allowing researchers to reuse data and specimens comfortably.

The difficulty of the technique was related to the huge number of images produced from each specimen. This led to difficulties in up-loading data and in managing image analysis. In order to improve the efficiency of image analysis, it was necessary to eliminate those images that were not relevant to the restoration by setting the lower grey threshold and upper grey threshold when images are screened (pages 47-48).

This is to say, from approximately 1000 images produced from each specimen, there were about 400 - 450 images which are out of the “region of interest” and were eliminated from the limited area of analysis. It should be noted that the technique was relatively expensive with the cost to scan one specimen being just under $100AUS. There was also the need to manually scan sections prior to running the appropriate software. In addition, the steps take time and labor to calculate the volumetric quantity of microleakage from such huge number of sections.

5.4 Main experiment and overall discussion of the methodology

In general, the project has introduced a new methodology for the study of micro-leakage around dental restorative materials and has partially overcome some of the problems faced by previous studies. This is to say, the results from the above experiments have partly met the research objectives.

5.4.1 Significance of the results of the main experiment

For the reasons discussed previously, different published research methodologies do not allow the results of this study to be easily compared. It should be emphasized that the main focus of the current research was the development of a technique to quantify micro-leakage in three dimensions and does not attempt to make a comparison between different restorative materials. There has always been controversy over the interpretation of the results of two dimensional studies of micro-leakage and the clinical significance. To simplify the investigation the experiment was confined to a comparison between conditioning and non-conditioning of dentine prior to the placement of a Fuji IX restorations and the application of Vaseline as a sealant after placement. The use of a mild solution of poly-acrylic acid as a conditioning agent has generally been recommended to remove the smear layer from the prepared cavity. The subsequent change in surface energy of the dentine is expected to increase the ability of glass-ionomer cement to wet the tooth surface, thereby increasing the adaptation of the material to the tooth (Mount and Hume 2005). The application of a sealant to maintain water balance is also customary but not essential. Whilst the results suggest no statistical difference between conditioning and non-conditioning, the large standard deviations around the means in both groups, may indicate that the sample size of 10 specimens was inadequate to give a meaningful result.

It should also be noted that there were some specimens with significant leakage making the volume of microleakage higher than the average level of leakage found within this study. This resulted in larger standard deviations. The increase in microleakage was not thought to be due to technical problems in specimen preparation but appeared to be related to the tooth restoration interface. It was shown that once the leakage has traveled along the whole restorative interface the severity of leakage into dentinal tubules dramatically increased (figure 25). This extra leakage was possibly included into the "region of interest" during drawing and subsequently influenced the calculation.

The second variable introduced was to test the influence of Vaseline on the uptake of the buffered silver nitrate solution. It is apparent from the analysis that Vaseline did inhibit the uptake. This is considered to be a positive result and demonstrates the capability of the MCT-1072 to differentiate between samples.

In terms of microleakage of glass ionomer cements, it has been reported that microleakage of glass ionomer cements was significantly consistent and that root surface margins exhibited much greater leakage than occlusal margins (Prati 1989; Hallett and Garcia 1993; Wilder, Swift et al. 2000; Quo and Drummond 2002; Corona 2005). These authors concluded that the chemical bond between Fuji IX and tooth structure is not continuous and thus cannot prevent the penetration of dye agents. Generally, the results from the main experiment demonstrated the same patterns of leakage of glass ionomer cements, although this reflection is highly relative as previous work has been conducted with different techniques. Nevertheless, this infers the MCT-1072 provided positive microleakage results.

5.4.2 Dye solution

It is important for this methodology that the dye solution should be highly opaque radio-graphically in relation to restorative materials and tooth structures so that even the little amount of dye leakage can be photographed.

Another important issue regarding dye solution in this methodology is that the dye leakage should not penetrate into dentinal tubules but rather stays stably at the tooth/restoration interface. The results showed that it seems impossible to locate any metal dye solution that cannot enter dentinal tubule as the dentinal tubule diameter is significantly larger than that of any metal molecules or metal atom. One suggestion can be made for future studies to limit the penetration of the dye solution into dentinal tubules is that dentinal tubule ends at the pulp chamber should be sealed with bonding agents so that it can stop the hydraulic movement mechanism.

Of the X-ray contrast dye solutions studied, it was found that 50% silver nitrate solution buffered to pH 6.5 can delineate micro-leakage satisfactorily as it allows suitable contrast to be produced when exposed to X-rays of the MCT-1072. Generally, the radiographic contrast enables a clear distinction to be made between tooth structure and restorative material.

Another finding in relation to silver nitrate solution was that it does not require the additional process of placing specimens into photo-developing solutions, which are corrosive by nature, to convert silver ions to metallic silver as other workers have done (Taylor and Lynch 1992).

It must be noted that silver nitrate solution readily diffuses into both dentine and glass-ionomer, making it difficult to quantify the true value of silver at the tooth-restoration interface. This problem will always be present, and will need to be taken into account with glass-ionomer and dentine.

It was also demonstrated that the severity of silver spreading into dentinal tubules varies among areas within the tooth and from tooth to tooth. This is likely due, as pointed out in the literature review, to the differences in size and number of dentinal tubules and these changes may be in accordance with tooth's ages and the degree of the secondary and tertiary dentine.

5.4.3 Three-dimensionally quantitative and qualitative analysis

One of the exciting parts of the work was the application to microleakage studies of the advanced computer programs, which had been written for image analysis with the MCT. It was shown that CT-an with the function of manual drawing of the outline of the tooth/restoration interface as the "volume-of-interest", could be used for the microleakage calculation. This allowed silver deposition at the tooth/restoration interface to be calculated, with the silver deposition at dentinal tubule areas to be partly eliminated from the "volume of interest" and hence from the microleakage calculation.

This research has introduced a new method to calculate volume leakage and offered a way forward for future studies into microleakage. In addition, a technique to allow the identification and elimination of dye leakage into the restoration and dentin from the overall calculation of microleakage represents a significant improvement, as the accuracy of the result is more rigorous. Having said that, there are always some areas where it was difficult to entirely separate between true microleakage and silver deposition at the dentinal tubules. This was because low levels of silver deposition produce poor x-ray contrast and hence an unclear image. Efforts were made to overcome the above problems by carefully taking every cross section into examination and the "volume of interest" was manually drawn slice-by-slice. In addition, there was always a need to carefully identify the deposition of silver ions in all areas from the immediate interface between the restoration and the dentine. The process was therefore laborious and time consuming.

Of note, although beyond the scope of this paper, was the appearance, in several specimens (e.g. figures 26, 27, 28, 29) of a radio-opaque space present along the restoration base, its cause and its significance is unknown.

5.4.4 Clinical relevance of microleakage results

It is generally accepted that micro-leakage can occur at the submicron level and in the present research the equipment was capable of achieving a maximum resolution of 4 microns only. That is to say, with a machine capable of a higher resolution the amount of micro-leakage that could be detected would be more precise.

Although there has been a recent shift to investigate microleakage at the submicron level or nano-leakage level, the clinical relevance in terms of recurrent caries is still controversial. Clinically, as discussed previously, no recurrent caries was found in restorations with marginal gaps below 50 microns (Jorgensen and Wakumoto 1968; Mjör and Toffenetti 2000). In the light of this report, with resolution of 4 microns, the results can be considered clinically relevant.

It was realized that volume and surfaces of the object could be calculated with the CT-an (pages 46-47). These are valuable variables for the investigation of the extent of leakage in relation to the whole cavity surface. In addition, the relation between the volume of microleakage and the volume of the restoration was also valuable in assessing the influence of the restoration volume on the extent of microleakage. However, these variables were not accurately achieved as the silver nitrate was invasive both to restoration and tooth structures, making this calculation unreliable.

The three dimensional models obtained from the reconstruction of two dimensional slice data gave very clear visual information about the pattern of micro-leakage and where it potentially may have initiated. As a result, the qualitative description of micro-leakage was undoubtedly improved by MCT. Moreover, the technique for the first time allowed quantification in three dimensions, and as a result, it opens the way for further investigations into this clinically important area.

CHAPTER 6 CONCLUSION

6.1 Restatement of research objectives

The major objective of this research was to introduce a new methodology into the study of microleakage, making it possible to analyze microleakage of restorations non-destructively. In addition, it aimed to present the ways in which microleakage results can be analyzed three-dimensionally.

6.2 Summary and general conclusions

The results provided a comprehensive analysis of the microleakage of the test materials. This was only possible with the use of the MCT to study microleakage non-destructively and three-dimensionally.

A non-destructive methodology using MCT scanning has proved to be innovative in the study of micro-leakage around dental restorations. MCT can provide sharp images of micro-leakage and with appropriate computer software enabled quantitative and qualitative analysis to be performed.

6.3 Suggestions and future directions

The current work highlights the need to further perform basic studies into microleakage methodology. Whilst the micro-CT and associated software produce some very nice images and allow quantitative results to be obtained, the inability to discern the “true microleakage” because of the reaction of the leakage dye with either the restoration or tooth structure or both, has implications for the wider use of the technique. It may be that glass ionomer restorative materials will always suffer from this reactivity problem and that the methodology presented in the current work will need to be applied to other materials such as resin composite. Another suggestion could be that an alternative dye solution, yet to be identified could replace the silver nitrate solution, which is extremely invasive.

Further developments in micro-CT technology have led to the construction of machines that can operate at the submicron level. Such a development would no doubt improve the resolution and accuracy of microleakage data and increase the understanding of the microleakage process.

To further advance the accurate analysis of microleakage, it is suggested that the methodology and techniques developed in the present work be applied to the study of various restorative materials. For example, the technique could have significance for the widely used method of bonding resin composite to tooth structure. There are a variety of adhesive systems employed in dentistry that are tested in terms of shear bond or tensile strength as there is no standard for microleakage.

Ultimately, further research should continue to develop the micro-computed tomography method with the eventual goal that it may be used in the development of a standard for microleakage.

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