

Aus der Poliklinik für Zahnerhaltung und Parodontologie
der Ludwig-Maximilians-Universität München

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Fluorescence-aided Caries Excavation

Dissertation

zum Erwerb des Doktorgrades der Zahnheilkunde
an der Medizinischen Fakultät der
Ludwig-Maximilians-Universität zu München

vorgelegt von

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aus

Nanjing, China

2014

Mit Genehmigung der medizinischen Fakultät
der Universität München

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Tag der mündlichen Prüfung: 22. 01. 2014

TO My Parents

For their endless love, care and support...

ACKNOWLEDGEMENT

I borrow the words that I read from one student's thesis long time ago. 'Writing a thesis is done by a single person who graduates. However, doing a research and gain knowledge can not be done without the help of other people.' First and foremost, I would like to thank my supervisor sincerely, Prof. Dr. Karl-Heinz Kunzelmann, for his constant support, creative ideas, constructive suggestions and endless patience during my studies. As a great mentor, he not only imparted me a large amount of knowledge, but also inspired my initiative and creativity. I am very grateful for all the supports I have received from Mrs. E. Köbele, Mrs. G. Dachs and Mr. Thomas Obermeier. I am also very grateful for the friendship of Dalia Kaisarly, Jian Jin, Xiaohui Xu and Dr. R. Takahashi. I would like to express my special gratitude to Dr. Indra Nyamaa, who has helped me a lot during my studies and even in daily life. And I also owe a debt of gratitude to Dr. Heinrich from W&H Dentalwerk Bürmoos GmbH for his technology support. I would like to thank my friends both in China and in Germany for their warm encouragement. Last but not least, I would like to express my deeply appreciation to Prof. Dr. Reinhard Hickel, the dean of department of Operative Dentistry, Faculty of Dentistry, at the Ludwig Maximilians University in Munich, Germany. Without his introduction, I could not meet my supervisor and pursue my doctoral study in our lab.

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Chapter 1

General Introduction

Dental caries is the primary cause of oral pain and tooth loss (Kidd et al., 2000). It is one of the most prevalent chronic diseases of people worldwide, which people are susceptible to throughout their lifetime (Featherstone, 2000; Pitts, 2004). Until now, a great deal of time and effort has been spent in clarifying its etiology and histopathology, preventing its progress and improving its diagnosis techniques and removal approaches. The purpose of this general introduction is to present a brief review of the basic knowledge of caries process and various caries diagnosis, detection, and removal techniques, along with the aim of our study on the fluorescence-aided caries excavation.

1.1 Mechanisms of Dental Caries — a dynamic process

Dental caries is the localized destruction of susceptible dental hard tissues by acidic by-products generated through bacterial fermentation of dietary carbohydrates (Fejerskov et al., 2008b). And the mechanisms of the caries process are similar for all types of caries (Selwitz et al., 2007). This disease process initiates within the oral microbial biofilm (dental plaque), which covers a tooth surface and is a place for bacterial colonisation (Fejerskov, 2004; Fejerskov et al., 2008a). Based on the previous studies, caries process has been demonstrated to be continuum (Featherstone, 2004). The following figure cited from an article by Featherstone concisely illustrates that dental caries process is a dynamic balance between demineralization caused by pathological factors and remineralization caused by protective factors.

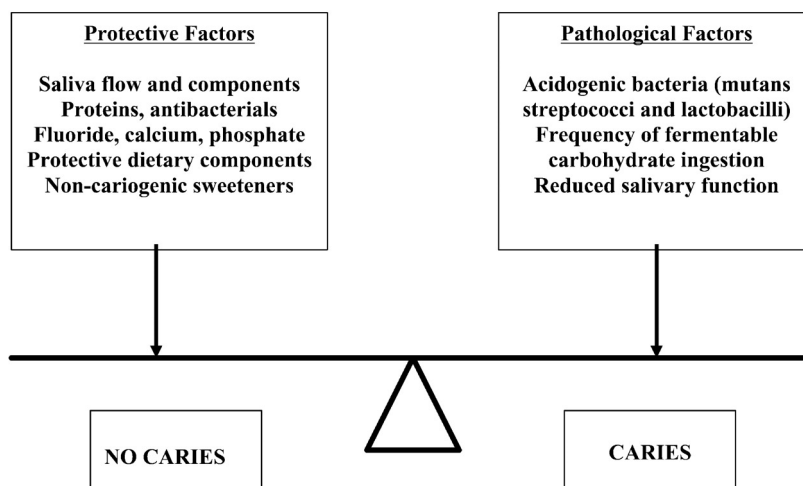


Figure 1.1 Schematic diagram of the balance between pathological factors and protective factors in the dental caries process (Featherstone, 2004).

The outline of dental caries process can be described as follows (adapted from Featherstone, 2000 & 2004):

(1) Acidogenic microorganisms in the biofilm ferment carbohydrates in the human mouth, and produce organic acids, such as lactic, formic, acetic and propionic.

(2) The acids diffuse into the dental hard tissues, including enamel, dentin and cementum, and partially dissolve the mineral crystals, which are mainly composed of carbonated hydroxyapatite. This is called demineralization.

(3) Calcium and phosphate, together with fluoride ions penetrate into the tooth tissues and deposit a new veneer on the crystal remnants. This is called remineralization. And the new mineral crystals surface is more resistance to acid as compared with the original carbonated hydroxyapatite mineral.

(4) If the demineralization process continues without effective remineralization, the amount of mineral loss can lead eventually to cavitation. The formation of a cavitated lesion can protect the biofilm, and unless the patient is able to cleanse this area, which can aggravate the carious attack (Kidd and Fejerskov, 2004).

(5) As the dentin tissues contain not only the mineral crystals but also the collagen fibrils, once the collagen is exposed after the mineral demineralization, it will be digested by

proteases and hydrolases produced by oral bacteria, leading to the breakdown of dentin and cavitation (Clarkson et al., 1986; Kawasaki and Featherstone, 1997; Kuboki et al., 1977).

(6) The dynamic process including demineralization and remineralization takes place frequently daily in the mouths of most people, and determines the eventual outcome, leading to either cavitation, or repair and reversal of the lesion, or maintenance of the status quo.

1.2 Histopathology of Caries Lesion (dentine caries lesion)

Since the bacteria in the dental plaque ferment the carbohydrates, attacking a tooth, the ultrastructure of dental hard tissue starts to change. Due to the aim of our study mainly related to dentin caries, here the histological changes of dentin tissues are the most interests.

Compared with the traditional pathology summarized by Furrer in 1922, carious dentin as described by Fusayama and his co-workers is consisting of two layers: an outer infected layer and an inner affected layer (Fusayama, 1993). In outline (Fusayama, 1979), the outer layer, which is bacteria-infected and irreversibly denatured, is considered to be physically unremineralizable. On the contrary, the inner layer is uninfected, retains the cross-band structure and intermolecular cross-links of collagen fibrils, and can remineralize. In terms of the microstructural differences, the inner layer is further subdivided into the turbid layer, the transparent layer and the subtransparent layer (Ogawa et al., 1983).

Table 1.1 Histological structure and characteristics of different layers of dentin caries. Adapted from the book "A simple pain - free adhesive restorative system by minimal reduction and total etching." by Fusayama (1993).

Furrer 1922 (traditional pathology)								Bacterial rich layer	Few bacteria layer	Pioneer bacteria layer	Turbid layer	Transparent layer	Vital reaction layer	Normal layer
Fusayama		Outer carious dentin			Inner carious dentin			Normal dentin						
					Turbid layer	Transparent layer	Subtransparent layer							
Bacteria invasion		Infected			Uninfected			Uninfected						
Structure	Apatite crystal	Minute granule Irregularly scatter			Small plate Periodicity on collagen fibers			Normal						
	Collagen	Irreversibly broken Crossband disappear			Reversible shift to a precursor state Crossband maintained			Normal						
	Crystal in tubules	None			None	rhomboid	Small granule	None						
	Odontoblast process	Disappear			Smooth	Pitted	Pitted	Smooth						
Vital reaction		Insensitive			Sensitive			Sensitive						
Remineralization		Impossible			Possible			----						
Clinical practice		Completely remove			preserve			----						



Figure 1.2 The different dentin caries zones are: 1 bacterial invasion; 2 demineralized layer; 3 transparent layer; 4 normal dentin; 5 tertiary dentin. (The image of the tooth was obtained from website: <http://www.dentaljuce.com/fruit/page.asp?pid=605>)

With regards to the transparent layer, although it is often used interchangeably with sclerotic dentin in cases by many people, it is worth paying attention to the discrimination between them (Fejerskov et al., 2008a; Fusayama, 1993). Due to the translucent characteristic of both, it is not possible to distinguish the different types of them at the light microscopic level (Fejerskov et al., 2008a). However, the investigations of the ultrastructure of carious dentin have reported that the transparent layer is formed as a result of the precipitation of the dissolved apatite into large rhomboid crystals, which are identified as whitlockite crystals, but not the hydroxyapatite crystals of sclerotic dentin (Fusayama, 1993; Ogawa et al., 1983; Zavgorodniy et al., 2008). The sclerotic dentin is a defence reaction of the vital pulp-dentinal organ to external insults. And the spread of enamel lesion should be taken into account to understand this reaction, because enamel is a microporous tissue and its porosity increases as

a result of enamel demineralization, leading to the stimuli passing through this tissue into the pulp-dentinal organ (Fejerskov et al., 2008a). There are two main factors that accelerate the tubular sclerosis. One is attrition, which is considered to be a mild stimulus from the oral cavity mediated through the enamel and contributes to age-related changes of teeth. The other is caries. The tubular sclerosis observed in conjunction with caries is a result of either initial mineralization of the peritubular space by calcification of the odontoblast processes or an initial intracytoplasmic calcification by a secondary periodontoblastic mineralization (Daculsi et al., 1987; Frank and Voegel, 1980; Schüpbach et al., 1992). If the carious lesion progresses rapidly, it is quite common to see "dead tracts" in the dentin, which means the odontoblast processes are destroyed without having produced tubular sclerosis, leading to the empty tubules (Fejerskov et al., 2008a).

Besides, the mechanical properties of the transparent layer have been studied for decades. Long ago, the transparent layer was considered as a hypermineralized layer with higher hardness value (Bradford, 1960; Fusayama, 1993). As a matter of fact, despite the tubular lumens are largely filled with mineral deposits, the transparent layer is much softer than the normal dentin (Marshall et al., 2001; Ogawa et al., 1983). On one hand, the whitlockite crystal of this layer has lower calcium content than the normal hydroxyapatite and is much softer (Frank and Voegel, 1980). On the other hand, the main contributor to the mechanical properties of dentin is the intertubular dentin, which is partially demineralized in this layer, but not the peritubular or the intratubular dentin (Marshall et al., 2001; Ogawa et al., 1983; Shimizu et al., 1981; Zavgorodniy et al., 2008). Therefore, even though the transparent layer has higher mineral content, but relatively lower mechanical properties than sound dentin (Angker et al., 2004).

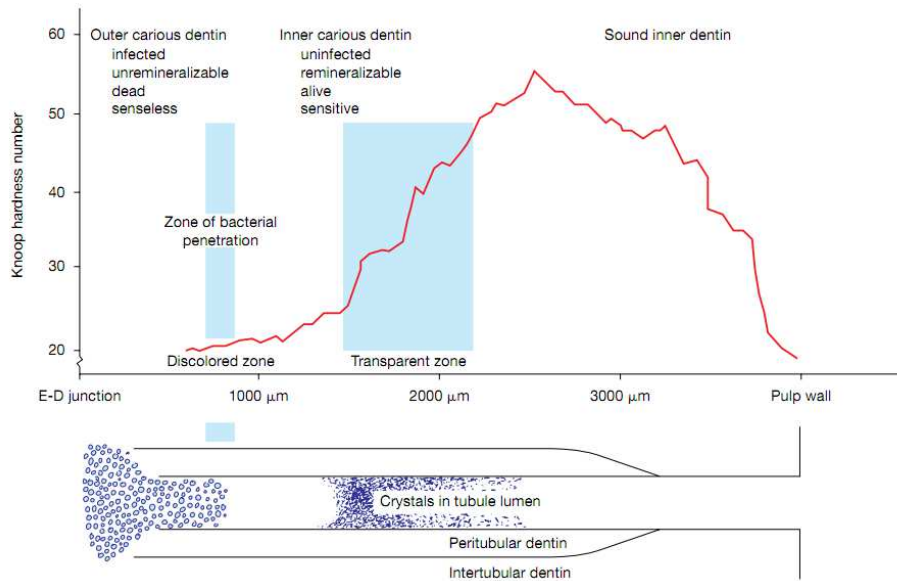


Figure 1.3 Schematic illustration of relationship between a Knoop hardness curve, the outer carious dentin, the transparent zone and the inner sound dentin. Besides, the bacterial invasion, and precipitation of mineral crystals in the dentinal tubules are shown (Fejerskov et al., 2008a, the original image is from Ogawa et al., 1983).

1.3 Caries Detective and Diagnostic Methods

Medical diagnosis is defined as "the art or act of identifying a disease from its signs and symptoms" or "the decision reached by diagnosis" (Merriam-Webster Online). In short, it includes both diagnostic procedure and opinion (Wikipedia: medical diagnosis). The objectives of diagnosis are: (1) detecting or excluding diseases, (2) contributing to further diagnostic therapeutic management, (3) assessing prognosis, (4) monitoring the clinical course of the disease, (5) measuring physical fitness in relation to requirements, (6) informing the patient (Nyvad et al., 2008). In caries diagnosis, dentists do not do the classical differential diagnosis, but detect the signs and symptoms that can be attributed to dental caries and classify carious lesions into categories (Nyvad et al., 2008). Moreover, an assessment of the patient's risk for caries is an important part of caries diagnosis (Kidd, 1998). These factors are the basic information of saliva, diet, fluoride, oral biofilm, oral hygiene, past caries experience and so on. Thus, the methods that will be described in this part might be precisely classified as caries detective methods rather than diagnostic methods.

1.3.1 Traditional methods

1.3.1.1 Visual-tactile method

Until the introduction of bitewing radiography in 1925, clinical diagnosis completely relied on a visual-tactile examination to look for caries lesions (Nyvad et al., 2008). This method requires good lighting and clean, dry teeth. Today, a tooth is dried with a gentle blast of air. Since the difference of the refractive index between carious and sound enamel becomes greater when water is removed from the pores in enamel, an initial non-cavitated enamel lesion can be more easily disclosed. And a sharp explorer with moderate pressure is used as an aid to remove the biofilm on the surface, check the break on tooth surface, and as well as "feel" the texture of tissues. Besides, a mirror is helpful when vision is difficult to reach areas on the teeth. In a word, results of this method rely on the assessment of color and texture change of dental hard tissues.

Nowadays, visual inspection and tactile probing of this method are considered to be quite subjective (Pretty, 2006). And the assessment of the two features is qualitative, only providing the information on the severity of teeth, but is short of quantification (Pretty and Maupomé, 2004). Besides, the sharper explorer seems to be an unwise instrument for caries detection. First, an explorer can not disclose approximal caries lesion easily. Second, it was shown in 1960s that using a sharper explorer can induce iatrogenic damage, particularly on initial caries within occlusal fissures, and favour continued lesion development (Bergman and Lindén, 1969). Some in vitro studies also found that the careless use of an explorer had a deleterious effect in terms of subsequent enamel demineralization, especially in pits and fissures (van Dorp et al., 1988; Yassin, 1995). Additionally, regarding the diagnosis of caries with filling, probing with an explorer could cause cavitation of an outer lesion, damage the margin of a restoration, or even induce a marginal defect which might then be misinterpreted as a carious lesion (Bergman and Lindén, 1969; Ekstrand et al., 1987). Thus, some researchers have claimed that "Given the potential for caries-inducing and caries-accelerating iatrogenic damage from the use of a sharp explorer, combined with lack of any evidence of additional diagnostic benefit, sharp explorers should no longer be used for coronal caries diagnosis" and "Educational initiatives will be needed to share the evidence on sharp

explorers and persuade those still using them to give them up" (Pitts, 2001).

However, since the visual-tactile method is very convenient and money-saving, it is still prevailing worldwide, particularly in countries where dentists have no easy access to radiography or other additional diagnostic technologies (Nyvad et al., 2008). Moreover, the visual-tactile method is the only way that allows a distinction between cavitated and non-cavitated caries lesions, as well as between active and inactive caries lesions (Nyvad et al., 2008).

1.3.1.2 Radiography

Dental x-ray was described as "the most revolutionary aid in dental diagnosis" in 1920s (Rochlen and Wolff, 2011). Bitewing radiography is the most common technique for caries detection, particular approximal lesions that the visual-tactile can not effectively search for. It has several main advantages as follows (Mejàre and Kidd, 2008):

- (1) Sites that are inaccessible for conventional visual-tactile examination can be studied.
- (2) The depth of approximal lesions can be assessed and the relation between the lesion and dental pulp can be estimated.
- (3) It is a non-invasive method and does not lead to iatrogenic damage to dental tissues.
- (4) It can be filed and re-examined. Thus, it can monitor the progression of a caries lesion.

Despite the above advantages, radiographic method has been complained about its low validity in diagnosing of early enamel lesions, its underestimation of lesion depth and other shortcomings. And the quality of radiographic images influences the diagnosis. One study showed an apparently higher detection rate of digital radiography with high quality (greater than 70%) than that of conventional radiography (less than 50%) in case of histological examination as detection criteria (Wenzel and Fejerskov, 1992).

1.3.2 Additional methods

Because of the scientific and technological advances, several non-invasive approaches

have been introduced in the field of caries detection and diagnosis. These novel methods generally have the physical principles respectively (Table 1.2). Except the laser-induced fluorescence (DIAGNOdent) and violet light-induced fluorescence (FACE), which are also used as caries excavation aid or method, other methods will be briefly introduced in this part.

Table 1.2 Overview of additional diagnostic methods (Adapted from literatures: Lussi and Angmar-Månsson, 2008; Pretty, 2006)

Physical principle	method
X-rays	Digital image enhancement Digital subtraction radiography
Light	Fiber optic transillumination (FOTI) Digital images fiber optic transillumination (DiFOTI) Quantitative light-induced fluorescence (QLF) Laser-induced fluorescence (DIAGNOdent) Violet light-induced fluorescence (FACE) Optical coherence tomography (OCT)
Electrical current	Electrical conductance measurement (ECM) Electrical impedance measurement
Ultrasound	Ultrasonic caries detector

1.3.2.1 Digital radiography

Traditional radiography has always been plagued by problems, such as improper exposure time and processing errors (Rochlen and Wolff, 2011). In recent years, digital receptors are brought into use in the dental radiography (Wenzel, 2004). There are two choices for digital image systems - charge coupled device (CCD) based and storage phosphor systems (SPS) (Macdonald, 2001). Compared with traditional radiography, this digital method has some advantages. One is the elimination of processing errors (Peker et al., 2007). Besides, the radiation exposure is lower (Haak et al., 2001). And the most noted advantage but also forensic limitation at the same time is that digital radiographs are easy to process and analyze (Lussi and Angmar-Månsson, 2008). Moreover, the quality of the original image is critical for further processing and analysis.

Since the spatial resolution of digital images and the range of gray shades are lower than

that of regular films, sensitivities and specificities of unenhanced digital images are confirmed to be lower when assessing small proximal lesions (Verdonschot et al., 1992 & 1999). However, original digital images can be enhanced by using a range of algorithms to improve the diagnostic perform. This is called digital image enhancement.

As described above, digital images offer opportunities for manipulation and further processing. Subtraction radiography is one of the most promising technologies for caries detection (Pretty, 2006; White et al., 1999). Two images of the same object are aligned together. Subsequently, the grey values of the first image are subtracted from that of the second image, any differences of grey values of the two images indicate the change in the object (Wenzel et al., 1993). As for dental caries, if no changes have occurred, the result of a subtraction is zero, otherwise, any non-zero results must be caused by the changes in dental tissues, such as demineralization (Lussi and Angmar-Månsson, 2008; Pretty, 2006). Laboratory studies have shown that subtraction technology could be capable of diagnosing primary and secondary caries (Halse et al., 1994; Minah et al., 1998; Nummikoski et al., 1992). Clinically, this method has not routinely used, since subtraction radiography requires extreme standardization of both dose and position to obtain a good registration of two images (Lussi and Angmar-Månsson, 2008; Pretty, 2006).

1.3.2.2 (Digital images) Fiber optic transillumination

Fiber optical transillumination employs high-intensity light, which attempts to penetrate dental tissues, and detects dental caries according to the varied densities of tissues (Neuhaus et al., 2009). The carious lesions appear as "shadows" as the light scatters passing through the lesion. This method is a simple, fast and cheap supplementary aid for diagnosing approximal caries in the anterior teeth and occlusal surfaces (Lussi and Angmar-Månsson, 2008). Digital images fiber optical transillumination replaces the human eyes with a CCD receptor to improve the sensitivity of this method (Keem and Elbaum, 1997).

1.3.2.3 Quantitative light-induced fluorescence (QLF)

In 1920s, Benedict suggested that the fluorescence of teeth be an alternative for diagnosing dental caries (Benedict, 1928 & 1929), since the mineral loss of a dental hard

tissue induces loss of its natural autofluorescence. After more than half a century, researchers began to report on the use of laser auto-fluorescence for qualitative and quantitative assessment of mineral loss in enamel both in vivo and in vitro (Bjelkhagen and Sundström, 1981; Bjelkhagen et al., 1982; Hafström-Björkman et al., 1992; Sundström et al., 1985). A tooth is illuminated and the demineralized areas appear as dark areas (Hall and Girkin, 2004).

This auto-fluorescence has been shown to rely on the enamel dentinal junction (EDJ). The excitation light passes through the transparent enamel and excites fluorophores within enamel dentinal junction (Pretty, 2006). When demineralization of enamel happens, the scattering effect of the lesion leads to the less excitation light reaching the EDJ (Bosch, 1996), and the fluorescence from EDJ is scattered as it attempts to pass through the lesion (Pretty, 2006). Thus, the presence of mineral loss reduces the fluorescence. As the light scattering in the lesion acts as a barrier for excitation light to pass through the underlying sound tissues, the detective depth of QLF is limited, up to 400µm (Ando et al., 2004; Neuhaus et al., 2009; Selwitz et al., 2007).

Interestingly, the validity of this method depends on the population (Pretty et al., 2003; Zandoná and Zero, 2006). In a high-risk population, it is highly predictive of the possibility that a patient has a caries lesion (the value varies between 0.9 and 0.98). On the contrary, in a low-risk population, it is much less predictive (the value is between 0.20 and 0.65). Besides, since stains, plaque and fluorosis can influence its accuracy, it is essential to use a visual examination to exclude non-hypoplastic areas (Stookey, 2004; Zandoná and Zero, 2006).

In all, QLF is an important advanced technology for early caries detection (primary enamel lesions, secondary lesions and the lesions around orthodontics brackets) (Stookey, 2004). And various studies have demonstrated its potential to be used in both scientific research and clinical practice (Ando et al., 2001; Ferreira Zandoná et al., 1998; Tranaeus et al., 2001). Further refinement and clinical validation need to be done on this method.

1.3.2.4 Optical coherence tomography (OCT)

Optical coherence tomography is an interferometric technique, typically employing near-infrared light (Wikipedia, OCT). It is notably used in ophthalmology (Lussi and

Angmar-Månsson, 2008). Light travels faster in material with a low refractive index and slower in media with a high refractive index. OCT measures the intensity of back-scattered light for imaging. The difference between sound dental tissues and demineralized tissues can be interpreted on the OCT images. To date, OCT has been demonstrated to be able to detect early enamel demineralization *ex vivo*, monitor lesion process, and artificial caries under composite sealants and restorations (Amaechi et al., 2001; Baumgartner et al., 2000; Fried et al., 2002; Ko et al., 2005), which may approve this method has the potential be an alternative in caries detection.

1.3.2.5 Electrical measurements

The mineral loss during caries process induces increased porosity in the tooth structure, which can be filled with many ions from the oral cavity. Afterwards, the increased ionic content in the pores leads to increased electrical conductivity or decreased electrical resistance or impedance (Longbottom and Huysmans, 2004). Based on this principle, Electrical Caries Monitor (ECM) and Electrochemical Impedance Spectroscopy (EIS) are employed for caries detection.

ECM uses a single fixed-frequency (23 Hz) alternating current to measure the "bulk resistance" of tooth tissue (Longbottom and Huysmans, 2004). It is normally proposed for occlusal caries detection, as the probe of ECM can easily reach the occlusal sites. And it has portable products already. In contrast, EIS employs a sinusoidal electrochemical perturbation (potential or current) to a sample that covers a wide range of frequencies. And the analysis of the system response contains information about the interface, the structure and reaction taking place on the sample (<http://www.gamry.com/application-notes>). Thus, EIS is assumed to be more appropriate than single frequency measurements (Kühnisch et al., 2006). Until now, a large number of laboratory studies have reported the use of EIS for caries detection and diagnosis (both occlusal caries lesion and approximal caries lesion), monitoring the demineralization/remineralization process, detection of smear layer and age-related change in dental hard tissues (Eldarrat et al., 2004 & 2010; Huysmans et al., 1996 & 1998; Kühnisch et al., 2006; Schulte et al., 1999; Xu et al., 2008).

As several factors, including the porosity of the tissues, the thickness of the tissues, hydration of the enamel, the surface area of the electrode contact with the tooth and temperature, can affect the measurement, the electrical measurements need to be well standardized and clinically identified, validated in the future (Longbottom and Huysmans, 2004).

1.3.2.6 Ultrasonic caries detector

Ultrasonic test makes use of the mechanical waves, which can pass through gases, liquids, solids and the boundaries between them (Hall and Girkin, 2004; Tagtekin et al., 2008). Compared with the densely packed sound tooth tissue, mineral loss during demineralization facilitates the transmission of ultrasound. The different reflected sound waves from sound tissue and caries lesion can be collected and interpreted (Pretty, 2006). Ultrasonic technique has been studied previously for the detection of enamel demineralization (Ng et al., 1988), approximal caries lesions (Ziv et al., 1998), and determination of thickness changes on enamel (Arslantunali Tagtekin et al., 2005; Huysmans and Thijssen, 2000). Despite these encouraging findings, the use of ultrasound for caries detection is developing slowly, and there is no more further reports on the application of this method in dental caries (Pretty, 2006).

1.4 Caries Excavation Methods

Not all caries lesions need operative treatment, namely caries excavation followed by the replacement of a restoration. Only active, cavitated caries lesions require operative treatment. Once a dentist considers operative treatment for a carious lesion, he must decide the endpoint of caries excavation in the first place, which has been discussed since 1880s. Conventional excavation applies the color and hardness as the main criteria. In 1970s, the concept of two layers of carious lesion (dentin lesion) was put forward and has been accepted by scientists and dentists, and the aim of caries excavation is to remove the outer infected layer staining red and preserve the inner affected layer, which stains pink, when a caries disclosing dye is used (Fusayama, 1979). Moreover, along with the development of adhesive dentistry, modern restorative dentistry has moved from the extension-for-prevention concept, the classical

principles of cavity preparation that were established by G. V. Black (Black, 1908), to minimal intervention dentistry (Tyas et al., 2000), which is more conservative and aims to save as much remineralizable and healthy dentin as possible. Because of the dentin-pulp complex, scientists have proposed the stepwise excavation and partial excavation rather than complete excavation for deep carious lesions. In fact, the dental education about operative dentistry between different countries, or even between different dental schools in the same country may be diverse, leading to the subjectivity of caries excavation more or less. Apparently, the endpoint of caries excavation is still under discussion without a precise definition. Here we are not struggling with this "slippery" topic of how much carious tissues must be removed, but paying attention to the caries excavation methods including the conventional and novel ones (Table 1.3). And the main interests of this part are conventional excavation, polymer bur, caries disclosing dyes-aided excavation, chemo-mechanical excavation, atraumatic restorative treatment (ART) and fluorescence-aided excavation, each of which has its own working principles

Table 1.3 Classification of caries excavation methods (Adapted from Banerjee et al., 2000)	
Category	Method
Mechanical and rotary	Conventional excavation with carbon, steel or tungsten-carbide burs Polymeric burs Ceramic burs Caries disclosing dyes-aided excavation (Caries Detector) with tungsten-carbide burs Laser-induced fluorescence-aided excavation (DIAGNOdent) with tungsten-carbide burs Fluorescence-aided caries excavation (FACE) with tungsten-carbide burs
Mechanical and non-rotary	Air-abrasion excavation Air-polishing Sono-abrasion excavation Chemo-mechanical methods Atraumatic restorative treatment
Chemo-mechanical	Caridex; Carisolv
Photo-ablation	Lasers

1.4.1 Conventional excavation with carbon, steel or tungsten-carbide burs

Traditional caries removal gains the access to the caries with a high-speed handpiece under water cooling, then change to a slow-speed one with a round bur to remove peripheral caries (Ricketts and Pitts, 2009). A dentist assesses the resultant surface with a explorer and stops excavation as soon as the surface "feels as hard as normal dentine" (Roberson and Sturdevant, 2002). This conventional method can not selectively remove the infected carious dentine while preserve the affected dentine. Furthermore, the heat generated by an inadequately cooled high-speed handpiece and the vibration from a slow handpiece can cause damage of the dental pulp (Ricketts and Pitts, 2009). The conventional excavation method is often plagued with over-excitation and unintentional pulpal exposure (Celiberti et al., 2006). However this method is still applied worldwide.

1.4.2 Polymer burs

Polymer bur is a selective caries-removal rotating instrument, which can remove demineralized dentin, but preserve sound dentin.. It is made of polyamide polymer. The most important advantages of these burs are removing less sound dentin, cutting fewer dentinal tubules and triggering less pain sensations (Dammaschke et al., 2006). SmartPrep and SmartBurs II (SS White, Lakewood, NJ, USA) are two generations of commercial products of these burs. Besides, polybur P1 is also a kind of commercial burs (Komet dental, Gebr. Brasseler GmbH & Co. KG, Germany). The cutting edges of these burs are not spiralled but shovel-like straight. The polymer material of Smartprep is harder than carious dentin (Knoop hardness 0 to 30), but lower than sound dentin (Knoop hardness 70-90) (Dammaschke et al., 2006; Silva et al., 2006). It is said that the SmartBurs II is harder than Smartprep. Once the bur touches caries-affected or sound dentine, it will wear down and make further cutting impossible finally (Dammaschke et al., 2006; de Almeida Neves et al., 2011). Thereby, these polymeric burs are self-limiting, single-use instruments for caries excavation.

Despite the polymeric burs with potential merits can be accepted by patients (Allen et al., 2005), more residual caries both in permanent and primary teeth were found in cavities with Smartprep burs compared to conventional tungsten-carbide burs (Dammaschke et al., 2006; de Almeida Neves et al., 2011). Other study reported that polymer burs and tungsten burs seemed to have similar effectiveness for caries removal (Meller et al., 2007). These may indicate that the polymer burs needs more studies or clinical trials to be validated, or even to be improved.

1.4.3 Caries disclosing dyes-aided (Caries Detector) excavation

In 1972, a 0.5% basic fuchsin in a propylene glycol base was initially used as the first caries disclosing dye and was suggested to aid in the differentiation of the two layers of dentine caries (Kuboki et al., 1983). Because of its carcinogenicity, a 1.0% acid red in propylene glycol was used as a substitute and marked as a commercial product "Caries Detector" (Kuraray, Tokyo, Japan) (Fusayama, 1988 & 1993).

The mechanism of using these dyes is based on the selective penetration of the solvent in

the dyes (Fusayama, 1993). Propylene glycol preferentially penetrates the loosened collagen matrices that have been irreversibly denatured by breakdown of the intermolecular cross-links in the outer carious dentin, but not the solid collagen matrices in the inner caries dentin or sound dentin (Fusayama, 1988 & 1993). However, the caries disclosing dyes are proved to be nonspecific for carious lesions, but also to stain the sound circumpulpal dentin and sound dentin at the enamel-dentinal junction which have a naturally lower mineral content and are relatively more porous (Banerjee et al., 2003; Yip et al., 1994). And excessive removal of dentin has been reported when using the dyes during caries excavation (Yip et al., 1994). In addition, incomplete removal of bacteria is considered as another drawback of the dyes (Boston and Graver, 1989). Some studies have demonstrated that the dye-stainable status is not a good predictor for presence or absence of bacteria (Anderson et al., 1985; Zacharia and Munshi, 1995) and the absence of stain can not ensure the elimination of bacteria (Boston and Graver, 1989). These findings would suggest that the caries disclosing dyes penetrate the more porous tissues (McComb, 2000) and stain the tissues with reduced mineralization rather than the demineralized carious lesions particularly (Ansari et al., 1999).

The size of the dye molecules affects penetrability (Fusayama, 1993). Dyes that were dispensed in higher-molecular weight carriers can reduce diffusion in porous tissues (Cvetkovic et al., 2005; Trampel et al., 2002). Recently a new staining dye (Caries Check, Nippon Shika Yakuhin, Shimonoseki, Japan) with a higher molecular weight polypropylene glycol component, has been developed to minimize over-staining of uninfected porous dentin (Hosoya et al., 2007). According to the manufacturer, compared to propylene glycol in Caries Detector (MW=76), polypropylene glycol in this dye has a higher molecular weight (300) and shows lower diffusion into carious dentin. Using DIAGNOdent as the monitor of residual caries, the in vitro study has shown that Caries Check can remove more infected carious dentin, but avoid excessive removal (Hosoya et al., 2007).

The use of a caries disclosing dyes is still under question and even seems to be unreliable by some researchers (Javaheri et al., 2010), however, these dyes are clinically helpful for caries diagnosis and excavation in many cases.

1.4.4 Chemo-mechanical methods

The idea of using chemicals to assist in caries excavation was first developed in the middle of 1970 by Goldman, an endodontist, while using sodium hypochlorite (NaOCl) in root canal therapy (Elkholany et al., 2009; Goldman and Kronman, 1976; Ricketts and Pitts, 2009). NaOCl is able to remove the organic materials in the root canals and dissolve the carious dentine tissues. However, as it is too corrosive and decompose non-necrotic tissues, NaOCl was diluted and buffered with sodium hydroxide, sodium chloride and glycine producing a solution of 0.05% N-monochloroglycine (NMG) having a pH of 11.4, which is also known as GK101 (Goldman and Kronman, 1976). The GK101 system was shown to only attack the degenerated collagen fibers and soften the inner layer of carious dentine (Goldman and Kronman, 1976; Kurosaki et al., 1977). After applying the solution onto the caries surface, a special hand instrument is used to scrape the surface and remove the lesion tissues. Later it was found that if the glycine is replaced by aminobutyric acid, the GK101 would be more effective (Schutzbank et al., 1978). Subsequently, the GK101E, which consists of a freshly prepared aqueous solution of N-monochloro-D, L-2-aminobutyrate (NMAB), was marketed in 1984 as “Caridex” system. The Caridex is a two-bottle system, one of which contains sodium hypochlorite and the other of which contains glycine, aminobutyric acid, sodium chloride and sodium hydroxide. The mixture of the two solutions produces a pH approximal to 11 and is stable up to one hour.

Because of the number of disadvantages associated with Caridex system, such as the large volumes of liquid used per cavity preparation and the increased time required (Ricketts and Pitts, 2009), Carisolv (MediTeamDental, Göteborgsvägen, Sweden) has been introduced as a replacement. It makes use of three naturally occurring amino acids (glutamic acid, leucine and lysine) with different charges, but works equally to the Caridex system (Elkholany et al., 2009). Compared to the Caridex system, it is simplified into two syringes, one containing sodium hypochlorite (0.5%) and the other containing the combination of glutamic acid, lysine, leucine, carboxymethylcellulose, sodium chloride, sodium hydroxide and a red dye. The mixed gel is applied onto the caries surface and causes proteolytic degradation of the already partially broken-down collagen in the outer carious dentine, followed by the removal of lesion

using specially designed non-invasive instrument. This procedure is repeated until all carious tissues are removed.

All the descriptions of the chemo-mechanical caries removal above show its ability of selectively removing the outer layer of dentin caries. Moreover, it does not attack the sound dentin as it is alkaline (Hossain et al., 2003; Yip et al., 1995). Over-excavation of the cavity, thereby, is prevented. After excavation, the cavity dentin is sound or normally calcified without the formation of smear layer compared with the excavation using rotary instruments (Ricketts and Pitts, 2009). This is beneficial for the enhancement of dentine-adhesive bonding strength to achieve longevity of restorations (Hosoya et al., 2001; Wolski et al., 1989). The last but not the least advantage of this method is the wide range of patients' acceptance due to no need for local anesthesia (Chaussain-Miller et al., 2003; Kakaboura et al., 2003), although its slowness is still an obvious shortcoming.

1.4.5 Atraumatic restorative treatment (ART)

The atraumatic restorative treatment (ART) technique is an alternative approach for outer carious lesions using hand instruments only. Subsequently, a cavity is restored with a glass ionomer restoration. It was first developed in Tanzania in the mid-1980s and introduced in remote areas of developing countries, where electricity supplies were limited and dental treatment was not readily available or affordable. And it has been implemented in a school oral health program in Zimbabwe in 1990s (Frencken et al., 1996).

It is certain that bacteria are left in the cavity using hand instruments, however, the total amount of bacteria is much reduced (Bönecker et al., 2003). Clinically, restorations tend to wear and fail due to loss of the glass ionomer restorations, it is of note that they have a much reduced rate of secondary caries compared with amalgam restorations (Mandari et al., 2003). In addition, clinical longitudinal studies showed high survival percentage for single-surface restorations in both primary and permanent teeth, but unsatisfactory survival percentage for multiple-surface restorations in primary teeth (van't Hof et al., 2006). A recent systematic investigation plus meta-analysis showed the survival rates of single-surface and multiple-surface ART restorations in primary teeth after 2 years placement were 93% and

62% respectively, while regarding ART restorations in permanent teeth, it was 85% and 80% for single-surface restorations over the first 3 and 5 years and was 86% for multiple-surface restorations respectively (de Amorim et al, 2012).

Nowadays, ART is becoming more accepted in developed countries (Pilot, 1999). In clinical practice, noise made by rotary instruments would frighten children, trigger unpleasant memories and cause discomfort. Besides, the needle used in local anesthesia also induces emotional discomfort, or even physical pain. Consequently, ART is recommended to be a suitable approach to be used in children, the elderly, special needs patients, or patients who have fear and anxiety towards dental treatment, and whose behavior may influence the operation by a dentist altogether (Carvalho et al., 2009).

1.4.6 Fluorescence-aided excavation

Fluorescence is the emission of visible light by a substance that has absorbed light of a different wavelength (Lakowicz, 2006). In 1927 Bommer first reported an orange and red fluorescence of the oral biofilm (Bommer, 1927). Since then, a larger number of research groups investigated the auto-fluorescence of dental plaque and carious lesions either under ultraviolet or laser irradiation (Benedict, 1928; Bjelkhagen et al., 1982; Hartles and Leaver, 1953; de Josselin de Jong et al., 1995; König et al., 1998 Sundström et al., 1985). Koenig and Schneckenburger found that compared to healthy dental tissues, carious lesions showed a distinguished red-orange fluorescence, which indicated the possibility of differentiation between healthy and carious tissue (Koenig and Schneckenburger, 1994). Metabolites (porphyrins) produced by several types of oral bacteria are supposed as the major contributor to this auto-fluorescence.

As it can be used as a marker for bacteria-infected dentine, auto-fluorescence signal have been applied as aid for diagnosing dentine caries and monitoring caries excavation.

A few years ago, fluorescence successfully induced by a red laser with a wavelength of 655 nm was reported to differentiate carious tissue from sound tissue (Hibst and Gall, 1998). Based on this principle, a laser fluorescence device (DIAGNOdent - KaVo, Biberach, Germany) was invented for caries diagnosis. This device does not produce an image, instead

it displays numerical values ranging from 0 to 99 presenting the intensity of fluorescence. The threshold between occlusal caries limited to enamel and caries into dentin is around 18, which is an arbitrary value, under humid conditions (Lussi et al., 1999 & 2001; Shi et al., 2000). It has been recommended as a promising device for detection of occlusal caries, measurement of carious lesions adjacent to orthodontic brackets, detection of recurrent caries and residual caries and so on (Aljehani et al., 2004 & 2006; Ando et al., 2004; Deery et al., 2006; Lennon et al., 2002; Staudt et al., 2004). This technique of laser-induced fluorescence was also employed by Er: YAG laser for caries excavation (Eberhard et al., 2005; Krause et al., 2007). As controlled by the fluorescence signal emitted from carious dentine as a result of introduction by a diagnostic laser, Er: YAG laser for caries removal is efficient and active. And the fluorescence cut-off values can be preset to avoid over-excitation.

In 2002, a novel caries removal system, fluorescence-aided caries excavation (FACE) was described as a direct method to distinguish between outer infected and inner affected carious dentin clinically (Buchalla and Lennon, 2002). Unlike DIAGNOdent and Er: YAG laser, this system uses fiber-optic violet light (370-430 nm) instead of the red laser as the excitation light. The light source is integrated into a slow-speed handpiece. The operator is allowed to see the fluorescent areas through a yellow glass filter of 530 nm. Only carious dentin emitting red-orange fluorescence is removed selectively, which is much more objective than Caries Detector or the conventional visual-tactile method. Since the introduction, FACE showed the highest sensitivity and specificity as evaluated by confocal microscopy (Buchalla and Lennon, 2002; Lennon et al., 2002). Other studies have found that FACE is more effective in removing bacteria-infected dentin without significantly increasing cavity size and requires shorter excavation time (Lennon et al., 2007), when compared to other excavation methods, including conventional techniques, caries detector dye-aided excavation and chemo-mechanical excavation. In addition, histological investigation has shown samples presenting bacteria after using FACE were significantly fewer than that with conventional excavation method (Lennon et al., 2006b). However, there is no clinical trial of testing this method until now.

In conclusion, all of the excavation methods described above are used with the aim of

removing carious tissues and keeping sound tissues. Although caries dentin is divided into several layers according to the histological investigation, it is a continuum, which can be easily interpreted from the schematic illustration by Ogawa et al.(Figure 1.3). Moreover, caries is a dynamic process. It is very hard for a dentist to decide where he should stop excavation. In fact, each excavation method is so plausible based on its own fundamentals that it is difficult for us to decide which one is the best. And maybe that is the reason why almost every method is questioned in some cases.

1.5 The Aim of This Study

As described above, fluorescence signals can be used as both an indicator for caries diagnosis and a monitor for caries excavation. This study intended to investigate a new version of FACE from W&H Dentalwerk Bürmoos GmbH. Compared with the original FACE system, the 370-nm excitation light was replaced by a violet light of 405 nm, which is an optimal wavelength for the fluorescence emission by porphyrins (Koenig and Schneckenburger, 1994). Besides, a 470-nm glass filter (GG495, Schott, Mainz, Germany) was added to the original yellow one, which is supposed to be helpful in differentiation the red-orange fluorescence of carious dentin from the green fluorescence of sound dentin by the manufacturer.

Previous studies on FACE normally investigated the bacteria presence after excavation, the treatment efficacy or sensitivity and specificity compared with other excavation alternatives. The aim of this study was to compare FACE and conventional excavation and Caries Detector-based excavation from other aspects and focused mainly on the conservative property of FACE. And the study can be divided into three parts as followed:

1. As both Caries Detector dyes and FACE are able to differentiate the two layer of carious dentin, and selectively remove the outer layer, the first experiment intended to compare the amount of carious dentin that indicated by Caries Detector and FACE.
2. Histological examination in previous studies is normally destructive to the samples. X-ray computed micro-tomography (microCT), a non-invasive and non-destructive technique, was applied for the comparison of the mineral content after removal by conventional

excavation and FACE.

3. Hardness is an important mechanical property of dental hard tissue. And the feeling of hardness is also one of the criteria for conventional excavation method. Thus, the microhardness values of cavity floor after removal by the two approaches were compared.

Chapter 2

Comparison between fluorochrome marked and caries disclosing dye stained carious dentin

2.1 Background and Significance

Dental caries, also known as tooth decay, is one of the most prevalent chronic diseases of people worldwide (Featherstone, 2000; Pitts, 2004). Modern restorative dentistry has moved from the extension-for-prevention concept, the classical principles of cavity preparation that were established by G. V. Black (Black, 1908), to minimal intervention dentistry (Tyas et al., 2000), which is more conservative. More precise diagnostic and detective methods of caries are required before a restoration.

Early observations showed that the superficial layer of carious dentin is heavily infected by bacteria, highly demineralized and contains irregularly scattered granular crystals and irreversibly denatured collagen fibrils. Underneath this layer, the deeper affected dentin layer exhibits decreased collagen crosslinks, but comprises needle-like apatite crystals, regularly attached to collagen fibrils with less bacterial invasion (Ohgushi and Fusayama, 1975; Fusayama, 1976).

The treatment objective of dentin caries prior to restoration is to remove the infected dentin and preserve the affected dentin. However, to assess the carious dentin lesion and determine the endpoint of caries removal before a filling is always troublesome for clinical practitioners. Sato and Fusayama proposed a special dye to ease this decision (Sato and Fusayama, 1976). Caries Detector (Kuraray Medical Inc., Japan) nowadays is a red dye, which is a mixture of propylene glycol. The molecular weight of propylene glycol is an important property. With its molecular weight of 76 it can penetrate into the lesion tissue. The pore within the carious lesion acts like a molecular sieve. The pores which can be infiltrated with propylene glycol are associated with the outer carious lesion which should be removed. The red stain is transported together with the propylene glycol into the lesion and represents a visual clue for the dentist to decide how much tissue he should remove. Using Caries Detector

the dentists are able to effectively distinguish the infected layer which has a deep red color from the affected layer which is stained just slightly red or pink (Kuboki et al., 1983). However, this approach to stain the carious lesion with Caries Detector as a marker of the active lesion was reported to be more or less subjective and could lead to over-excitation (Yip et al., 1994).

Several decades ago, an increased autofluorescence has been found in the carious dentin lesion. Moreover, a red auto-fluorescence of the carious dentin, which was supposed to be emitted by the bacterial metabolites, was detected and recommended to be an indicator of infected dentin for caries excavation (Alfano and Yao, 1981; Koenig and Schneckenburger, 1994). Buchalla and Lennon (2002) developed a novel caries excavation device based on the fluorescence properties of carious dentin. They named this method fluorescence-aided caries excavation (FACE). Carious lesions are excited with a special light at 405 nm and can emit a red-orange fluorescence, which allows dentists to detect the bacteria-infected tissue conveniently. And this red-orange fluorescence is claimed to be a visible aid to differentiate infected and affected dentin (Buchalla and Lennon, 2002).

Based on the working principles of the two approaches described above, it is concluded that the Caries Detector was developed taking the advantages of mineral loss and the change of dentin structures caused by the acidogenic activities of oral bacteria, while FACE focuses on fluorochromes associated with the presence of bacteria in the carious lesion. According to previous in vitro studies conducted on both primary and permanent carious teeth, FACE has shown to be more caries selective, effective and efficient than other methods (Lennon et al., 2006b & 2009). However, the differences between the carious lesion stained by Caries Detector and the infected tissue as indicated by FACE have not been investigated yet. The objective of this study is to compare the lesion depth and area of the carious lesion with planimetric tools, when carious lesions are determined (1) with FACE and (2) Caries Detector in the same tooth specimen. As both methods are based on a sound theoretical fundament, the null hypothesis is that there would be no difference of the lesion depth and areas as marked with both methods.

2.2 Materials and Methods

2.2.1 Sample preparation

30 freshly extracted permanent human teeth with soft dentin caries were collected and stored in 0.01% thymol solution in the dark. The experimental procedures were approved by the ethics committee of medical faculty, Ludwig Maximilians University. Within one month, each tooth was cut longitudinally through the lesion center to get one nearly 300 µm thick slice (Leica SP 1600-Saw Microtome, Leica Microsystems Nussloch GmbH, Nussloch Germany). Each slice was mounted on a plastic slide and subsequently polished to obtain slices with a thickness of approximately 150 µm and parallel surfaces as established by a polishing machine (EXAKT 400CS plus EXAKT AW110 Control, EXAKT Apparatebau GmbH, Germany). Polishing papers with P 1000, 2500 and 4000 (LECO Corporation, St. Joseph, Michigan, USA) were used in succession under running water for this purpose. Finally the surface was polished with a diamond spray (DP-Spray p 1µm, Struers A/S Denmark) and polishing discs (Polishing disc, LECO Corporation, St. Joseph, Michigan, USA).

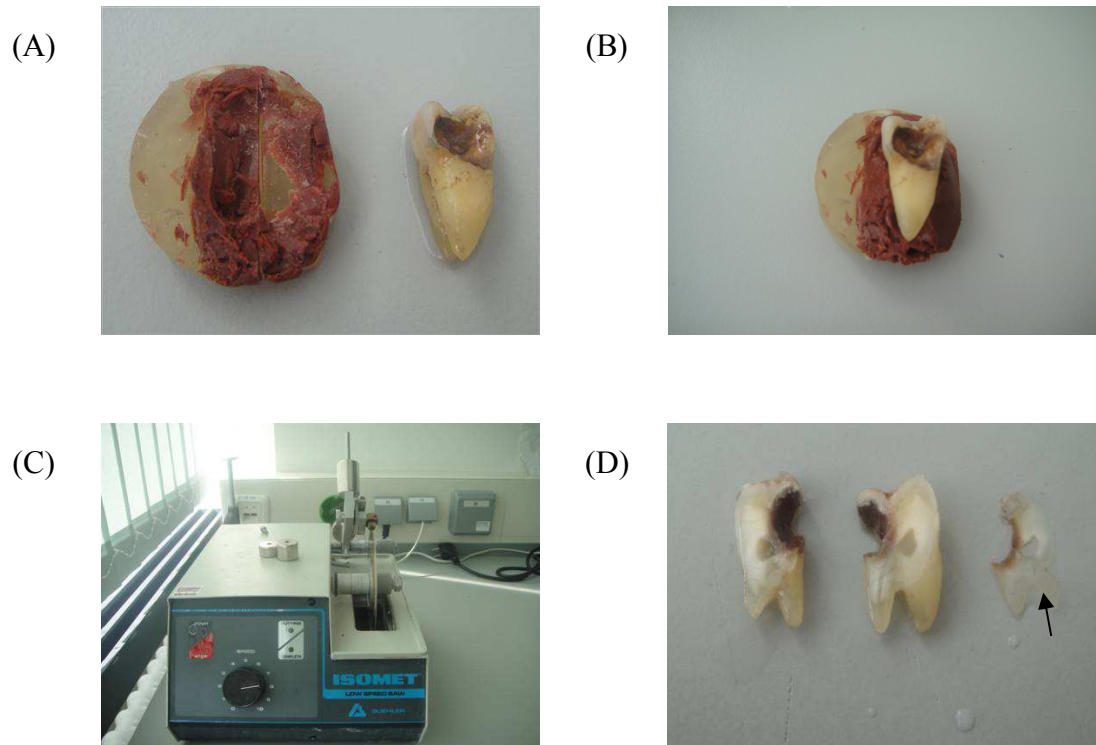


Figure 2.1 (A): Teeth with dentin caries were selected (A); (B) and (C): With the cutting machine (Leica SP 1600-Saw Microtome, Leica Microsystems Nussloch GmbH, Nussloch Germany), one nearly 300 μm thick slice (D) was cut from each tooth.

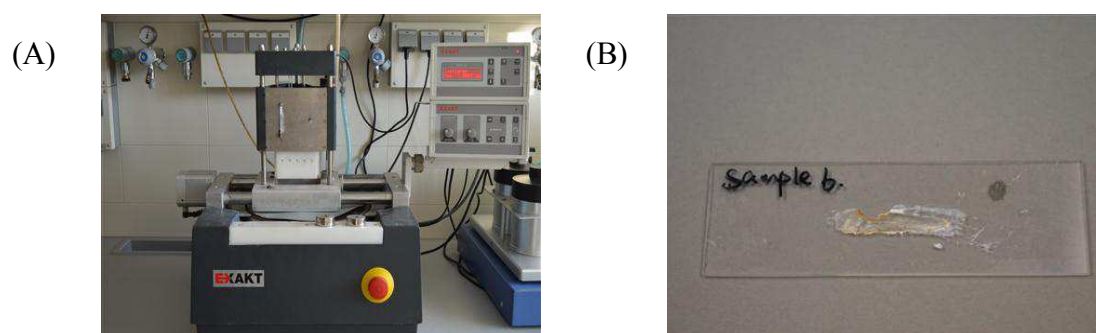


Figure 2.2 Each slice was mounted on a plastic slide and subsequently polished to obtain slices with a thickness of approximately 150 μm and parallel surfaces as established by a polishing machine (EXAKT 400CS plus EXAKT AW110 Control, EXAKT Apparatebau GmbH, Germany).

2.2.2 Investigation of FACE and Caries Detector staining

In the FACE group, each slice was mounted on the stage of a microscope (Axioskop 2 MAT, Plan-NEOfluar 2.5x/0.075 Objective, AxioCam MRc 5, AxioVision, Rel., 4.8, Carl Zeiss AG, Germany). Then digital images were taken under specific FACE illumination conditions. The HBO illumination light was filtered to get an excitation wavelength of 405 nm. The emitted light was filtered with two glass filters (Schott BG36+Schott GG495, Schott AG, Mainz, Germany). During the investigation, the room was kept dark to exclude stray light.

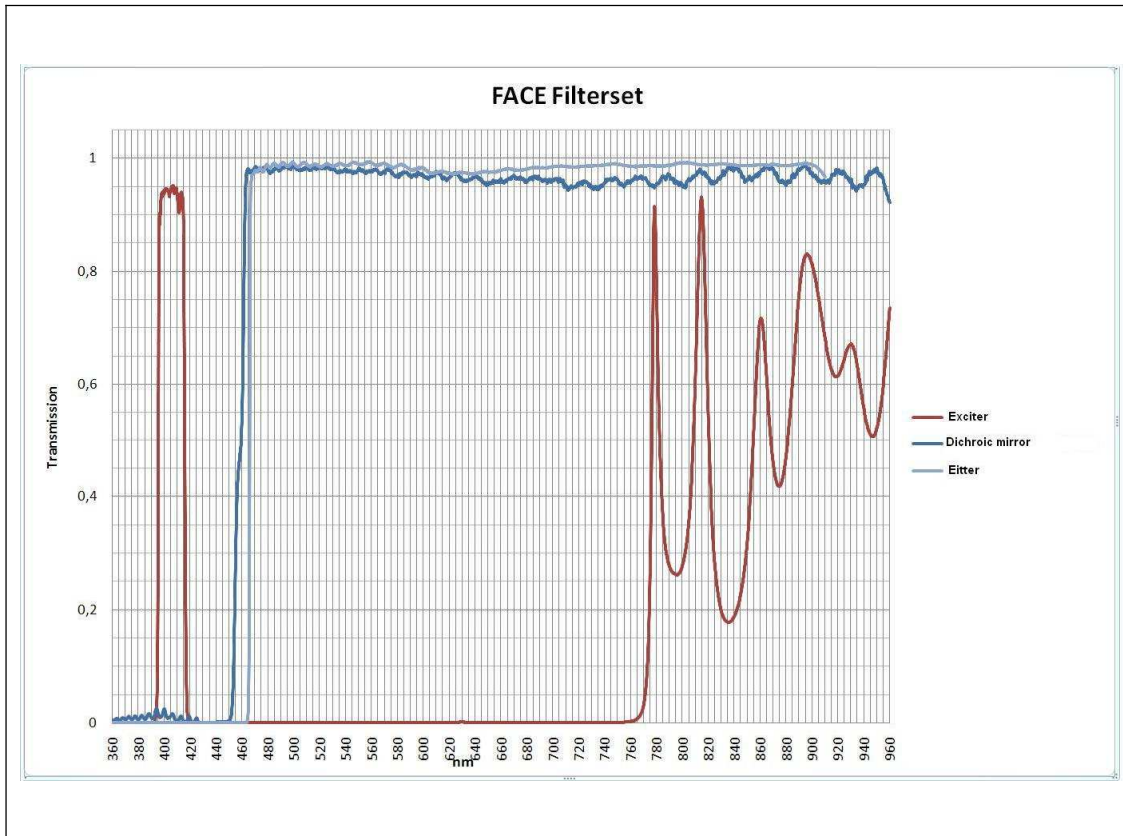


Figure 2.3 The spectrum of the filter in FACE system.

Then each tooth slice was stained for 10 s with Caries Detector (Caries Detector, Kuraray Medical Inc., Japan), rinsed with distilled water and dried gently with compressed air. Reflected light was used to examine and photograph the red stained areas on the slices. All of the images were separately saved in the proprietary Zeiss zvi format for further processing.

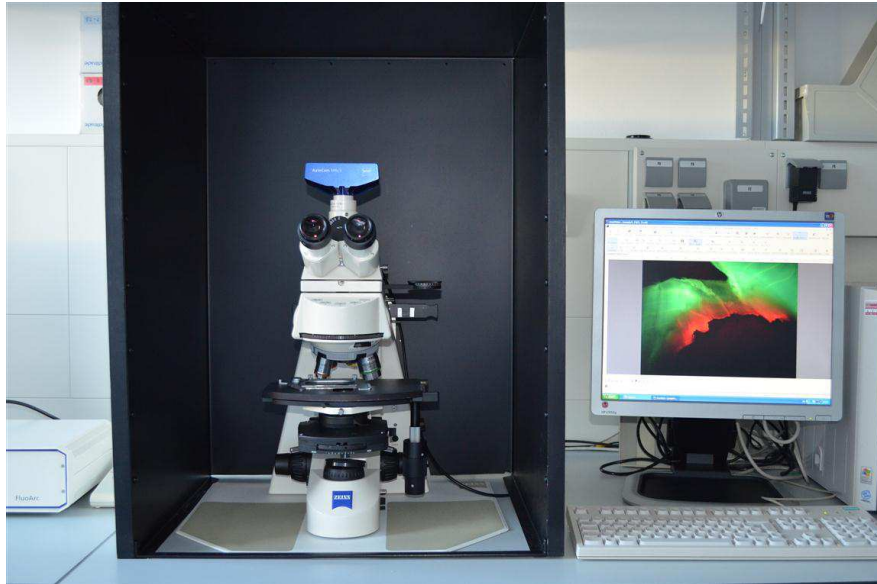


Figure 2.4 A microscope (Axioskop 2 MAT, Plan-NEOfluar 2.5x/0.075 Objective, AxioCam MRc 5, AxioVision, Rel., 4.8, Carl Zeiss AG, Germany) was employed to investigate the FACE and Caries Detector staining. During the investigation of FACE images, the room was kept dark to exclude stray light. Besides, the box that covers the microscope was used to overcome the influence of the computer screen light on the images.

2.2.3 Image analysis

The Image J distribution FIJI was used for further image analysis (Fiji is just ImageJ: <http://fiji.sc/wiki/index.php/Fiji>). Because of the special image format, all the images were opened with the Image J plugin Bio-Formats. The color evaluation comprised a series of steps. 1) Each image data was interpreted into a RGB stack. 2) The background around the tooth slice on the image was removed with the ROI manager tool to exclude the noise of the image background. 3) Each RGB image stack was converted into CIELAB format. The CIE L*a*b*(CIELAB) is the most complete color space. The coordinate a represents the position of color between red/magenta and green (a* negative values indicate green while positive values indicate red) and was used to obtain a region of interest (ROI) for the evaluation of lesion depth and area.

In the FACE group the carious dentin is marked with a red-orange fluorescence, which is definitely different from the sound dentin which is marked with a green fluorescence. All the images were automatically segmented using the threshold algorithm of Image J. However, in the Caries detector group, an arbitrary but visually meaningful value of 25 was used as a cut-off value to differentiate the red-colored area that should be removed from the pink/slightly pink stained area that should be preserved. In the resulting white and black image the number of segmented pixels was defined as the carious lesion area, as the size of each pixel was constant due to the identical magnification settings throughout the whole experiment. After the additional metric calibration of every image, the distance from the lesion surface to the bottom of the lesion was defined as the depth of the lesion. The lesion depth was measured at 10 different locations evenly distributed over each lesion.

2.2.4 Statistical analysis

The Shapiro-Wilk test and the graphical plot test showed that both the lesion area and depth were not normally distributed in both groups. As a consequence, the Mann-Whitney U test was used for the comparison of the mean lesion area and depth. All of the statistical analyses were conducted using SPSS, version 11.0 (IBM SPSS, Chicago, Illinois, USA), and the significance level was $p \leq 0.05$.

2.3 Results

Table 2.1 shows the statistical data. There was no significant difference of the lesion area between the two groups ($p=0.883$). In addition, the lesion depth in FACE group was not significantly different from that in Caries Detector group ($p=0.064$).

Figure 2.5 & 2.6 shows a representative example with carious lesions, which are marked with the red-orange fluorescence by FACE or the differential staining based on Caries Detector.

Table 2.1 Mean and standard deviation (SD) of the lesion area and depth in the FACE and Caries Detector group

Group	lesion area	lesion depth (μm)
FACE n=20	196850 ± 183633	215 ± 216
Caries Detector n=20	235060 ± 273227	216 ± 254

* Both the lesion area and the lesion depth have no significant difference between the two groups.

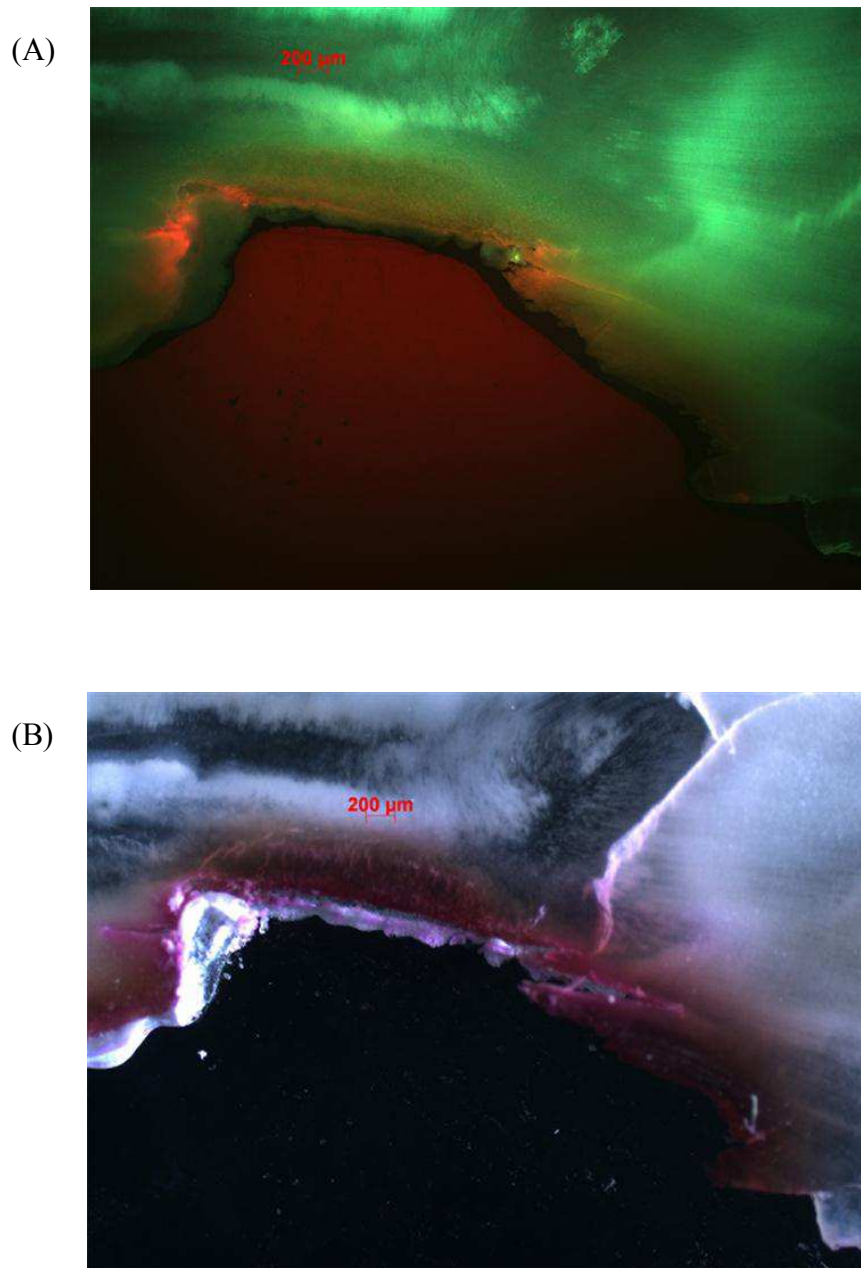


Figure 2.5 The images of one tooth slice after using FACE and Caries Detector. (A) The original image in the FACE group (the red-orange fluorescence indicates the lesion); (B) the original image in the Caries Detector group (the red stain indicates the lesion).

(A)



(B)



Figure 2.6 After image process, the images of the tooth slice after using FACE and Caries Detector showed in figure 2.5 were converted into black-white images. The white area showed the caries lesion on both images. (A) The carious lesion in the FACE group after image processing; (B) The carious lesion in the Caries Detector group after image processing.

2.4 Discussion

In clinical practice, generally, the color of caries lesions evaluated by visual inspection and the degree of hardness detected with an explorer are typical criteria employed for the diagnosis of dentin caries and the assessment of the caries-absent status of the lesion. Although Kidd et al., have shown that this conventional method is satisfactory (Kidd et al., 1993), the judgements of the color and hardness of carious dentin are quite subjective and have low reproducibility. And Fusayama and his colleagues have already mentioned that the visual and tactile criteria might not be reliable for the clinical removal of caries (Fusayama et al., 1966). Therefore, caries disclosing dyes have been recommended to facilitate the clinical discrimination between infected and affected dentin and to increase the objectivity of caries evaluation with the naked eyes. At the very beginning, 0.5% fuchsin in propylene glycol was used. Later, due to the carcinogenicity of fuchsin, a 1% acid red in propylene glycol dye such as the commercial product Caries Detector used in this study was launched as an alternative for clinical use (Kuboki et al., 1983). After application of this dye, the carious dentin which stains red should be removed while the dentin which stains light pink is suggested to be preserved. It has been reported that the slightly pink-stained dentin does not show any bacterial invasion (Sano, 1987). According to the previous study, only the typical acute or active caries clearly stains with the dye, while the typical arrested or chronic caries is very weakly stained (Fusayama, 1979). Due to the standard for choosing samples that only slices obviously stained with Caries Detector were kept, it can be presumed that only samples of the typical active caries were included in this study.

The original rationale of using the detecting dyes was based on the preferential staining of demineralized dentin collagen matrices that have been irreversibly denatured by the breakdown of the intermolecular cross-links (Fusayama, 1988). In another words, the dye solution does not stain the bacteria directly. The red stain of Caries Detector is primarily associated with pores where the stained glycol solution accumulates sufficiently to be visible to the eye. In addition, not only the demineralized infected dentin lesion but also the sound dentin including dentin at enamel-dentin junction and circumpulpal sites can be stained with Caries Detector, which may be interpreted as a false positive signal potentially causing

over-excavation (McComb, 2000). Therefore, in our experiment sound dentin at both the enamel-dentin junction and the circumpulpal sites were avoided as much as possible to enhance the objectivity of Caries Detector, as well as to simplify the experimental conditions for an automatic evaluation. However, because the interpretation of light pink stain of dentin is quite subjective in clinical situations, a significant inter-operator difference has been showed (Iwami et al., 2007). In contrast to the experiment by Iwami and his colleagues, in which teeth samples after excavation were used, instead, the whole carious lesion before excavation and the software FIJI for the evaluation were used to minimize the influence of any subjective color interpretation by the operator. Furthermore, the CIE $L^*a^*b^*$ approach and the definition of reproducible cut-off values for the segmentation reduced the subjective interpretation by operators as much as possible.

FACE is taking the advantage of the red-orange autofluorescence of carious dentin, which is totally different from the green color of sound dentin (Alfano and Yao, 1981). This kind of fluorochromes was supposed to be emitted by the metabolites of oral microorganisms mainly the porphyrins (Alfano and Yao, 1981), when the carious lesion was inspected under a violet light source (Buchalla and Lennon, 2002). For this reason, FACE can be used as a direct method to differentiate between intact dentin and dentin with a substantial demineralization clinically.

The auto-fluorescence of carious dentin has been already used in DIAGNOdent, which is a diagnostic device based on laser-induced fluorescence. It was demonstrated that the DIAGNOdent values of both active and arrested dentinal carious lesion were closely related to the rates of bacteria detection (Iwami et al., 2004 & 2011), which might confirm that the auto-fluorescence can be used as an indicator of the carious dentin lesion on some level. Since its introduction, FACE has been compared with other methods in terms of the cavity size and remaining bacteria after caries removal (Lennon et al., 2006b & 2007). In this experiment, the images of slices were captured without caries removal and the extent of the red-orange fluorescence color was obtained with the approach using a^* from the CIE $L^*a^*b^*$ color space, which is absolutely reproducible. The subjectivity when operators discriminate the red-orange color from green during the caries removal in other studies is eliminated. Based on the above

information and other studies, some researchers observed a poor correlation between the color of lesions stained with a caries detecting dyes and the rates of bacterial detection, while the red-orange fluorescence color is closely related to the bacterial invasion. FACE was suggested to be a better alternative than Caries Detector for caries removal (Lennon et al., 2006b & 2009).

In this study Caries Detector is applied to a thin tooth slice. This may cause some differences when compared to the clinical situation where Caries Detector is applied to the surface of a cavity. In a cavity the red dye has to diffuse from the surface into the deep lesion while on a slice all the areas of the lesion are stained at the same time. The diffusion in a cavity may influence the intensity of the red stain at the transition zone into sound dentin. However, clinically, Caries Detector can be applied several times to the cavity which should compensate the diffusion induced differences. For this reason, in fact the fuchsin dye, which was used to stain and identify carious dentin in early publications, was also applied to tooth slices (Kuboki et al., 1983), thus the application of Caries Detector to tooth slices in our experiment should be totally valid. Moreover, the teeth included in this study have the lesions that are far away from the enamel-dentinal junction to avoid false positive signals with Caries Detector. The excellent agreement between Caries Detector and FACE in our study can be explained in part with this selection of lesions. Another explanation for this agreement is the underlying mechanism of both methods. Caries Detector stains dentin with reduced mineral density. FACE identifies fluorochromes with an extrinsic origin. Extrinsic origin refers to the fact that these fluorochromes are not present in sound dentin. They may be metabolic derivatives of bacteria. But it is also possible, that they just diffused into the less mineralized lesion from any other external fluorophore source such as food. The molecular size of fluorochromes is in the order of magnitude as the dye carrier in Caries Detector or even larger. For this reason the fluorophore front might not be too much different than the dye front of Caries Detector. Lennon et al. found a good agreement between the absence of bacteria and the fluorescence intensity (Lennon et al., 2006b & 2009). However, at the current state of knowledge about the fluorochromes, it is also possible that the fluorochromes diffuse into less mineralized dentin even without the presence of bacteria just as Caries Detector does.

If the association between Caries Detector and FACE is so close, what is the advantage of FACE? At present, based on our findings, the most important advantage should be that FACE indicators are strictly related to bacteria-infected dentin. They should not show false positive signals due to unspecific protein staining known for Caries Detector (McComb, 2000). And it should not be the over-excavation where Caries Detector still stains dentin due to this unspecific protein staining reaction, such as the dentin close to the pulp.

However, FACE has some limitations itself as well. The fluorochromes are prone to be bleached. Under intensive light the fluorescence intensity diminishes as a result of dye bleaching even without excavation. The room was kept dark in this experiment to reduce this source of error so as to prove the validity of the FACE approach. But in a clinical setting, it is impossible to exclude the intervention under intensive illumination, which should be considered when FACE is used. Besides, the robustness of the fluorochromes under the light of dental operation has to be investigated in a subsequent study.

Besides, it is known that the fluorescence is influenced by storage conditions and time periods (Iwami et al., 2011). The extracted teeth stored less than one month, much shorter than that other experiments (Lennon et al., 2006b & 2009), were used in this experiment. Therefore, the influence of the storage time and conditions on FACE as a potential source of error should also be further investigated.

Nevertheless, even with a few limitations, an agreement between Caries Detector and FACE was shown in this study. And it is valuable to mention that there is no arbitrary value during the image evaluation, which might indicate FACE has high reproducibility and the advantage of less false positive findings.

Chapter 3

microCT based comparison between fluorescence-aided caries excavation (FACE) and conventional excavation

3.1 Background and Significance

In the context of the change from Black's principle 'extension for prevention' to the concept of 'minimal intervention dentistry', clinical practitioners are recommended to remove the heavily infected and irreversibly demineralized dentin and preserve the affected dentin which has the potential to remineralize as much as possible (Tyas et al., 2000). Therefore an optimized approach of caries excavation is required to accurately differentiate the "infected" from "affected" dentin as well as minimally destruct the healthy dentin. However, until now the differentiation of the two terms "infected" or "affected" dentin is based on differential staining of the carious lesion with an unspecific dye (Fusayama et al. 1979). The red stain of Caries Detector is primarily associated with pores where the stained glycol solution accumulates sufficiently to be visible to the eye. The demarcation line to differentiate between "infected" and "affected" dentin is influenced by subjective interpretation of the stain intensity. In addition, the concept of differential staining suggests the existence of a clear threshold to differentiate "infected" from "affected" dentin. This interpretation is not accurate, however, as caries with all the associated changes in tissue (amount of bacteria, dissolution of mineral, presence of fluorochroms etc.) is a diffusion controlled process which is influenced by dentin tubuli and the active repair mechanisms of the pulp. Consequently, a continuous gradient exists between the center of the caries lesion and sound dentin. Along this gradient the degree of destruction changes from "no potential to remineralize" to "be potentially remineralizable". The amount of microorganisms along this gradient is reduced but some bacteria can be identified even within the sound dentin tubules. This gradient leading to lack of a clear and exactly defined therapeutic endpoint makes it so complicated for a dentist to determine how 'clean' a cavity should be before a restoration (Kidd, 2004).

Currently, as the soft and wet carious dentin lesions harbor significantly more bacteria than samples from hard or dry lesions (Kidd et al., 1993 b), dentists are advised to remove the

carious dentin to a level where it is 'firm' (Kidd, 2004). Therefore, the classical criterion for caries excavation, which still is widely used, is the dentin hardness as it is detected with a dental explorer. This conventional method has shown to be clinically satisfactory (Kidd, 1993a). However, not only because of the variation of hardness between teeth or even sites within one tooth, but also the experience and tactile assessment between different clinicians, the estimation of the hardness of carious dentin is quite subjective and has a low reproducibility. Some researchers mentioned that the visual and tactile criteria might not be a reliable guide for the clinical removal of caries (Fusayama et al., 1966). Several alternatives such as Caries Detector, the chemo-mechanical method Cariosolv or polymer instruments (Boston 2003) have been developed in the past few decades. Although the claim of these methods is to make the caries excavation more objective, over- or under-excitation or other problems such as long treatment time or higher treatment costs have been shown in various in vitro studies (Kidd, 1993a, Neves et al., 2011b).

Recently, a novel caries excavated technique 'fluorescence-aided caries excavation (FACE)' has attracted a certain degree of attention. FACE uses the red-orange fluorescence originating from by-products of bacterial metabolism in carious dentine as the marker of infected dentin (Buchalla and Lennon, 2002). Previous studies reported that FACE has a better effectiveness and working efficiency in the excavation of infected dentine of both permanent and primary teeth than other excavation methods (Lennon et al., 2006b, 2007 & 2009).

Previously, histological examinations after excavation were used as the gold standard and regarded as the evaluation parameter. However, those histological investigations were normally destructive to the samples and the sample preparation is time consuming. X-ray computed micro-tomography (microCT), is a non-invasive and non-destructive technique, which allows the recording of internal features of samples based on the evaluation of mineral density and three-dimensional reconstruction (Lin and Miller, 1996). Additionally, it also allows the longitudinal evaluation of the alteration in the same tooth (Clementino-Luedemann et al., 2006a; Neves et al., 2011a, b). To date, microCT has been used in dental research (Swain and Xue, 2009) and recommended to be applied for the study of dentin caries

excavation techniques (Neves et al., 2010). The aim of this in vitro study was to compare the excavated effectiveness of FACE to that of conventional excavation method using the microCT evaluation.

3.2 Materials and Methods

3.2.1 Sample selection and excavation

Freshly extracted human permanent teeth with dentin caries were collected and stored in 0.01% thymol solution at 4 °C in the dark. The experimental procedures were approved by the ethics committee of medical faculty, Ludwig Maximilians University. Each tooth was cut longitudinally through the center of the carious lesion into two parts (Leica SP 1600-Saw Microtome, Leica Microsystems Nussloch GmbH, Nussloch, Germany). Through visual detection, only samples without dental pulp involvement were included into the experiment. Finally, twenty teeth were selected. Two halves of each tooth were randomly assigned into two treatment groups. One half was excavated using the conventional excavation method, while the other half was excavated using FACE.

Caries excavation for all the samples was carried out by the same operator. Caries was removed using Tungsten carbide round burs (HISEM, GEBR BRASSELER GmbH & Co. KG, Lemgo, Germany) in a slow-speed handpiece without water cooling. The sizes of the round burs were selected according to the cavity size and geometry.

In the conventional excavation group, softened dentin were detected with a sharp explore. Only soft dentin was removed by an ordinary slow-speed handpiece (GENTLEpower LUX 7LP, KaVo Dental GmbH, Biberach, Germany) with Tungsten carbide round burs. Hard dentin was preserved, even when it was stained.

In the FACE group, carious dentin was detected and removed by using a special slow-speed handpiece with an integrated LED (Demoinstrument UV-LED, W&H Dentalwerk Burmoos GmbH, Austria), which emits light at a wavelength of 405 nm. Round burs were used. The operator inspected the lesion through goggles with specific lenses (BG36 + GG495, Schott, Mainz, Germany). Only the red-orange-colored area was removed. Because the emitting light is powered by a dynamo in the handpiece and its brightness is influenced by the

speed of the handpiece (brightness ~ speed), for the sake of ensuring that all of the red-orange carious lesions were removed, a battery-powered 405 nm LED probe (Proface RB-405, Demoinstrument UV-LED, W&H Dentalwerk Burmoos GmbH, Austria), the emitted light of which is relatively stable, was used to check the cavity after excavation until only a green color could be observed. During the excavation, the room was kept dark.

(A)



(B)



Figure 3.1 In the FACE group, carious dentin was detected and removed with (A) a special handpiece (Demoinstrument UV-LED, W&H Dentalwerk Buermoos GmbH, Austria). A LED light, which is emitting light 405 nm, is integrated into this handpiece (see the yellow arrow). The operator inspected the lesion through a goggle with specific glass filters (BG36 + GG495, Schott, Mainz, Germany). (B) A battery-powered LED, whose emitting light intensity is constant, was used to check the cavity after excavation.

3.2.2 MicroCT scanning procedures

A high resolution X-ray micro-computed tomography (μ CT 40, Scanco Medical AG, Basserdorf, Switzerland) was used to determine the therapeutic endpoint for this investigation. The acceleration voltage was 70 kVp and the cathode current was 114 μ A. A 0.5mm aluminum filter was installed in the beam path to cut off the softest x-rays, resulting in a detector response close to 31 keV. All the samples were scanned with the 20 μ m resolution using an integration time of 300 ms. Datasets consisted of 395 slices each. During the scanning procedure, all the samples were kept in a humid environment using Parafilm (Parafilm M, Pechiney Plastic Packaging, Chicago, USA) to seal the upper side of the sample holder in order to prevent the desiccation of the tooth. The samples were kept humid to avoid shrinkage of the dentin, which happens when dentin dries, as this would have influences the measured calcium concentration per volume element.

(A)



(B)



Figure 3.2 (A) a sample was put on the sample holder in a humid environment using Parafilm (Parafilm M, Pechiney Plastic Packaging, Chicago, USA) to seal the upper side of the sample holder in order to prevent the desiccation of the tooth. (B) A high resolution X-ray micro-computed tomography (μ CT 40, Scanco Medical AG, Basserdorf, Switzerland) was used for this investigation.

3.2.3 Images processing and evaluation

Mineral concentration

In order to allow a quantitative and reproducible comparison of the samples the microCT was calibrated weekly with the Scanco solid calibration phantom (HA-Scanco, Bassersdorf, CH). This phantom is based on the European sine and forearm calibration phantoms (Nazarian et al. 2008). It consists of different concentrations of hydroxyapatite (HA) crystals embedded in epoxy resin. The overall HA densities and the corresponding bone equivalent densities are known (Nazarian et al. 2008). According to the manufacturer, the HA density of the calibration samples are 0.099, 0.199, 0.399 and 0.8 g cm⁻³. After imaging the phantom with the microCT image cross sections were reconstructed and the mean grey value which is equivalent to the linear attenuation coefficient was determined for each HA sample in a circular region of interest. Based on the four calibration points a calibration curve could be calculated with a fitted linear equation of the form $y = ax + b$ where y represents the mineral density, x represents the linear attenuation coefficient, while a and b are the constants of the linear equation. Based on the determined parameters a and b the mineral density could be calculated using the linear attenuation coefficient. It should be mentioned that due to the linear correlation of both values the same conclusions can be drawn from both measures. It would have been totally enough to summarize the data based on the linear attenuation coefficient alone. However, in dental publications the mineral concentration is more common, which is the reason for the calibration procedure. Based on the calibration approach described above, the data of calibration throughout the whole experimental period were collected. The mean grey values of the four phantoms were calculated, as well as the mean the linear attenuation coefficients. The mean linear attenuation coefficients of the HA phantoms with four different mineral concentrations were 0.762, 1.023, 1.5 and 2.395 cm⁻¹ respectively. Figure 3.3 shows the calibration curve and the equation for the resin embedded solid HA phantoms.

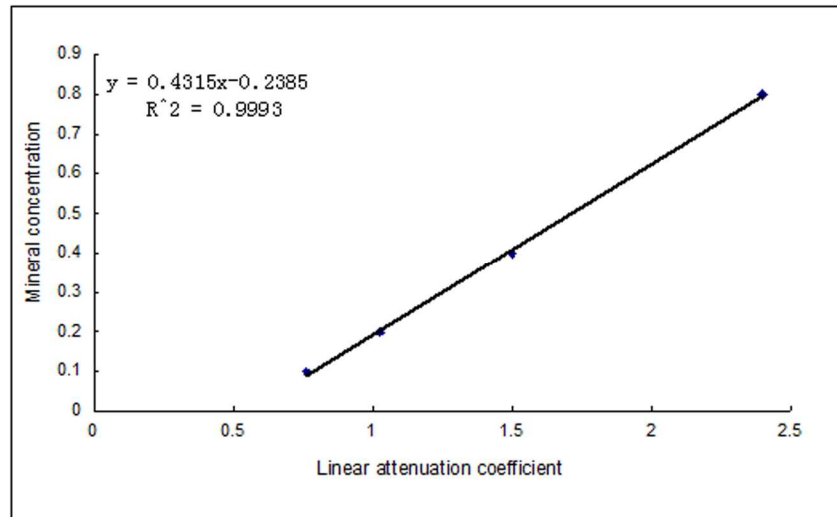


Figure 3.3 Calibration curve and the equation for the resin embedded solid HA phantoms

Images processing

Using Fiji, an ImageJ distribution (Fiji is just ImageJ: <http://fiji.sc/wiki/index.php/Fiji>), the raw data were imported and stored as 16-bit binary for further evaluation. The slices were filtered with a median filter (radius 1 pixel) and a data reduction along the z-direction was performed by averaging two neighboring slices throughout the whole stack of images.

Data evaluation

The mineral concentration respectively the linear attenuation coefficient at the surface of the lesion after excavation was used as the statistical parameter. The lesion surface was determined based on a statistical measure: the background of the image was selected on a slice with the freehand selection tool at several locations and the mean background value was calculated to serve as the threshold value for the lesion surface.

Then a line of interest was selected from the background into sound dentin perpendicular to the treated surface. A series of five grey values larger than the threshold value were obtained and the mean value was calculated as the grey value of the lesion surface after excavation. According to the information from the manufacturer, the ratio between the grey value and the linear attenuation coefficient was 4096, and the linear attenuation coefficients

were calculated. Finally the linear attenuation coefficient was converted into the mineral concentration value using the equation obtained above.

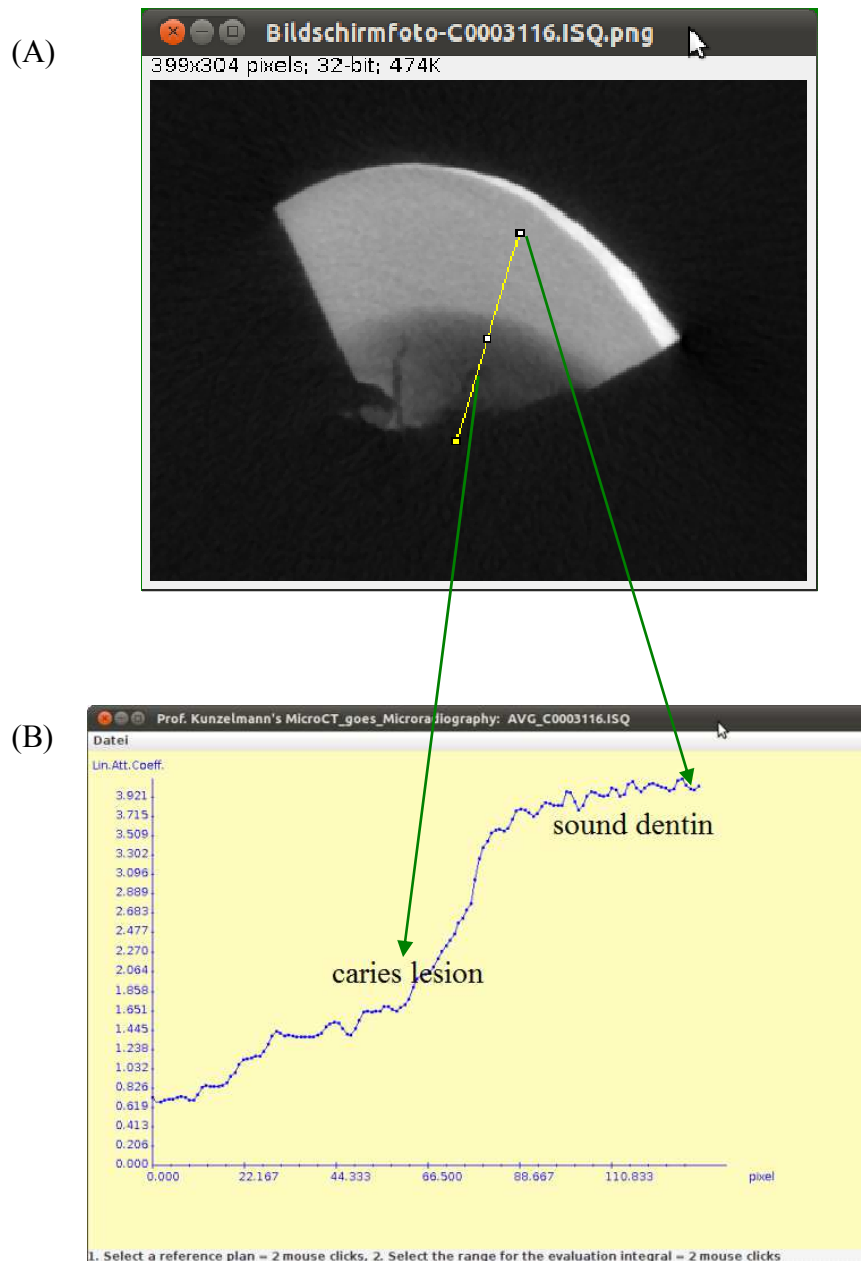


Figure 3.4 A line is drawn onto a tooth slice with caries lesion (A); the linear attenuation along this line is plotted and it decreases due to caries process (B). The x-axis scale is given in pixels. Each pixel is 20 μm wide. The y-axis-values are the linear attenuation values. The unit is cm^{-1} .

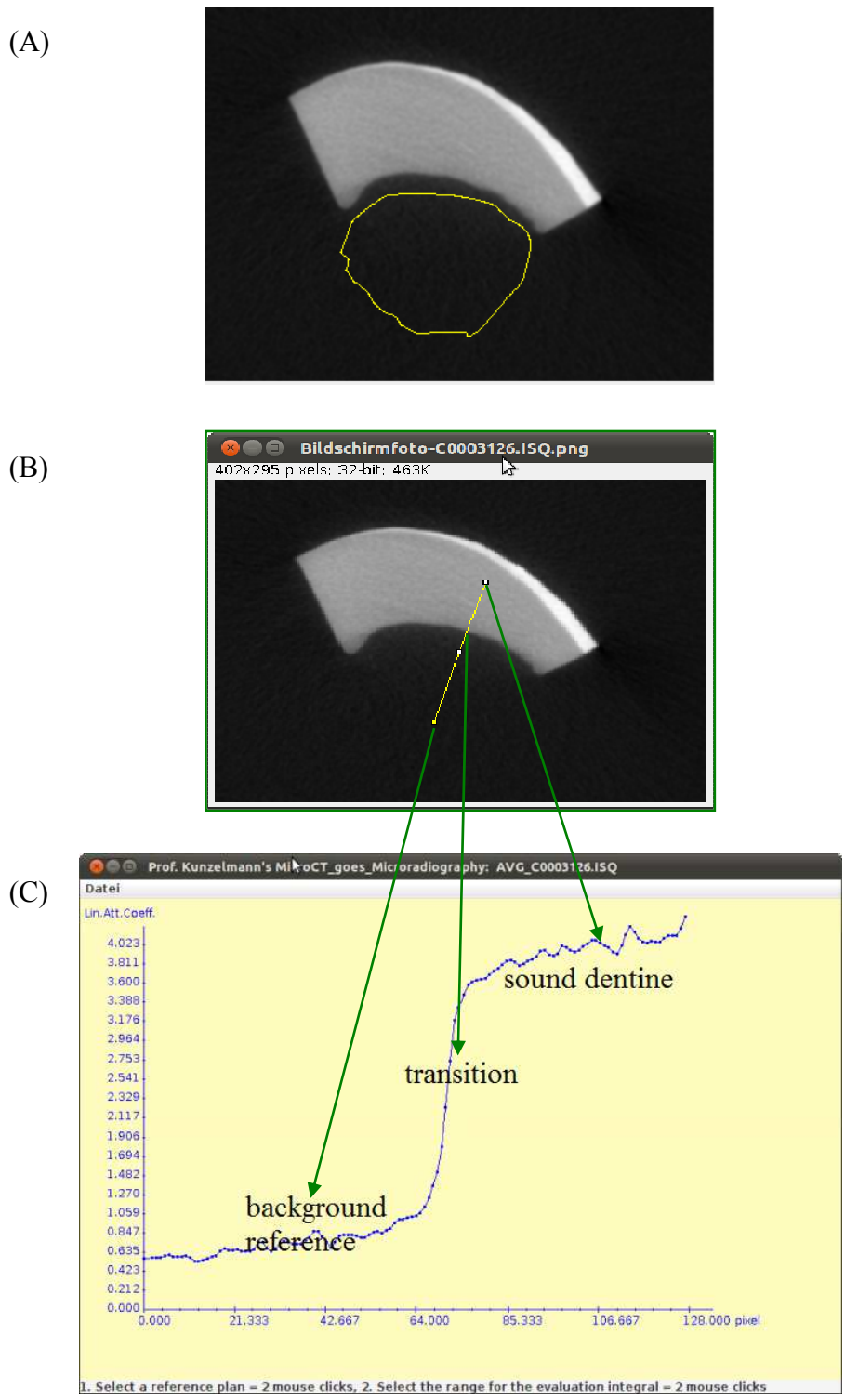


Figure 3.5 (A) an image of the previous tooth slice after caries excavation. The freehand selection (area with yellow boarder) is an arbitrary selection of background pixels, which are used to calculate the background reference data (mean, sd) within the outlined area. A line is drawn onto this slice (B) and the line is plotted (C).

3.2.4 Statistical analysis

The Shapiro-Wilk test and graphical plots showed that the values of the calculated mineral concentration in the FACE group were not normally distributed while the calculated values in the conventional excavation group were normally distributed. Therefore the Mann-Whitney U test was used for the statistical analysis. All of the statistical analyses were conducted using SPSS, version 11.0 (IBM SPSS, Chicago, Illinois, USA), and the significance level was $p \leq 0.05$.

3.3 Results

As shown in Table 3.1, the mean linear attenuation coefficient at the lesion surface was $2.80 \pm 0.12 \text{ cm}^{-1}$ after using the conventional excavation method. In comparison, the value was significantly lower in the FACE group, which was $1.93 \pm 0.39 \text{ cm}^{-1}$ ($p < 0.001$). With the calibration, the mean mineral concentration of the superficial tissue after the conventional excavation was $0.97 \pm 0.05 \text{ g/cm}^3$, which was significantly higher than the value in the FACE group, which was $0.59 \pm 0.17 \text{ g/cm}^3$.

Table 3.1 Mean value with standard deviation of the linear attenuation coefficient and mineral concentration of tissue at the surface after excavation in FACE and conventional excavation group.

Treatment Group	Linear attenuation coefficient (cm^{-1})	Mineral concentration at the superficial surface (g/cm^3)
FACE N = 20	1.93 ± 0.39	0.59 ± 0.17
Conventional excavation N = 20	2.80 ± 0.12	0.97 ± 0.05

3.4 Discussion

The mineral content has been suggested to be associated with the mechanical properties of calcified tissue (Pugach et al., 2009). Mineral density has also been considered as a standard parameter for the assessment of demineralization and remineralization in dental research. Thus investigation on mineral concentration is of great importance for dental caries (Zou et al., 2011). Mineral concentration in dental hard tissue can be measured either directly using chemical analysis, or indirectly by polarizing microscopy or transverse microradiography (Ten Bosch and Angmar-Månsson, 1990). However, these techniques are normally carried out on a single sample or a thin sample resulting in the destruction of sample and the time-consuming sample preparation (Ten Bosch and Angmar-Månsson, 1990). In addition, it is not always guaranteed that the sample surface is projected perpendicular onto the high resolution plate. Even slightly oblique surfaces result in an intensity gradient from the background into sound tissue at the surface of the sample.

On the contrary, microCT systems allow the non-destructive evaluation of an object through the volumetric x-ray attenuation measurement (Davis and Wong, 1996). The raw data from microCT measurement can be reconstructed three-dimensionally and provide the internal structure of experimental samples. Besides, this technique allows the longitudinal evaluation of the sample before and after an experimental procedure. The grey levels of the reconstructed microCT images represent spatial distribution of linear attenuation coefficients within a sample which is determined by the energy of the x-ray source. And the atomic composition of the material sample and the x-ray attenuation coefficient is reported to have a linear relationship with the mineral concentration (Elliott et al., 1998; Nuzzo et al., 2002; Swain and Xue, 2009). In the past few years, the microCT has been employed for the evaluation of the mineral concentration of dental hard tissue (Wong et al., 2004; Clementino-Luedemann and Kunzelmann, 2006) and also for the investigation of carious lesions and caries removal (Willmott et al., 2007; Neves, et al., 2010). Figure 3.4 shows a representative example that the change of the linear attenuation coefficient is corresponding to the change of the mineral concentration, which can indicate different tissues and the alteration of the calcified tissue.

However, the calibration of the microCT to determine the mineral density is remaining a major challenge (Zou et al., 2009).

First, beam-hardening effect which can lead to artifacts is a primary practical problem for the polychromatic x-ray resources used in this study. And the only way to eliminate beam hardening is using monochromatic x-ray resources like for example a synchrotron, which is totally unrealistic for dental applications. To reduce the effect of beam hardening, the microCT machine in this study is installed with 0.5-mm aluminum in the beam path for the selective removal of low-energy x-rays. In addition, the manufacturers implement sophisticated beam hardening corrections into their systems which are applied during the reconstruction of the 3D data. Another detail, which is important to keep the beam-hardening induced variations of quantitative measurements with the microCT as low as possible is to place the samples at a comparable position during the whole experiment and to use a comparable volume under investigation throughout the whole series of measurements. These additional criteria were considered in our experiment.

Second, because each machine has slightly different settings in the machine setup, sensor characteristics, x-ray-spectrum, filtering, beam hardening correction or volume under investigation (= sample size, especially concerning the sample cross-section and the ratio of dentin to enamel) etc, the mineral densities determined with a microCT vary between different machines and different publications. Moreover, due to the intrinsic structure of dental hard tissue, it is a little complicated to calculate the mineral concentration. Except a small amount of inorganic component, enamel is a tightly packed mass of hydroxyapatite. Previous studies concerning on the mineral density of enamel and enamel caries often ignored the attribution of inorganic component to the linear attenuation coefficient and assumed the mineral content of enamel to be pure hydroxyapatite (Angmar et al., 1963; Wong et al., 2004; Clementino-Luedemann and Kunzelmann, 2006). Dentin is composed of 70wt% inorganic materials, 20 wt% organic matrix and 10 wt% water. The contribution of water and organic materials were considered by some researchers who assumed that the contribution of collagen, the main inorganic material in dentin, to the measured linear attenuation coefficients was constant (Willmott et al., 2007). And sometimes the contribution was neglected

(Clementino-Luedemann et al., 2006; Neves et al., 2011a). Thus the mineral densities of dentin reported in previous studies are more or less different. Clementino-Luedemann and Kunzelmann have shown the density values between 1.36 and 1.45 g/cm³ of sound permanent dentin (Clementino-Luedemann and Kunzelmann, 2006), while Willmot et al. found a mean modal value of 1.42 g/cm³ for sound deciduous dentin with the upper limit of 1.77 g/cm³ and the lowest limit of 1.27 g/cm³ (Willmott et al., 2007). In addition, a recent study used a dentin-caries threshold based on hardness measurements showed a value of 1.11 g/cm³ as the cut-off value for the endpoint of dentin caries removal (Neves et al., 2011a). To avoid the consideration of the complexity of the dentin and the alteration of the mineral concentration as a result of the carious attack, the calibration approach suggested by Zou (Zou et al., 2009) was applied in this study. The calibration curve and the obtained equation in our study demonstrated a very good linear correlation between the linear attenuation coefficient and the mineral density during the whole experiment. As described above, it might not be appropriate to compare the mineral concentration of the superficial dentin after conventional excavation with the mean value of 0.97 g/cm³ to the value from other experiments, however, the small standard deviation value (0.05) indicates the stability of conventional excavation during the experiment and the small inter-sample variation.

The primary objective of caries excavation before restoration is to completely remove the infected dentin and preserve affected dentin as much as possible. According to the investigation on the microstructure of dentin caries, the affected dentin still has mineral loss resulting in the reduction of mechanical properties especially the decrease of hardness (Pugach et al., 2009). Thus the conventional method using the tactile feedback of hardness to determine if tissue should be removed was considered to excavate much affected dentin leading to over-treatment. The mineral concentration of the dentinal tissue at the surface after conventional excavation in the present study is lower than the value of sound dentin with 1.36 and 1.45 g/cm³ described above, which indicates that the operator in this study succeeded in her approach to be as tissue preserving as possible even with the conventional excavation method. Besides, it is necessary to mention that the tungsten carbide burs used in this study

has been shown the capability of reducing the risk of over-excitation and facilitate dentists to remove infected tissue (Neves et al., 2011a).

FACE makes use of the visible red-orange fluorescence of carious dentin which is reported to originate from the by-products of bacterial metabolism. With FACE, only the infected dentin as indicated by the fluorescence should be removed and dentin with the potential to remineralize should be preserved (Buchalla and Lennon, 2002). Compared to caries detector or the conventional method to determine the excavated endpoint, FACE showed the highest sensitivity, specificity and predictive values for residual caries detection based on the evaluation using confocal microscopy (Lennon et al., 2002). Histological investigation found fewer samples presenting bacteria in dentin after excavation with FACE than using the conventional excavation method (Lennon, 2003). In the present study, using the microCT evaluation, the mineral concentration at the surface after excavation with FACE is significantly lower with a mineral concentration value of $0.59 \pm 0.17 \text{ g/cm}^3$. Due to the complexity of caries process and the calibration of microCT for dentin caries, until now, there is no accurate value of mineral concentration to establish the excavated threshold which can preserve the affected dentin. Only few study showed the mineral concentration of typical carious dentin with the value of 0.55 g/cm^3 (Kinney et al., 1994), while other researchers claimed that the mineral density of carious region was 0.27 g/cm^3 (Neves et al., 2010). Thus in this study it is not appropriate to claim that FACE can preserve the affected dentin. But the statistical results might imply that FACE is more conservative compared to conventional excavation.

Although, the results in the present study with previous studies altogether might imply that FACE is a conservative excavation method, until now, FACE is investigated only in in vitro studies. Clinical trials need to be done to validate the high sensitivity, specificity of FACE before it hits the market and is applied clinically.

Chapter 4

Microhardness testing on the cavity floor after fluorescence-aided caries excavation and conventional excavation

4.1 Background and Significance

Modern restorative dentistry has moved from the extension-for-prevention concept, the classical principles of cavity preparation that were established by G. V. Black (Black, 1908), to minimal intervention dentistry (Tyas et al., 2000), which is more conservative and aims to save remineralizable and healthy dentin as much as possible.

Cariou dentin, as described by Fusayama, is arbitrarily divided into two layers (Fusayama, 1979). The outer infected layer, which is heavily infected by bacteria, irreversibly denatured and physiologically unremineralizable, should be removed. The inner affected layer is only partially demineralized and has the potential to remineralize. This layer can and should be preserved during caries excavation (McComb, 2001). To preserve the affected layer, alternative dentin caries removal methods and aids, such as chemo-mechanical methods and caries-disclosing dyes, have been developed.

FACE (fluorescence-aided caries excavation), a novel caries excavation system, claims to be selective about removing infected dentin (Buchalla and Lennon, 2002). Using the FACE system, sound dentin fluoresces green after being illuminated with violet light, whereas bacterially infected dentin emits red-orange fluorescence. This red-orange fluorescence in carious dentin is associated with porphyrins, which are metabolites of oral bacteria (Alfano and Yao, 1981). During the excavation, only the red-orange fluorescing dentin is removed.

While red-orange fluorescence is used as an indicator for excavation with FACE, hardness is applied as one of the principal criteria for the therapeutic endpoint of caries removal using the conventional excavation method. A dentist assesses the resultant surface with an explorer and stops the excavation as soon as the surface "feels as hard as normal dentine" (Roberson and Sturdevant, 2002). However, in 1983, an illustration by Ogawa et al. (Ogawa et al., 1983) showed that compared with sound dentin, the inner affected dentin

showed significantly lower hardness values, which would imply that if the affected dentin tissues are preserved, the hardness at the cavity floor would be decreased compared to the hardness of the sound dentin. This observation would explain how over-excitation and unintentional pulpal exposure occur during conventional excavation (Celiberti et al., 2006).

Until now, little attention has been paid to the hardness of dentin after excavation, and there is limited information about the hardness of dentin after excavation when FACE is compared with other caries excavation methods. The main aim of this study was to determine whether FACE can preserve the affected dentin. Hardness testing was selected as an outcome measure to compare the hardness value of the cavity floor after FACE excavation with that following conventional caries excavation.

4.2 Materials and Methods

4.2.1 Tooth selection and excavation

Freshly extracted permanent human teeth with dentin caries (D3/D4) were collected and stored in 0.01% thymol solution at 4 °C in the dark up to one month. The experimental procedures were approved by the ethics committee of medical faculty, Ludwig Maximilians University (Munich, Germany). Each tooth was cut longitudinally through the center of the carious lesion into two parts (Leica SP 1600-Saw Microtome, Leica Microsystems Nussloch GmbH, Nussloch, Germany). Using visual detection, only samples in which the dental pulp was not involved or only partly involved in the caries were included in the experiment. Finally, 20 teeth were selected. Each tooth was sectioned through the lesion center into two halves. A cutting plane of each sample was obtained. Half of each tooth was excavated using the conventional excavation method, and the other half was excavated using FACE. Therefore, the conventional excavation and FACE groups each had 20 samples.

Caries excavation for all of the samples was undertaken by the same operator (GL). Caries was removed using round tungsten carbide burs (H1SEM, GEHR BRASSELER GmbH & Co. KG, Lemgo, Germany) in a slow-speed handpiece. The sizes of the round burs differed according to the cavity size.

In the conventional excavation group, visual and tactile criteria were used for caries detection. Softened dentin was detected using a sharp explorer. Only soft dentin was removed using an ordinary slow-speed handpiece (GENTLEpower LUX 7LP, KaVo Dental GmbH, Biberach, Germany) with round tungsten carbide burs. Hard dentin was preserved, even when it was stained.

In the FACE group, carious dentin was detected and removed by using a special slow-speed handpiece with an integrated LED (Demoinstrument UV-LED, W&H Dentalwerk Burmoos GmbH, Austria), which emits light at a wavelength of 405 nm. Round burs were used. The operator inspected the lesion through goggles with specific lenses (BG36 + GG495, Schott, Mainz, Germany). Only the red-orange-colored area was removed. Because the emitting light is powered by a dynamo in the handpiece and its brightness is influenced by the speed of the handpiece (brightness ~ speed), for the sake of ensuring that all of the red-orange carious lesions were removed, a 405 nm LED probe (Proface RB-405, Demoinstrument UV-LED, W&H Dentalwerk Burmoos GmbH, Austria), the emitted light of which is relatively stable, was used to check the cavity after excavation until only a green color could be observed. During the excavation, the room was kept dark.

4.2.2 Sample preparation

After excavation, each sample was embedded in transparent, cold-curing methyl methacrylate (Technovit 4004, Heraeus Kulzer GmbH, Hanau, Germany), with the cutting plane towards the bottom of a rectangular mold. After the methyl methacrylate hardened, each sample was removed from the mold. The surplus methyl methacrylate on the surface opposite the cutting plane was ground flat. Each sample was then mounted on a plastic slide, which served as an attachment for the subsequent polishing of the cutting plane (Figure 4.1). The cutting plane was polished (EXAKT 400CS plus EXAKT AW110 Control, EXAKT Apparatebau GmbH, Germany) using polishing papers (P 1000/2500/4000, LECO Corporation, St. Joseph, Michigan, USA) under running water. Finally, the surface was polished with diamond spray (DP-Spray p 1 µm, Struers A/S, Denmark) and polishing discs (Polishing disc, LECO Corporation, St. Joseph, Michigan, USA).

4.2.3 Hardness test

A Fischerscope® H100C XYp microhardness indenter (Helmut Fischer GmbH & Co. KG, Sindelfingen, Germany) was used to obtain precise hardness profiles of the dentin. A load of 500 mN was applied for 20 s to produce the indentations (Figure 4.3). The Martens hardness was used as the statistical parameter. Since the indenter is a square diamond pyramid with an opening angle of 136° according to Vickers, the approximate value of the Vickers hardness can be calculated using the software that is packaged with the machine. This value was considered as Vickers equivalent in this experiment and was also used for the comparisons. Each sample was mounted on the stage of the machine under the microscope with an adhesive tape (Tesa AG, Hamburg, Germany). A line was made from the pulp across the sound dentin area to the cavity floor (Figure 4.4). Each line of indentations was placed in an area where the distance between the cavity floor and the pulp was wide enough to ensure presence of sound dentine. Because the teeth were embedded in methyl methacrylate, a point 30 μm below the cavity floor was regarded as the hardness of the dentin at the cavity floor after excavation in order to avoid the indentation on the interface between the cavity floor and the methyl methacrylate. Each line had three segments: 1) the first segment ran from the pulp to a point 300 μm away from the pulp, with a distance between indentations of 30 μm ; 2) the second segment started at an average distance of 100 μm ; 3) the last segment ran from the cavity floor to a point 300 μm away, with a distance between indentations of 30 μm . The mean value of the three points in the sound dentin area that were closest to the point at the subsurface of the cavity floor was calculated, and this value was used as the control value. The relative microhardness of the cavity floor was obtained from the proportions of the microhardness of the cavity floor and the sound dentin described previously.

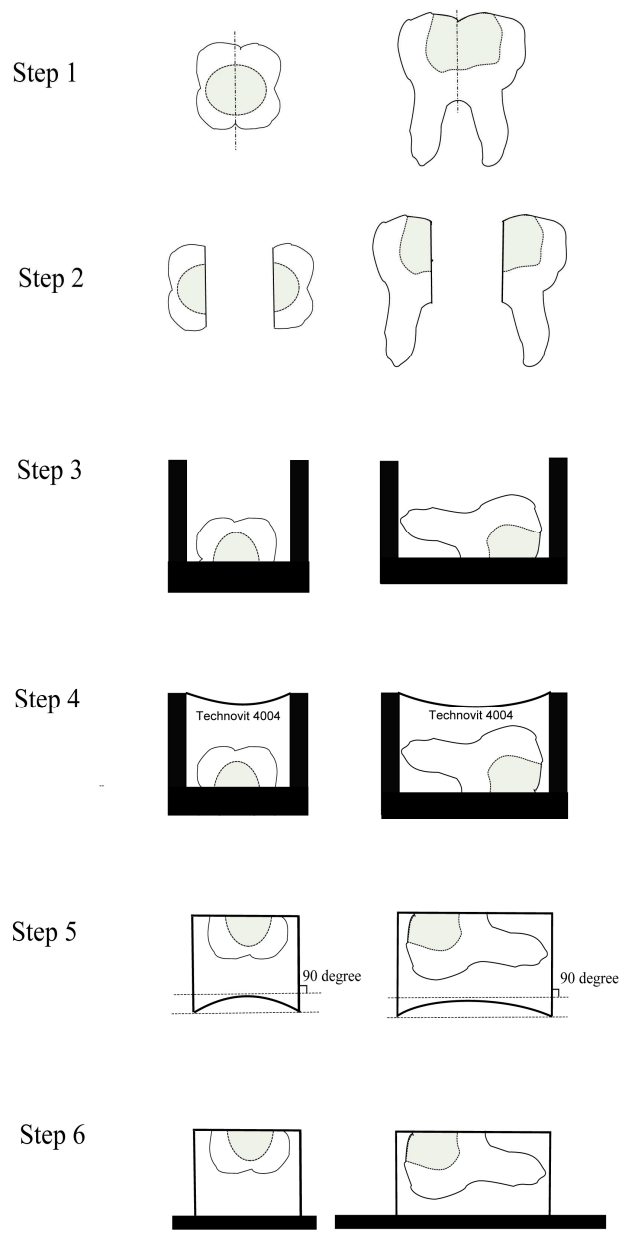


Figure 4.1 An illustration of the sample preparation. Step 1: a tooth with caries lesion was bisected through the center of the lesion in inciso-apical direction; step 2: half of the tooth was excavated with FACE, the other half was conventionally excavated; step 3: each sample was placed in the mold so that the cutting plane was parallel to the bottom of the mold; step 4: each sample was embedded with Technovit 4004; step 5: the surface of Technovit was slightly concave after the polymerization due to the curing contraction. It was ground to be perpendicular to the sample sides and parallel to the cutting plane; step 6: the cutting plane was polished.

(A)



(B)

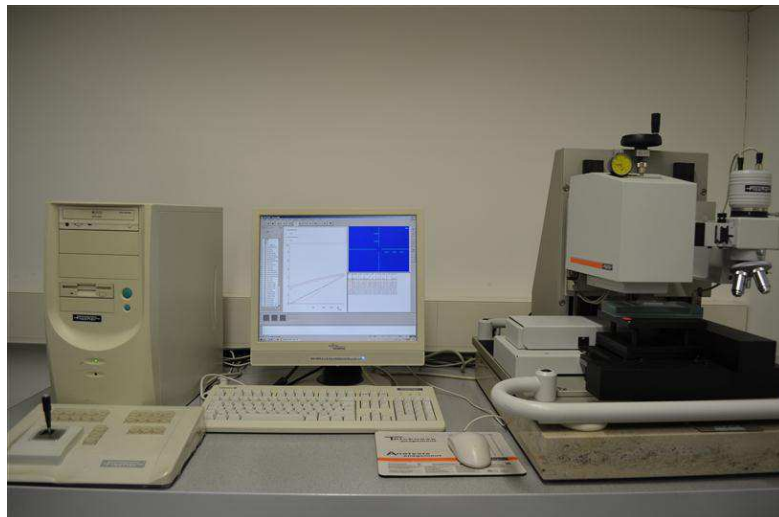
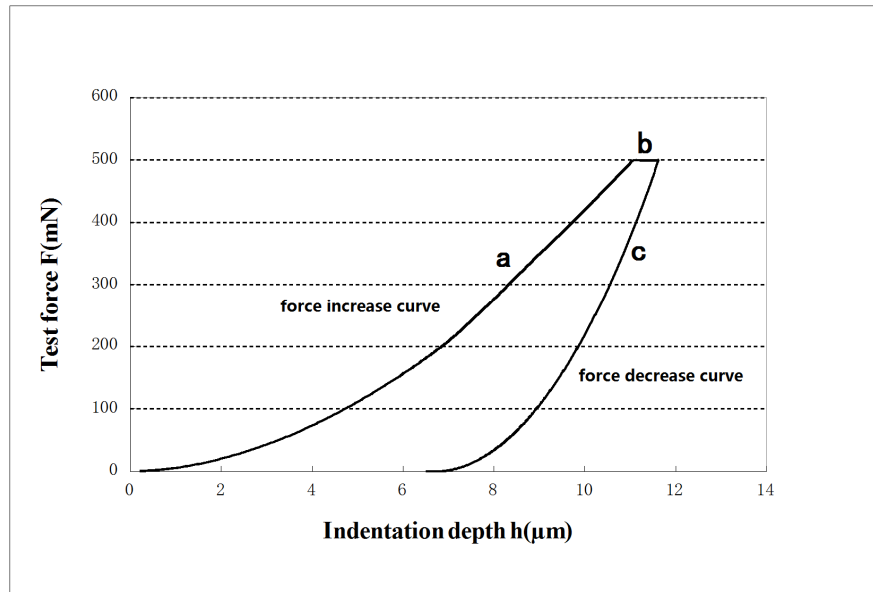


Figure 4.2 An embedded sample was mounted on a plastic slide (A), after being polished (The tooth bulk was polished using the same process of polishing tooth slice in chapter 1), the microhardness of the cavity floor was investigated using a Fischerscope® H100C XYp microhardness indenter (B).

(A)



(B)

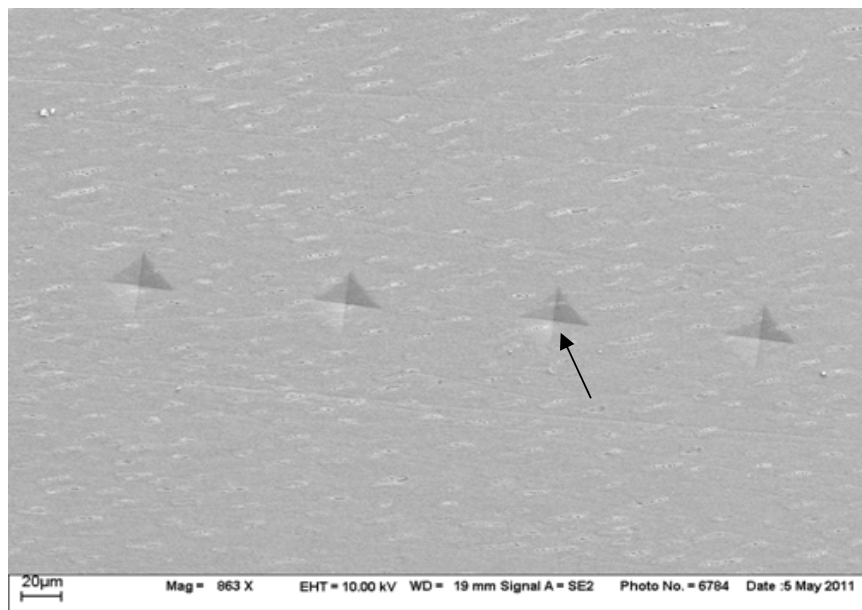


Figure 4.3 (A) Test force/indentation depth curve of one indentation. The test force increased steadily until reached the Maximum force 500mN within 20s(a), and maintained for 5s(b), then decreased steadily(c); (B) The indentations (the arrow) of the hardness test observed under SEM.

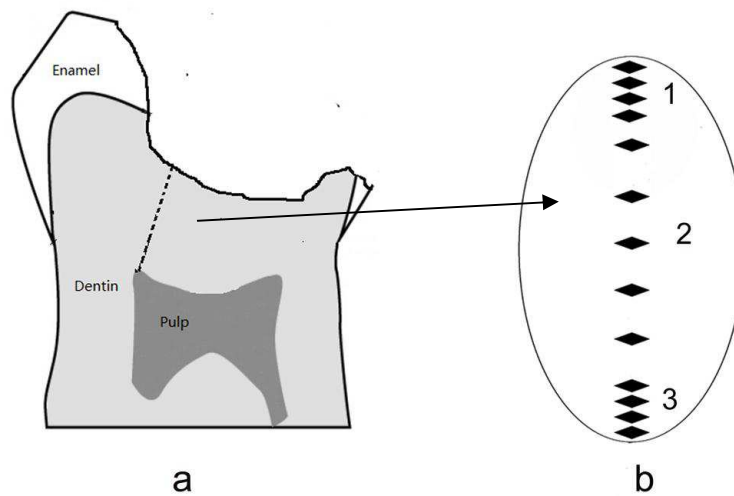


Figure 4.4 a) A line of indentations was made from the pulp across the sound dentin to the cavity floor. b) Based on the average distance between two indentations, the line was divided into 3 segments.

4.2.4 Statistical analysis

Both the Shapiro-Wilk test and graphical plot tests showed that the microhardness values of the sound dentin were normally distributed, but the absolute and relative microhardness values of the cavity floor in the FACE group were not normally distributed. The comparison of sound dentin between the FACE and conventional caries excavation groups was conducted using a Student's t test. Both the absolute and relative microhardness values of the cavity floor were analyzed with the Mann-Whitney U test. All of the statistical analyses were conducted using SPSS, version 11.0 (IBM SPSS, Chicago, Illinois, USA), and the significance level was set to 5% for all analyses.

4.3 Results

Figures 4.5 shows the line plots of the Martens hardness values from the two groups. The mean Martens hardness and the calculated Vickers equivalent of the cavity floor and the sound dentin, as well as the relative microhardness of the cavity floor are shown in Tables 4.1 and 4.2. The results indicated that neither the Martens hardness ($p=0.432$) nor the Vickers

equivalent ($p=0.518$) of the sound dentin showed significant difference between the two groups. Both the absolute Martens hardness and Vickers equivalent of the cavity floor in the FACE group were significantly lower than those in the conventional excavation group (both $p<0.00001$). Using either Martens hardness or Vickers equivalent as the statistical parameter, the relative microhardness of the cavity floor in the conventional excavation group was significantly higher than that in the FACE group (both $p<0.00001$).

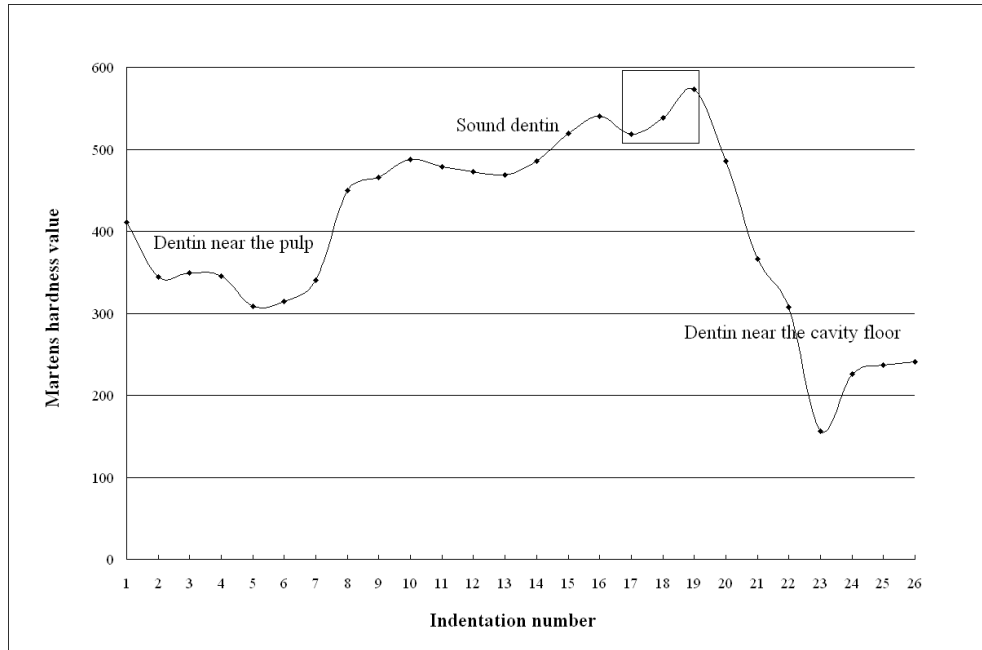
Table 4.1 Mean and standard deviation (SD) of the Martens hardness of the cavity floor and the sound dentin after excavation.

Group	Microhardness of the cavity floor (N/mm ²)	Microhardness of the sound dentin (N/mm ²)	Relative microhardness of the cavity floor (%)
FACE n=20	224±93*	495±81	46±17*
Conventional Excavation n=20	412±75	513±60	81±14

Table 4.2 Mean and standard deviation (SD) of the Vickers approximation of the cavity floor and the sound dentin after excavation

Group	Microhardness of the cavity floor (kg/mm ²)	Microhardness of the sound dentin (kg/mm ²)	Relative microhardness of the cavity floor (%)
FACE n=20	23±11*	57±10	41±17*
Conventional Excavation n=20	47±10	59±8	81±16

(A)



(B)

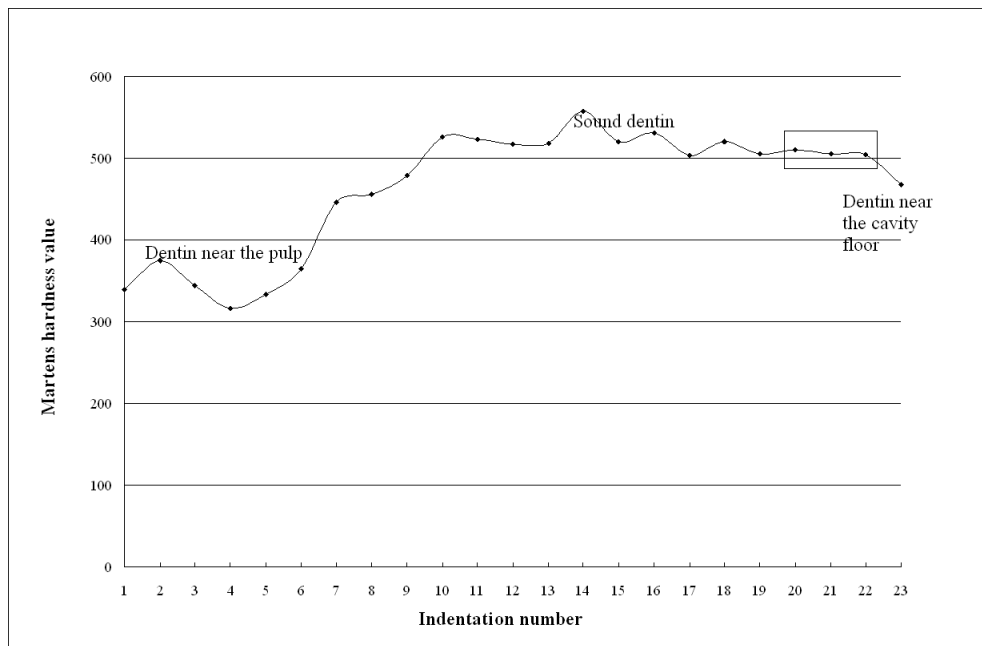


Figure 4.5 (A) A representative plot of the Martens hardness values after excavation with FACE. The three indentation points that are marked on the graph within the frame were chosen to calculate the mean value, which is regarded as the control value. (B) A representative plot of the Martens hardness values from the conventional excavation group. Again, the three indentation points that were chosen to calculate the control value for sound dentin are marked with a box.

4.4 Discussion

In the past, hardness tests were performed on sound and carious tooth tissues (Chuenarrom et al., 2009; Craig and Peyton, 1958; Fuentes et al., 2003; Fusayama et al., 1966; Marshall et al., 2001; Ogawa et al., 1983). The most commonly used approaches were the Knoop and Vickers hardness tests. In more recent publications, nanoindenters have also been used to evaluate the nanomechanical properties of tooth tissue.

The optical evaluation of the Knoop and Vickers indenter impressions is subjective when transparent materials, such as dentin and enamel, are evaluated (Shahdad et al., 2007). In addition, at lower loads, the already small measurement errors increase the data variation. The major shortcoming of the Knoop and Vickers hardness tests is that the measurements are performed after unloading the indenter. In particular, viscoelastic materials exhibit a time-dependent elastic recovery, which leads to additional variations in the results (Shahdad et al., 2007). At present, nanoindenters with liquid cells have been demonstrated as a better alternative (Marshall et al., 2001). Their high price, however, limits their availability. Automatic hardness testers, which measure Martens hardness, are another viable alternative and are recommended for hardness testing by some researchers (Shahdad et al., 2007). Although Martens hardness test devices cannot keep samples hydrated, they are superior to the Knoop or Vickers testers, as the applied load is much lower, and both the speed and precision of the measurements are higher. The Martens hardness test shares the same indenter geometry with the Vickers hardness test. It measures the depth of the indentation while the sample is under a test load and therefore includes the elastic and plastic deformations (Wilde and Wehrstedt, 2000). Thus, the Martens hardness test is less affected by the viscoelastic behavior of the materials and lacks the limitations of an optical measurement (Wilde and Wehrstedt, 2000).

Depending on the location of the measurement, the dentin hardness varies because of different mineralizations as well as the density and orientation of the dentin tubules. The dentin hardness values at locations close to the pulp were significantly lower compared to other areas (Craig and Peyton, 1958; Fusayama et al., 1966). The cavities in this study were

preselected to ensure that the cavity floor after excavation was at a comparable distance to the pulp. Even the indentation points where the control values for sound dentin were determined were at a certain distance from the pulp. Thus, the variability in the hardness values caused by the cavity location after excavation was decreased. Although all of the samples were collected without considering their histories (i.e., the age of the teeth, size of the cavity and activity status of the caries), all of which might have influenced the standardization of the excavation, the statistical results showed that there was only slight variation among the control values. In addition, the average Vickers equivalent of sound dentin (HV_{eq} 57 in the FACE group and HV_{eq} 59 in the conventional excavation group) in our experiment was close to the traditional Vickers hardness values of sound dentin (50-60) as reported by Fuentes et al. in 2003 (Fuentes et al., 2003). Moreover, each tooth was bisected into two halves and divided into two separate treatment groups to further minimize the variation.

In contrast to the clear definition of the two layers of carious dentin, changes in dentin properties, such as hardness and color, are continuous and thus do not have clear demarcation lines (Fusayama, 1993). Ahead of the bacterial invasion, acid produced by the bacteria in the biofilm penetrates the dentinal tubules and softens the surrounding dentinal substance, and demineralization starts at the junction of normal dentin and the subtransparent layer (Fusayama, 1993; Zavgorodniy et al., 2008). Because of the demineralization of intertubular dentin, the deeper carious dentin tissues, such as the transparent and subtransparent layers, has relatively high mineral concentrations due to the largely crystals filled dentinal tubules, but relatively weaker mechanical properties, including hardness and elastic modulus (Marshall et al., 2001; Zavgorodniy et al., 2008). Several studies have shown that the transparent zone can be softer than normal dentin (Fusayama, 1993; Marshall et al., 2001; Ogawa et al., 1983). As illustrated by Ogawa et al. (Ogawa et al., 1983), the lower limit of the hardness value of the transparent zone is less than half of the value of sound dentin. Clinically, the conventional method uses hardness and coloration as the main excavation criteria. It cannot objectively differentiate the inner affected dentin from the outer infected layer and cannot selectively preserve the inner affected dentin. In this study the hardness value of the cavity floor in the conventional excavation group is around 80 percent of the value of sound

dentin. This is much higher than the lower limits of the value of the transparent layer described before, which indicates that much inner affected dentin has been removed. Thus, the conventional method, which uses a dental explorer to determine whether the resultant surface is as hard as normal dentin, would already represent an overtreatment. In the FACE group, only infected carious dentin appearing as the red-orange-fluorescing region was removed. As previously described, this red-orange fluorescence is associated with the metabolites of oral bacteria (Alfano and Yao, 1981). It can be argued that FACE identifies a volume of dentin in which a high bacterial load is or was present. A high bacterial load can be considered equivalent to infected dentin. Thus, if the red-orange-fluorescing dentin is removed, the infected dentin will automatically be removed. The significantly lower hardness values of the cavity floor in this group prove that FACE offers the potential to save more dentin compared to the conventional excavation method.

FACE is a promising system for caries excavation. In addition to the tissue-preserving property of FACE as demonstrated in this study, other studies have demonstrated that FACE is more effective in removing bacteria-infected dentin without significantly increasing cavity size (Lennon et al., 2007), and requires shorter excavation time (Lennon et al., 2006), when compared to other excavation methods. These benefits of effective removal of bacterially infected dentin achieved by this technique need to be validated by further clinical investigations.

Summary

Dental caries is a bacteria-infected disease. After applying an excitation light, the by-products of oral bacteria, mainly porphyrins, can emit visible auto-fluorescence, which can be used as a marker for bacteria-infected dentin. Thus the auto-fluorescence signals have been employed as an aid for the differentiation between infected and affected dentin and monitoring caries excavation. In 2002 fluorescence-aided caries excavation (FACE) was invented as an excavation device to improve the efficiency and accuracy of caries removal. This study has investigated a recent version of FACE from the company W&H Dentalwerk Buermoos GmbH (Austria).

The study was divided into three parts:

1. The carious dentin indicated by FACE was compared with the lesion stained with Caries Detector dye using planimetry. The statistical results showed there was no significant difference between the areas of carious lesion marked using FACE and stained with Caries Detector, as well as the depth/thickness of the carious lesion in the two groups. Based on logical deduction, the clinical benefits which have been reported on Caries Detector could be expected from FACE. It is important to note that there was no arbitrary value in FACE group, which might indicate FACE has high reproducibility and the advantage of less false positive diagnosis.

2. The mineral concentration of the treated dentinal surface after caries removal with FACE and conventional excavation was compared using micro-computed tomography (microCT), a non-invasive and non-destructive technique for experimental objects. The mineral concentration of superficial dentin after using FACE was significantly lower than that with conventional excavation method. With some limits, it was very hard to compare the data with those from previous studies. However, under the conditions of this part, the results could imply that FACE is more conservative than conventional excavation method.

3. As hardness is one of the classic criteria of the conventional excavation method still widely used, this part compared FACE and conventional excavation based on the Martens and Vickers hardness of dentin at the cavity floor after caries removal. The microhardness values

of the cavity floor after FACE were significantly lower than values obtained using conventional excavation. Based on an illustration of the relationship between hardness values and different layers of carious dentin by Ogawa et al. (Ogawa et al., 1983), FACE showed the tissue-preserving property and was more conservative than conventional excavation.

Results showed in this study along with previous studies may imply that FACE is a promising device for caries excavation. However, a potential limitation of FACE was observed. During the sample collection, it was found that not every tooth with carious lesion could be detected in the form of the FACE signals, which would also cause false negative results in the clinical situation. So far it is impossible to find an explanation for this observation, as the teeth were collected at different sites and the history of every individual tooth was unknown. Besides, some researchers or dentists may doubt the practical applicability of FACE and complain about the inconvenience when using FACE, such as the treatment room needs to be darkened and dentists need to wear goggles. If the clinical studies in the future could validate its good efficiency and conservative property, the inconvenience would be just a small sacrifice when compared to the benefits of tissue preservation or even keeping the pulp vital by avoiding unnecessary pulp exposures.

Zusammenfassung

Karies ist eine bakterielle Infektionskrankheit der Zähne. Nach Aufbringen von Anregungslicht können die Nebenprodukte von Bakterien, wie die Porphyrine, Primärfluoreszenzen emittieren, die als Marker für das Bakterien infizierte Dentin verwendet werden. Deshalb ist dieses Autofluoreszenzsignal als Hilfe zur Unterscheidung zwischen infiziertem und betroffenem Dentin und Überwachung für die Kariesexkavation benutzt geworden. Im Jahr 2002 wurde "fluorescence-aided caries excavation (FACE)" erfunden, um die Effizienz und Genauigkeit der Kariesexkavation zu verbessern. Eine aktuelle Version von FACE von der Firma W&H Dentalwerk Bürmoos GmbH (Österreich) wurde in dieser Studie untersucht.

Die Studie wurde in drei Teile aufgeteilt:

1. Die Dentinkaries, die durch FACE angedeutet wird, wurde mit der Läsion, die mit dem Farbstoff "Caries Detector" gefärbt wird, mit Planimetrie verglichen. Die statistischen Ergebnisse zeigten, dass es keinen signifikanten Unterschied zwischen dem Bereich der kariösen Läsion in den beiden Gruppen, sondern auch der Tiefe/Dicke der kariösen Läsion gab. Als logische Folgerung könnten die klinischen Vorteile, die Caries Detector hat, von FACE erwartet werden. Es ist wichtig zu beachten, dass es keine beliebigen Werte in der FACE-Gruppe gibt, die zeigen, dass FACE eine hohe Reproduzierbarkeit und den Vorteil der weniger falsch-positiven Diagnose haben könnte.

2. Die Mineral-Konzentration der behandelten Dentinoberfläche nach der Kariesentfernung mit FACE und der konventionellen Exkavation wurde mit Mikro-Computertomographie (Mikro-CT) gemessen und verglichen. Mikro-CT ist eine nicht-invasive und nicht-destruktive Technik für experimentelle Objekte. Die Mineral-Konzentration des oberflächlichen Dentins in der FACE-Gruppe war deutlich niedriger als die in der konventionellen Exkavation-Gruppe. Bei einigen Beschränkungen, ist es sehr schwer, die Daten mit denen aus früheren Studien zu vergleichen. Trotzdem implizieren die Ergebnisse dieses Teils, dass FACE konservativer als konventionelle Exkavation ist.

3. Weil Härte eine der klassischen Kriterien der konventionellen Exkavation ist, wurden die Martens und Vickers-Härte des oberflächlichen Dentins nach der Kariesentfernung zwischen mit FACE und konventionellen Exkavation verglichen. Die Mikrohärtewerte des oberflächlichen Dentins in der FACE-Gruppe waren signifikant niedriger als die Werte in der konventionellen Exkavations-Gruppe. Basierend auf einer Darstellung der Beziehung zwischen Härte und den unterschiedlichen Schichten der Dentinkaries von Ogawa et al. (Ogawa et al., 1983) zeigten die Daten, dass FACE den Charakter der Gewebekonservierung hatte und konservativer als konventionelle Exkavation in dieser Studie war.

Die Ergebnisse in dieser Studie, zusammen mit früheren Studien implizieren, dass FACE ein vielversprechendes Gerät für Kariesexkavation ist. Jedoch wurde eine mögliche Einschränkung der FACE beobachtet. Als die Zähne gesammelt wurden, hatte nicht jeder Zahn mit Karies Primärfluoreszenz emittiert, die zu falsch-negativen Diagnosen aus klinischer Sicht führen würden. Es ist unmöglich, eine Erklärung für diese Beobachtung zu finden, weil die Zähne an unterschiedlichen Standorten gesammelt wurden, und wir nicht die Geschichte jedes einzelnen Zahnes wussten. Außerdem können einige Forscher oder Zahnärzte an der praktischen Anwendbarkeit von FACE Zweifel haben und sich über die Unannehmlichkeiten bei der Verwendung von FACE beschweren, weil das Behandlungszimmer abgedunkelt werden müsste, und Zahnärzte eine Schutzbrille tragen müssten. Falls die klinischen Studien in der Zukunft die gute Effizienz, die Effektivität, und die Gewebekonservierung von FACE überprüfen, sind die Unannehmlichkeiten nur ein kleines Opfer.

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