Cariological Studies on Endodontically Treated Teeth

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UNIVERSITY OF GOTHENBURG

وزارة التعليم العالي
Ministry of Higher Education

MINISTRY OF HIGHER EDUCATION SAUDI ARABIA

Gothenburg 2011

Abstract

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Caries might jeopardize the long-term successful outcome of endodontic therapy. Therefore, it is of an interest for the endodontist to evaluate caries susceptibility of root-filled teeth (RFT). In the present thesis, several studies were conducted to explore this relationship. In the first study, caries risk profile of 200 Saudi adults, using the Cariogram, and the frequency of recurrent caries in RFT were evaluated. All individuals were interviewed about their oral health, dietary habits and use of fluoride. Caries was registered both clinically and radiographically. Salivary and microbiological data were obtained using chair-side tests. The findings from this study did not show any significant difference in caries risk profile, at the individual level, except for the mutans streptococcus count. A significant difference was detected, however, in the proportion of recurrent caries, which was higher in RFT compared to vital teeth. Caries susceptibility of RFT can be attributed to both extrinsic and intrinsic factors. In the second study, caries susceptibility of RFT was compared with contra-lateral non-root-filled teeth (NRFT) regarding plaque-related factors. This study was carried out on a sub-sample (20 patients) with two or more RFT, recruited from the participants in the first study. Each patient was examined regarding cariogenic microflora of proximal plaque, in situ plaque pH-drop after a sucrose rinse (the Stephan curve) and de novo plaque formation. Recurrent caries and the quality of the coronal fillings/crowns of the teeth were also evaluated. The results showed that the endodontically treated teeth had an increased susceptibility to caries, ascribed either to alteration in their biological environment, or to inadequacy of the marginal fit of the dental restoration. In the third study, the frequency of recurrent caries in RFT versus NRFT was evaluated, retrospectively. The material consisted of totally of 11,554 teeth in 832 subjects, pooled from a large cross-sectional epidemiological study conducted in Jönköping, Sweden. The findings showed a significant association between endodontically treated teeth and recurrent caries. The fourth study assessed the effects of sodium hypochlorite (NaOCl), ethylenediaminetetraacetic acid (EDTA) and chlorhexidine (CHX) in various strengths and combinations on the demineralization of dentin, considering their use as irrigation solutions. Thirty-five single-rooted teeth were extracted and randomly allocated into seven groups. The teeth were analyzed with micro-computed tomography (micro-CT), before and after the treatment. Volume measurements, to assess the demineralization effect, were carried out with software. The data showed that NaOCl and EDTA irrigation solutions changed the quality of dentin, in a way that it may increase the caries susceptibility. To conclude, the results from this thesis should raise the awareness among dental clinicians regarding the potential increase in caries risk following endodontic treatment, and accordingly, precautionary measures should take place.

Key Words: Caries risk. Caries susceptibility. Cariogram. Endodontic treatment. Jönköping, Sweden. Micro-CT. Recurrent caries. Saudi Arabia.

ISBN: 978-91-628-8239-6

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Contents

Original papers

This thesis is based on the following four papers, which are referred to by their Roman numerals in the text:

I. Merdad K, Sonbul H, Gholman M, Reit C, Birkhed D. Evaluation of the caries profile and caries risk in adults with endodontically treated teeth. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;110:264-269.

II. Merdad K, Sonbul H, Bokhary S, Reit C, Birkhed D. Caries susceptibility of endodontically versus nonendodontically treated teeth. J Endod 2011;37:139-142.

III. Frisk F, Merdad K, Reit C, Hugoson A, Birkhed D. Root-filled teeth and recurrent caries - a study of three repeated cross-sectional samples from the city of Jönköping, Sweden. Submitted.

IV. Merdad K, Al-Hezaimi K, Al-Fouzan K, Birkhed D, Reit C. Micro-computed tomography (micro-CT) analysis of the effect of different irrigation solutions on dentin quality. In manuscript.

Introduction

Dental caries is of interest for the endodontist, since it is considered to be the main cause of irreversible pulp inflammation and subsequent treatment (1). After endodontic treatment, it contributes to coronal leakage; a possible cause for failure of endodontic treatment (2). Moreover, recurrent caries is considered to be a threat to the longevity of root-filled teeth (RFT) (3, 4). Recently, non-restorable carious destructions were reported as the main reason for extraction of RFT (5).

The focus of this thesis was “whether endodontically treated tooth is more susceptible to develop caries or not?” To answer this question, one has to understand the caries process, via the pulpal response to a caries lesion, and the possible changes of the dentin after endodontic treatment. Other important questions to answer for a better understanding of the disease are: “how does the caries lesion threaten the outcome of endodontic treatment, “what causes a tooth to be susceptible to caries” and “what are the characteristics of root-filled teeth from a cariological point of view”

What is dental caries?

Dental caries takes place in the tooth surface-adherent biofilm and is caused by acid-producing (cariogenic) bacteria. The acid-producing bacteria in the dental plaque metabolize fermentable carbohydrates, such as sucrose, fructose, glucose, and starch (6). The process entails a constant back-and-forth demineralization and remineralization between the tooth and the surrounding saliva.

Dental hard tissues (enamel and dentin) are sensitive to low pH levels. Demineralization takes place when the pH at the surface of the tooth drops below 5.7 (enamel) or 6.2 (dentin). This may be illustrated by the so called “Stephan curve” (Fig. 1) (7). Initially, demineralization may be reversed by remineralization from calcium and phosphate in saliva. However, if the acidic conditions persist for along period of time, with repeated consumption of sugars and/or impaired salivary flow, a caries lesion will develop (8).

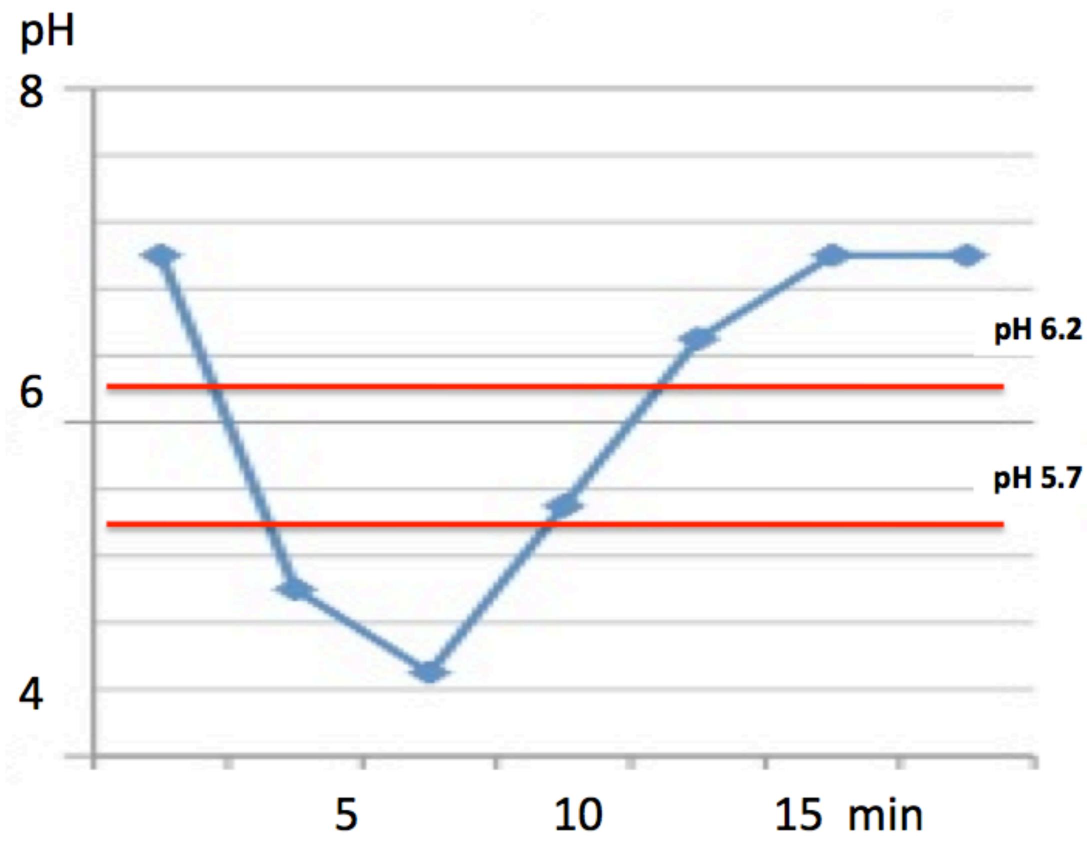


Figure 1. Stephan curve named after its "inventor" (7). It indicates changes in the hydrogen ion concentration on tooth surfaces. Only after a few minutes, pH may drop below the "critical pH" (red lines) i.e. a level at which tooth is demineralized (around pH 5.7 for enamel and 6.2 for dentin).

Pulp tissue has considerable reparative potential, particularly in young teeth with open apices and a good blood supply (9). However, the caries process can lead to marked changes within the pulp-dentin complex, which can vary considerably depending on the severity of the disease and the age of the pulp. Early in the caries process, the pulp reflects changes within the lesion. Thus, the initial pulp response is reversible. Later, the progression rate of caries is manifested by the quality of the dentin. Slowly progressing lesions create “tertiary dentin” resembling normal tubular dentin. Rapidly progressing lesions lead to the production of a tubular dentin or complete absence of tertiary dentin, as well as pulp necrosis and apical pathology might occur (10).

It has been reported that different immunoglobulins are produced in response to the destructive stimuli of caries, particularly when bacterial invasion reaches the dentino-enamel junction (CEJ) (11). The source of these antibodies is blood supply of the pulp, which further supports the role of the dental pulp in caries susceptibility (12). Also, studies have shown that suppression of dentinal fluid transport, significantly increased dental caries, whereas normal fluid transport was associated with little or no caries (13, 14).

It seems to be accepted that if the pulp has been eliminated, all physiological reactions, including tertiary dentin formation, will be inhibited and the histological picture and the progression rate of caries might be different. Recently, in a pilot study, Bjørndal et al. (unpublished) showed different histological caries pattern associated with root-filled teeth (Fig. 2).

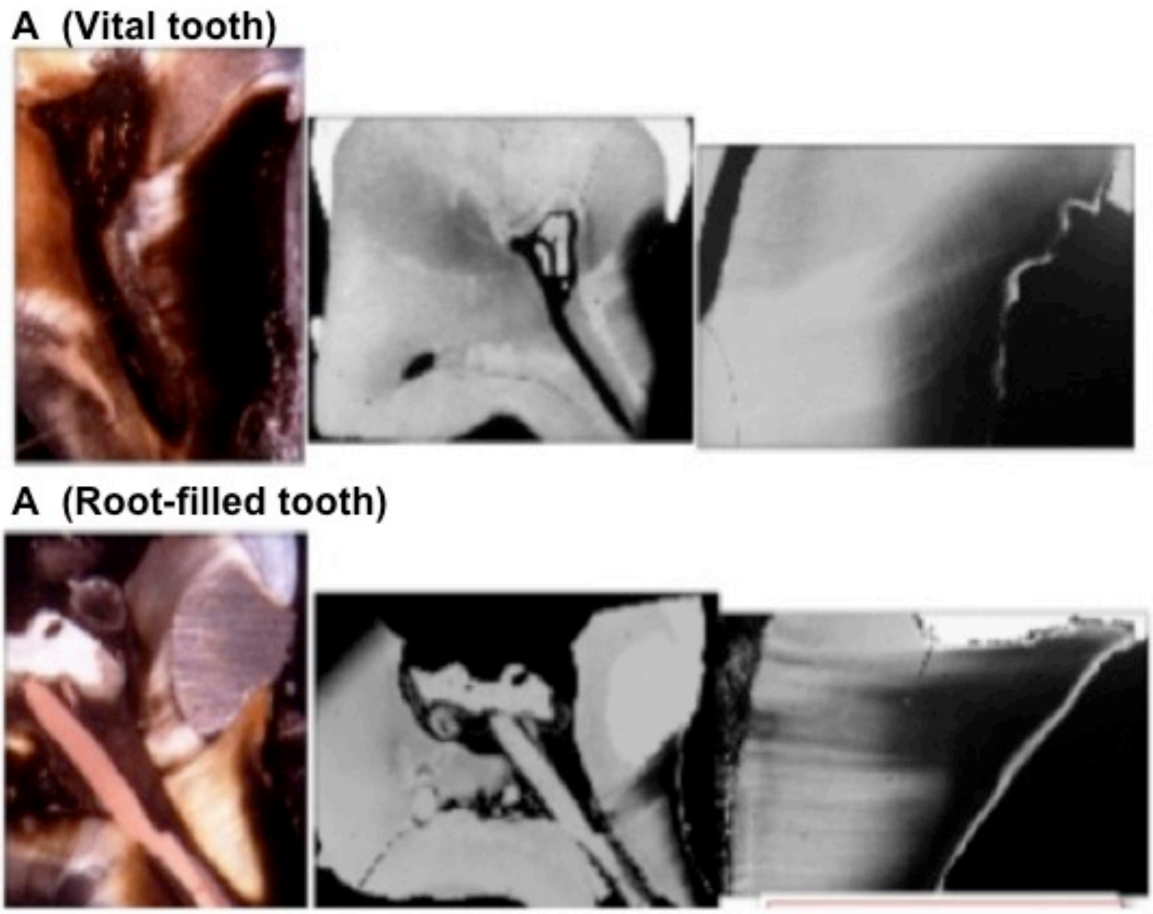


Figure 2. Longitudinal histological sections shows different caries pattern A) vital tooth, caries pattern is confined by tertiary dentin B) root-filled tooth in the same patient is a aggressively invading the tubules without dentin reaction. Courtesy Lars Bjørndal, Copenhagen.

What are the expected changes when the pulp is eliminated?

Endodontic treatment may result in changes in tooth structure due to both *extrinsic* and *intrinsic* factors. The extrinsic factors include, for example, changes in plaque accumulation, tooth surface tension, number of acid-producing microorganisms, and pH level in the dental plaque. Intrinsic factors that might influence the progress of the caries lesion, include defense mechanisms provided by the vital dental pulp as well as factors altering the dentin quality after root canal treatment (15, 16).

The literature is rich with examples on the role of pulp in the development and progression of dental caries. Animal and human studies have confirmed that the physiologic activity of the dentin-pulp complex has an effect on the overall health of the tooth (13, 17, 18). Loss of pulp vitality deprives the dentin of several defence mechanisms, such as the ability to deposit tertiary dentin and the production of antibodies against caries-related microorganisms. Additionally, loss of the intra-pulpal pain signalling system, which makes it possible for a lesion to progress undetected for a long period of time, changes in tooth moisture, and presence of microorganism within the root canal may also affect the caries process in dentin.

Internal factors related to endodontic treatment procedure include loss of tooth structure, cracks after cavity preparation, and lack of integration between the root canal filling and the coronal filling. In addition, materials used during endodontic treatment, such as sodium hypochlorite (NaOCl), chelators, and zinc oxide eugenole, can negatively affect the bonding strength of the restorative material and consequently, increas the risk for recurrent caries.

Caries susceptibility in root-filled teeth

The effect of pulp on caries susceptibility is controversial, Brewer et al. (17) found that ligation of blood vessels significantly increased dental caries in the rat. Another study showed that root canal treatment decreased dental caries (19). Mascres et al. (18) found that hindered vascularisation increased caries, when compared to teeth with a normal blood flow in same animals. They concluded that reduced blood supply increased the incidence of caries.

How caries affect the outcome of endodontic treatment

Studies have shown that microorganisms from the carious lesion can penetrate into the root canal seal and cause or sustain an existing apical inflammation following root canal treatment (20-22). It is generally accepted that the outcome of endodontic treatment affects the quality both of the root filling and the coronal fillings. Ray and Trope (23) suggested that the quality of the coronal restoration have an impact on the periapical health of RFT. Zadik et al. (5) reported that extractions of endodontically treated teeth were attributed mainly to deep carious lesions. This is not surprising, since caries is the most common reasons of tooth loss in general (4, 5). The lack of pulpal sensation often allows the carious process to continue without the patient seeking dental care.

Caries risk assessment and use of Cariogram

Dental caries is a multifactorial disease, caused by an interplay of several factors, including past caries experience, oral hygiene, use of fluoride, dietary habits, cariogenic bacteria, and the saliva (24). The risk of developing caries varies from one individual to another and is related to the balance between the various "attacking" and "resistant" factors. All these factors have been studied using different pedagogic models; the most recent is a computer-based program developed by Bratthall (25), referred to as the Cariogram. This interactive program analyzes different caries-related factors (Table 1) and presents the results as a pie chart, illustrating the "the chance of avoiding caries" as a percentage value (Fig. 3), which represent individual caries risk profile (26).

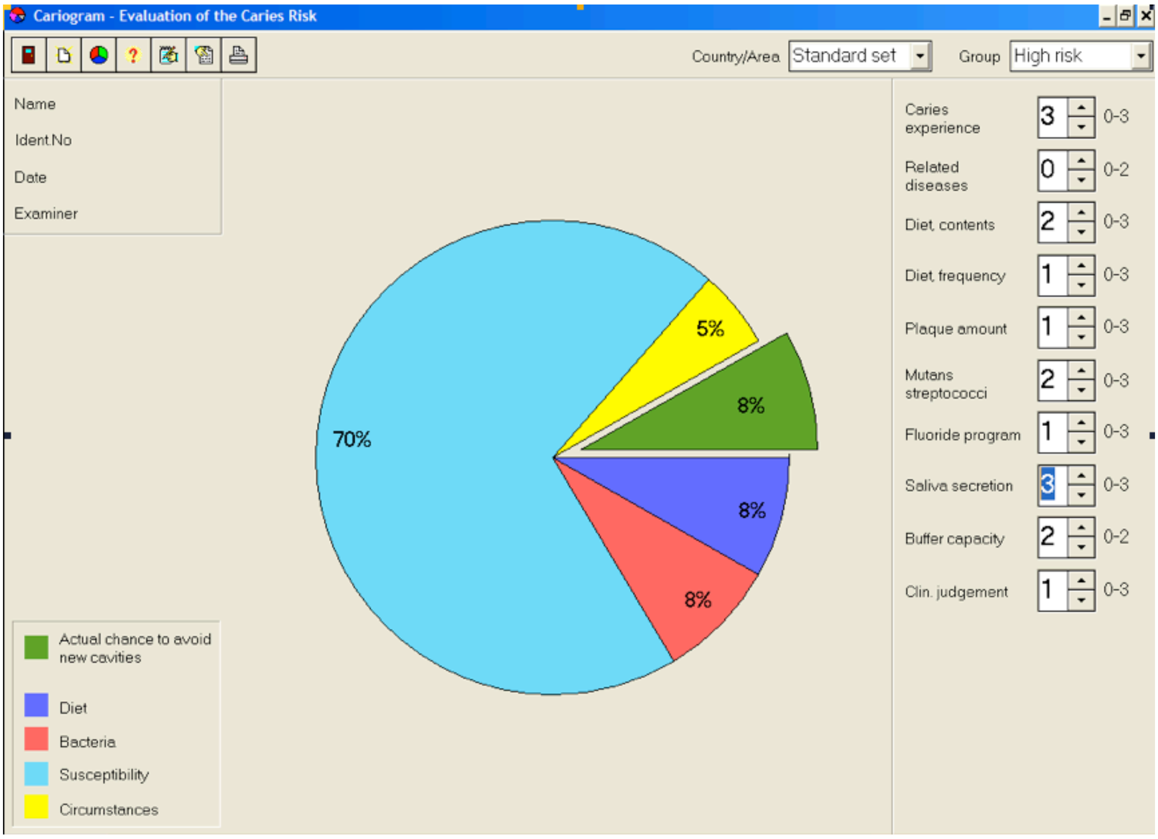


Figure 3. A Cariogram (as it appears in the computer) showing a high-risk patient with a low percentage (8%) of ‘‘actual chance of avoiding new cavities’’ (green sector). On the lower left, the five Cariogram sectors are explained in different colors. On the right, all nine factors plus clinical judgment are giving a score from 0 to 2 or 0 to 3.

The program has been validated as a prediction model and a significant correlation has been found between the Cariogram results and the caries increment over time in both children, elderly and orthodontic patients (27-33). A recent study found that the risk assessment using the Cariogram is in agreement with the opinions of dentists and dental hygienists (34). No studies have used Cariogram specifically to evaluate the caries risk in endodontically treated patients.

Table 1. Caries-related factors and the data needed to create a Cariogram, adapted from Bratthall et al. (26).

Factor *	Comment	Information/data needed
Caries experience	Past caries experience, including cavities, fillings and missing teeth because of caries. Several new cavities definitely appearing during preceding year should give a high score even if number of fillings is low	DMFT, DMFS, new caries experience in the past year
Related diseases	General disease or conditions associated with dental caries	Medical history, medications
Diet, contents	Estimation of the cariogenicity in food, in particular sugar contents	Diet history, lactobacillus count
Diet, frequency	Estimation of number of meals and snacks per day, mean for 'normal days'	Questionnaire results, 24-hour recall or dietary history (3 days)
Plaque amount	Estimation of oral hygiene: for example, according to Silness-Löe Plaque Index (PI). Crowded teeth leading to difficulties in removing plaque interproximally should be taken into account	Plaque index
Mutans streptococci	Estimation of levels of mutans streptococci (Streptococcus mutans, Streptococcus sobrinus) in saliva	Strip mutans test or other laboratory tests giving comparable results
Fluoride programme	Estimation of to what extent fluoride is available in the oral cavity over the coming period of time	Fluoride exposure, interview patient
Saliva secretion	Estimation of amount of saliva: for example, using paraffin-stimulated secretion and expressing results as millilitre saliva per minute	Stimulated saliva test (secretion rate)
Saliva buffer capacity	Estimation of capacity of saliva to buffer acids	Dentobuff or other laboratory tests giving comparable results

*For each factor, the examiner gathers information by interviewing and examining the patient, including salivary tests. Each factor is given a score, ranging from 0 to 3 (or 0 to 2) according to predetermined criteria. The score 0 is the most favourable value and the maximum score 3 (or 2) indicates a high, unfavourable value.

Hypotheses

- The hypotheses of the first study were that: (1) at *patient* level, individuals with multiple RFT are at a higher caries risk than individuals without RFT, and (2) at *tooth* level, RFT are at a higher caries risk than NRFT.

- The hypothesis of the second study was that there is a difference in caries susceptibility between RFT and NRFT.

- The hypothesis of the third study was that RFT are at a higher caries risk than NRFT.

- The hypothesis of the fourth study was that there is difference in the effects of NaOCl, EDTA and CHX on demineralization of dentin when used as irrigation solutions.

Aims

The present thesis consists of four parts. The first and second parts evaluate the caries risk profile using the Cariogram, the frequency of recurrent caries, and the caries susceptibility of RFT versus non–root-filled teeth (NRFT) in a Saudi population (Papers I & II). The third part examines the frequency of recurrent caries in RFT versus NRFT in a Swedish population (Paper III). The fourth part evaluates the effect of endodontic irrigants on dentin using micro-computed tomography (micro-CT), (Paper IV). The specific aims of this thesis were:

- to compare the caries risk profile of individuals with a minimum 2 RFT versus individuals without root fillings using the Cariogram, and to compare the frequency of recurrent caries in RFT versus NRFT (Paper I),

- to evaluate the caries susceptibility of RFT vs. NRFT in relation to dental plaque-related factors (Paper II),

- to compare the frequency of recurrent caries in RFT versus NRFT in three large Swedish epidemiological samples, obtained in 1983, 1993 and 2003, respectively (Paper III), and

- to assess the effects of sodium hypochlorite (NaOCl), ethylenediaminetetraacetic acid (EDTA), and chlorhexidine (CHX) on the demineralization of dentin, considering their use as irrigation solutions (Paper IV).

Material and Methods

An outline about the four studies design and topic are illustrated in Table 2.

Table 2. The four papers (I-IV) included in the present thesis.

Study	Design	Population	Title
I	Cross-sectional	200	Caries risk profile using the Cariogram model in Saudi adults with endodontically treated teeth
II	In-situ study	20	Caries susceptibility of endodontically versus non-endodontically treated teeth in Saudi adults
III	Cross-sectional	832	Root-filled teeth and recurrent caries – a study of three repeated cross-sectional samples from Jönköping, Sweden.
IV	In-vitro study	35	Micro-computed tomography (micro-ct) analysis of the effect of different irrigation solutions on dentin quality

Studies I & II

Study Population

The population was selected from a randomized list, using the permuted block strategy of adult patients, attending the screening clinic at the Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia. Of 612 patients attended, 200 were selected and divided into two groups of 100 each. In Paper I, individuals allocated to the endodontic group (EG) had a minimum of two endodontically treated teeth, while individuals in the control group (NEG; non-endodontic group) had no endodontically treated teeth. Medically compromised patients, pregnant women and nursing mothers, and individuals with only one root filled tooth were excluded from the study. Paper II was carried out on a sub-sample (20 patients) recruited from the EG. The population represents middle socio-economic Saudi adult patients. Patients who met the inclusion criteria signed a consent statement. The study protocols for both studies follow the ethical rules of research, with the general principles described in the Helsinki declaration (35). These studies were approved by the local ethics committee at King Abdulaziz University.

Baseline data

Figure 4 outlines the baseline data collected for Papers I & II including interviews, bitewing radiographs, photographs, plaque scores, salivary tests, and caries registration. De novo plaque formation, cariogenic microflora and pH-drop in proximal plaque in situ (Stephan curve). The quality of the coronal fillings/crowns of these teeth, including recurrent caries, were also examined in patients who participated in Paper II.

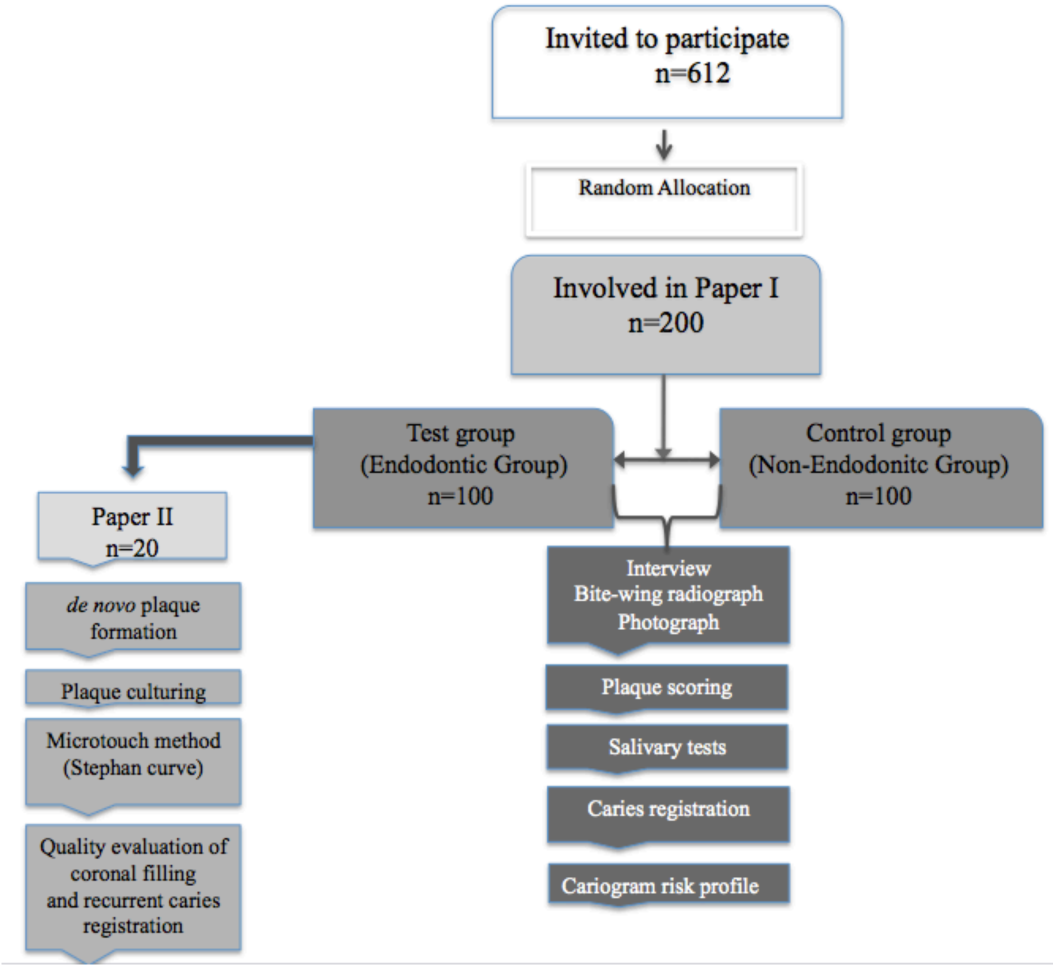


Figure 4. Study I and II CONSORT flow chart.

Questionnaire

Patients were interviewed using the standardized structured questionnaire, described in the Cariogram manual (36). Information on medical and dental history, dietary habits, and use of fluoride products were also collected.

Plaque index

Before thorough cleaning and saliva sampling, Plaque Index (PI) was scored, according to Silness and Löe (37) (Table 3). Four surfaces of six teeth were examined (16, 12, 24, 36, 32 and 44). No disclosing solution or tablet was used in order not to interfere with the caries registration.

Table 3. Plaque Index according to Silness and Löe (37).

Score	Criteria
0	No plaque
1	A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen in situ only after application of disclosing solution or by using the probe on the tooth surface.
2	Moderate accumulation of soft deposits within the gingival pocket or on the tooth and gingival margin, which can be seen with the naked eye.
3	Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin.

Salivary tests

Paraffin-stimulated whole saliva was collected for five minutes and the secretion rate expressed as ml/min. The saliva was analysed regarding buffer capacity and number of mutans streptococci and lactobacilli, using chair-side tests (CRT, Ivoclar-Vivadent, Schaan, Liechtenstein). The buffer capacity was determined using CRT Buffer (Ivoclar-Vivadent).

Clinical recording of caries

Teeth were cleaned with a rubber cup, pumice and dental floss. The teeth were then dried with compressed air and then examined using a mirror, number 17 explorer (Zepf, Seitingen, Germany) and standard light.

Caries was scored according to the criteria described by the World Health Organization (WHO) (38) (Fig. 5). Consequently, the number of decayed, missing and filled tooth surfaces (DMFS) were calculated for each patient. Molars (excluding third molars) and premolars were considered to have 5 surfaces and remaining teeth having 4 surfaces. Caries in a filled surface was scored as recurrent caries. Crowned tooth was scored as five filled surfaces. Laminate veneered tooth was considered one surface filled.

D1: clinically detectable enamel lesions with intact (non-cavitated) surfaces.

D2: clinically detectable cavities limited to the enamel.

D3: clinically detectable lesions in dentin (with and without cavity).

D4: lesions into pulp.

Figure 5. Caries index adapted from WHO criteria (42)

Radiographic recording of caries

Four bitewing radiographs were taken to score approximal caries. For primary caries, the Gröndahl's index was used (39) (Fig. 6). Recurrent caries was diagnosed as present or absent. All surfaces from the distal surface of the first premolar to the mesial surface of the second molars (24 surfaces/patient) were evaluated using a light desk and magnifying viewer. The kappa value for the radiographic recordings, based on 20% of the patients, was 0.90.

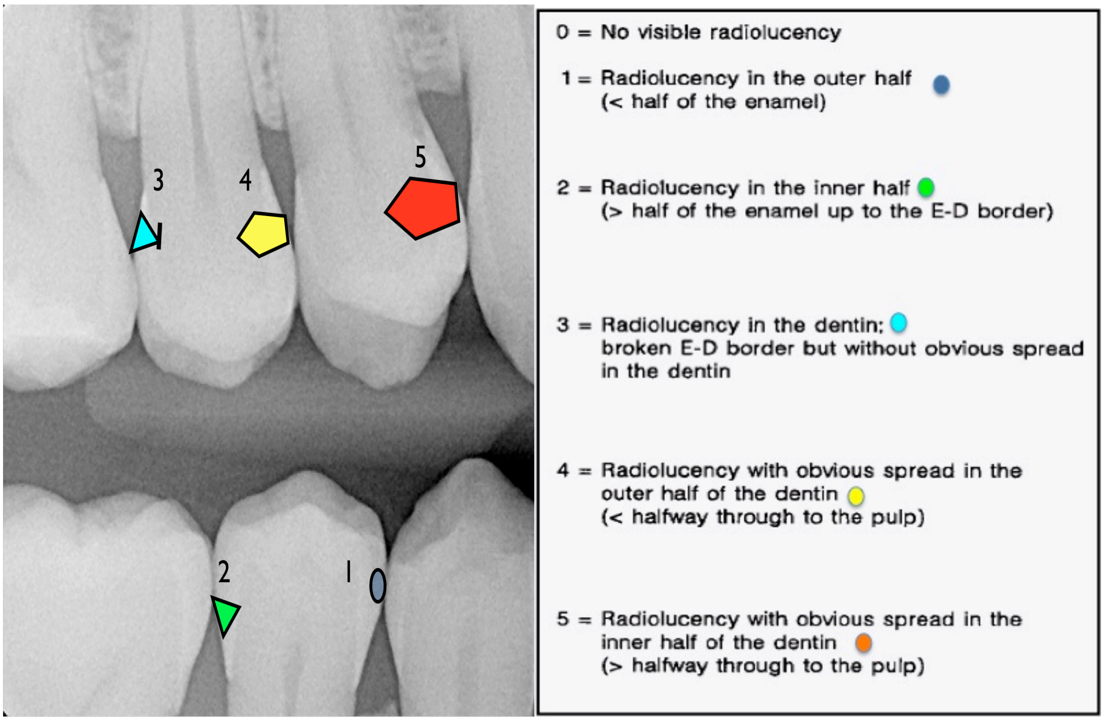


Figure 6: Gröndahl´s index adapted from Mejàre et al. (40).

Assessment of caries risk profile (Cariogram)

The Cariogram program (Fig. 3), with a built-in algorithm, creates an individual caries risk profile (26). Data on nine relevant caries-related factors are scored and entered into the program (Table 4). The scores are based on a numeric scale from 0 to 3 (or 0 to 2), with 0 as the most favourable score. The factor "Clinical judgment" was set to 1 in all patients. The individual caries profile was estimated and presented in a pie chart with five sectors, expressed in percentages: 1) “diet”, based on a combination of sugar intake and number of lactobacilli (dark blue sector), 2) “bacteria”, which is a combination of oral hygiene and number of mutans streptococci (red sector), 3) “susceptibility”, including fluoride program, salivary secretion rate and buffer capacity (light blue sector), 4) “circumstances”, is the past caries experience and general diseases (yellow sector), and finally 5) “the chance of avoiding caries” (green sector).

Each patient in Study II was examined for de novo plaque formation, cariogenic microflora, pH-drop in proximal plaque in situ (Stephan curve) and the quality of the coronal fillings/crowns of these teeth, including recurrent caries.

de novo ***plaque formation***

All teeth were cleaned on Day 0, and then participants were instructed not to brush their teeth for 48 hr. At the end of this plaque accumulation period, no disclosing solution or tablet was used in order not to interfere with the plaque-pH measurements. Plaque was scored both on test and control teeth, using the Plaque Index (PI) according to Silness and Löe (37).

Plaque culturing

Plaque samples were collected from the mesial and distal proximal surfaces of all examined teeth to evaluate the levels of mutans streptococci and lactobacilli, using the CRT chair-side test (Ivoclar-Vivadent). Cotton rolls were placed in the vistibule and the tooth surface dried with compressed air. Samples were collected using the tip of a sterile wooden toothpick (TePe Röd, Munhygienprodukter AB, Malmö, Sweden) to avoid contamination. Samples were cultured for 48 hr at 37ºC. Levels of mutans streptococci and lactobacilli were scored in four classes: A) 0, B) 1-10, C) 11-100 and D) >100 colony-forming units (CFU) according to the manufacture's manual.

Table 4. Cariogram sectors, variables and their corresponding scores adapted from Al-Mulla thesis (41).

Sector	Variable	Score
Circumstances (Yellow sector)	Caries experience	0: DFS = 0, caries-free and no fillings 1: DFS = 1–2, better than normal for age group 2: DFS = 2–4, normal for age group 3: DFS > 4, worse than normal for age group
Circumstances (Yellow sector)	Related Diseases	0: No systemic disease 1: Diseases/condition, mild degree 2: Severe degree, long lasting
Diet (Dark blue sector)	Diet content, Lactobacillus count (LB)	0: LB < 10^2 CFU/mL 1: $10^2 \le$ LB < 10^3 CFU/mL 2: $10^3 \le$ LB <10^4 CFU/mL 3: LB ≥ 10^4 CFU/mL Diet
(Dark blue sector)	Diet frequency, number of intakes per day (meals and snacks)	0: Maximum 3 intakes per day 1: Maximum 5 intakes per day 2: Maximum 7 intakes per day 3: More than 7 intakes per day
Bacteria (Red sector)	Plaque amount	0: No plaque 1: Film of plaque adhering to the free gingival margins and adjacent area of the tooth 2: Moderate accumulation of soft deposits in the gingival pocket, or on the tooth gingival margins 3: Abundance of soft matter within the gingival pocket and/or on the tooth gingival margins
Bacteria (Red sector)	Mutans streptococci count (MS)	0: MS < 10^3 CFU/mL 1: $10^3 \le$ MS < 10^4 CFU/mL 2: $10^4 \le$ MS < 10^5 CFU/mL 3: MS ≥ 10^5 CFU/mL
Susceptibility sector)	Fluoride program	0: Fluoride toothpaste plus fluoride tablets, (Light blue rinsing and varnishes (frequently) 1: Fluoride toothpaste plus fluoride tablets, rinsing and varnishes (infrequently) 2: Only fluoride toothpaste 3: No fluoride
Susceptibility (Light blue sector)	Saliva secretion rate	0: 1.1 mL/min or more 1: from 0.9 to less than 1.1 mL/min 2: from 0.5 to less than 0.9 mL/min 3: less than 0.5 mL/min
Susceptibility (Light blue sector)	Saliva buffer capacity	0: pH > 5.5 1: pH < 5.5–4.5 2: pH < 4.5
	Clinical judgment	0: More positive than what the Cariogram shows based on the scores entered 1: Normal setting, risk according to the other values entered 2: Worse than what the Cariogram shows based on the scores entered 3: Very high caries risk, examiner is convinced that caries will develop, irrespective of what the Cariogram shows based on the scores entered -

Microtouch method (Stephan curve)

The Stephan curve describes the changes in dental plaque-pH in response to a challenge over time. pH was measured using the microtouch method (42). A palladium microelectrode with a diameter of 0.1 mm (Beetrode, MEPH-1, W.P. Instruments, Inc., New Haven, CT, USA), was connected to an Orion SA 720 pH/ISE Meter, equipped with a porous glass reference electrode (MERE 1, W.P. Instruments, Inc.). pH was calibrated prior to the reading of each test as described by Scheie et al. (43). The subject's finger and the reference electrode were immersed in a 3 mol/L KCl solution. Resting pH was first registered (0-min value). The electrode was inserted interdentally just apical to the contact point on the natural tooth surface without touching any filling. The patient was the asked to rinse with 10 ml of a 5% sucrose solution for one minute and pH was measured after 2, 5, and 10 min. The individual Stephan curve was plotted and the area under the curve (AUC_{0-10}) was measured at pH 6.2, using a computer program (44).

Quality evaluation index

The teeth were cleaned with rubber cup and pumice and then examined using a mouth mirror and an explorer. The quality of the coronal fillings/crowns of these teeth, including recurrent caries, was evaluated according to modified United States Public Health Service (USPHS) Ryge criteria (45), regarding marginal integrity, anatomic form, surface texture and recurrent caries presence (Table 5).

Table 5. Modified USPHS-Ryge criteria (45).

Criteria	Satisfactory	Unsatisfactory
Marginal integrity	No visible evidence of ditching along the margin.	Visible evidence of ditching along the margin in which the explorer will penetrate or catch. Visible evidence of ditching along the margin in which the explorer will penetrate, the dentin is exposed. Bottom of the cavity exposed. The restoration is movable or fractured or tooth structure fractured
Anatomic form	The restoration is continuous with existing anatomic form (contours, cusps, planes, marginal ridges and proximal contact).	The restoration slightly under or over contoured or slightly deviated from normal or functional anatomy, or the material not sufficient to expose dentin, negligible or easily adjusted. The restoration is under or over contoured severely, sufficient material is lost to expose dentin, or some deviation from normal and/or functional anatomy, cannot be adjusted. Restoration is missing partly or totally.
Surface texture	Surface restoration is smooth.	Surface restoration is slightly rough or pitted, can be refinished. Surface restoration is deeply pitted, cannot be refinished. Surface is flaking or there is fracture on the surface of the restoration.
Caries	No caries contiguous with the restoration.	Evidence of decalcification contiguous with the restoration. Caries contiguous with the restoration, loss of tooth substance.

Study III

In 1983, subjects aged 3, 5, 10, 15, 20, 30, 40, 50, 60, 70 and 80 years in the city of Jönköping, Sweden, were examined. In each age group, 130 randomly selected individuals were invited to undergo clinical and radiographic examinations. It was repeated in the same manner and in the same geographical area in 1993 and 2003. The participation rate for all these age groups was 77% in 1983, 75% in 1993 and 69% in 2003. The attendance rate for those aged 20-70 years was approximately 65-80%; for details, see Hugoson et al. (46, 47). In Study III, only dentate individuals aged 20-70 years were included (Fig. 7).

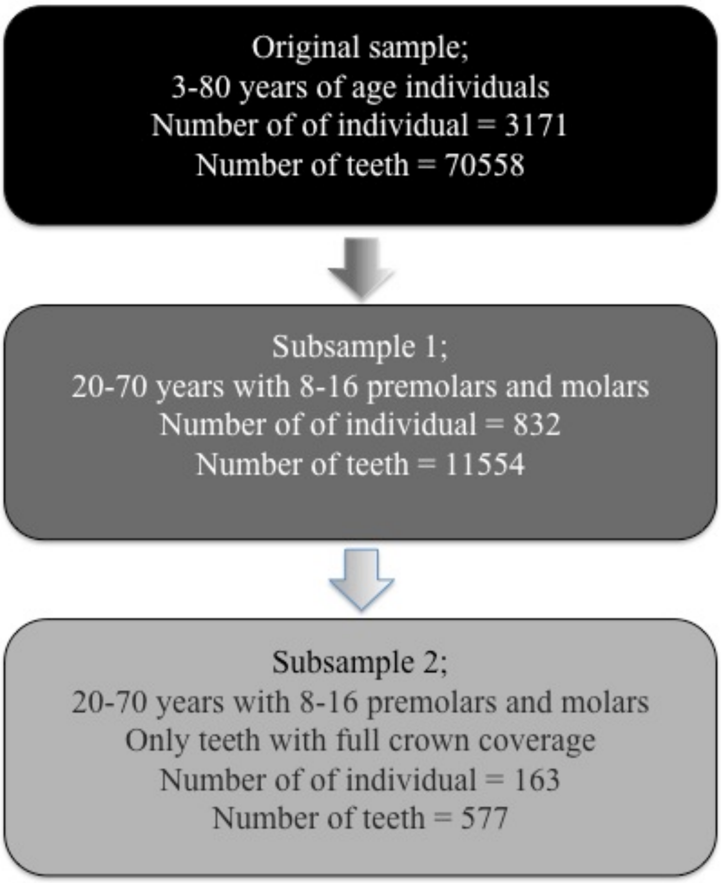


Figure 7. Study III CONSORT flow chart.

Clinical examination and diagnostic criteria

All examinations were carried out by calibrated examiners (46). Primary caries was recorded when the lesions could be verified as cavities by probing on a surface not previously filled and recurrent caries a restored surface.

Radiographic examination and diagnostic criteria

1983. Subjects aged 20-80 years were examined with both full mouth radiographic examination (FMR) and an orthopantomogram (OPG). If an individual recently had had a radiographic examination, the films were obtained from the dentist and if necessary supplemented with additional apical radiographs.

1993. In subjects aged 15-30 years, 6 bitewing radiographs and an OPG were taken. In cases with deep caries lesions and RFT, the examination was supplemented with apical radiographs. Subjects aged 40 years and older were examined with FMR and OPG.

2003. In subjects aged 10-40 years, an OPG and 6 bitewing radiographs were taken. In cases with deep caries lesions and RFT, additional periapical radiographs were taken. Subjects aged 50-80 years were examined by means of FMR, consisting of 16 periapical and 4 bitewing radiographs, as well as an OPG. Caries lesions on the radiographs were recorded when a clearly defined reduction in mineral content of the proximal surfaces could be seen. A tooth was considered endodontically treated when it was root-filled or amputated.

Study IV

Micro-CT technique

Thirty-five freshly extracted sound, single-rooted human lower premolars were used. The teeth were extracted as part of orthododontic treatment. Teeth were stored in a saline solution in a 4°C cooler. Each tooth was inserted into a customized sample holder to standardize the specimen's position during scanning, a damp sponge was placed in the sample holder to maintain a humid environment. Pulp chamber access was carried out using Endo access bur (Dentsply/Maillefer, Ballaigues, Switzerland). Then, the teeth were divided into 7 groups (five teeth each), canals were continuously irrigated using 27-gage needle attached to 10 ml syringe, the needle was inserted into the apical part of the canal.

- Group 1 rinsed only with 5.5% of NaOCl, 20 ml for 30 min
- Group 2 rinsed only with 2.25% of NaOCl, 20 ml for 30 min
- Group 3 rinsed only with 17% EDTA, 2 ml for 2 min
- Group 4 rinsed only with 2% of CHX, 10 ml for 10 min
- Group 5 (Mix 1) rinsed with a combination of 5.5% of NaOCl (20 ml for 30 min), 17% EDTA (2 ml for 2 min), saline 5 ml for 5 min, 2 % of CHX (10 ml for 10 min)
- Group 6 rinsed with a combination of 2.25% of NaOCl (20 ml for 30 min), 17% EDTA (2 ml for 2 min), saline 5 ml for 5 min, 2% of CHX (10 ml for 10 min)
- Group 7 rinsed only with saline (negative control), 20 ml for 30 min

Micro-CT scanning

All teeth were scanned over 360°, with Skyscan 1172 (Skyscan, Kontich, Belgium), using a 12 μm resolution filter (0.04mm Cu + 0.5mm Al), and 70 kV voltage.

Specimens were measured and evaluated as follows: (1) root length was measured starting from just below the CEJ up to the apical foramen and then divided into three equal parts, (coronal, middle and apical), then 70 µm horizontal cross-sectional slices were taken; and (2) each part was then divided into three regions of interest (ROI) at the same cross section including inner, central and outer (Fig. 8); (3) all scanned images were imported and 3D dataset images were reconstructed; (4) the 'attenuation coefficient' was calculated using software package CT Analyser (Version 1.5.0.0, SkyScan N.V., Aartselaar, Belgium); (5) nine regions per tooth were evaluated prior to and after irrigation using the image processing software; and (6) mineral content of the ROI was determined using the attenuation coefficient unit which is a direct reflection of the density of the selected region.

One of the strength of the micro-CT technique for dentistry is for evaluation of mineral content and changes in dental hard tissue. The results from scanning procedures are expressed as attenuation coefficient, which measures the absorption of a beam of light as it travels through an object, the equivalence of this value to mineral content.

The percentage loss/gain of mineral content was calculated using the following equation: attenuation coefficient after treatment minus attenuation coefficient of original sample divided by attenuation coefficient of original sample multiplied by 100. The percentages are displayed in Table 13.

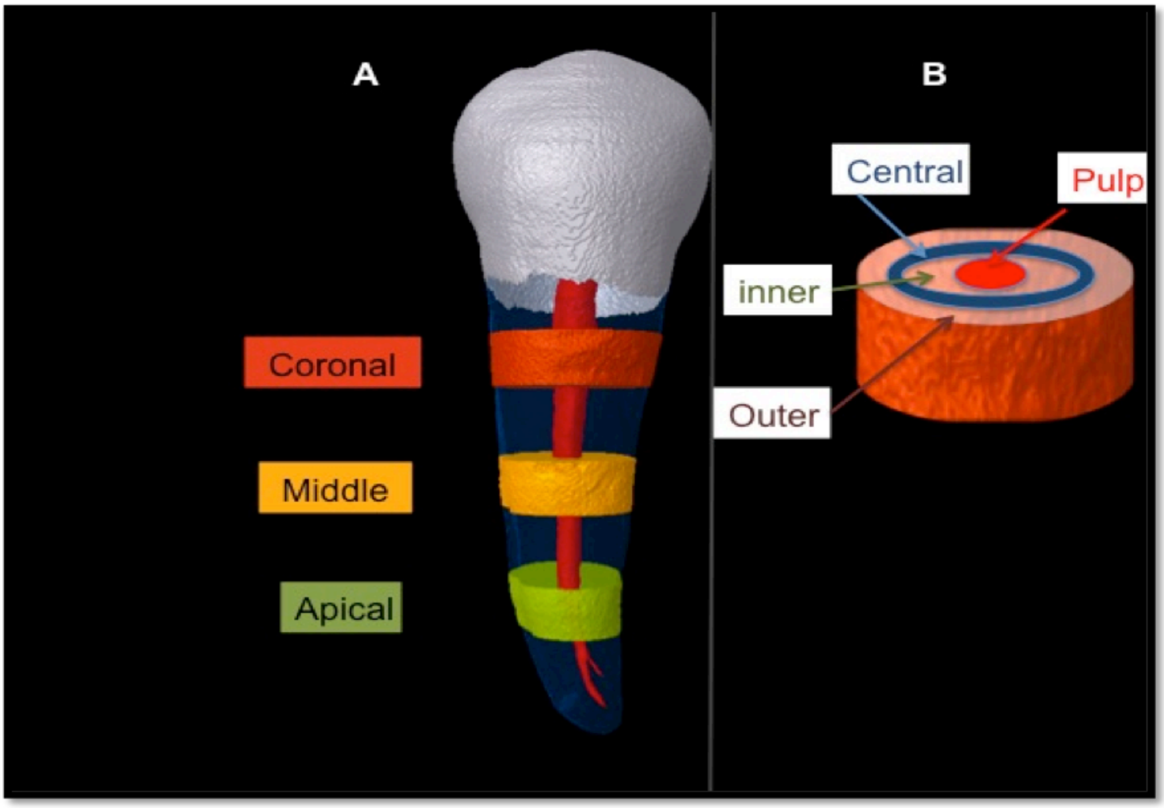


Figure 8. Schematic representation of the three different levels (coronal, middle and apical) in a premolar (A). The cross-section (B) shows three regions of interest (ROI) including inner, central and outer**.**

Statistical analysis

All data were analysed using the SPSS statistical package (version 11.0, 17.0 SPSS Inc., Chicago, Illinois, USA).

Study I. Descriptive statistics, including means, standard deviations, and range of all factors, were calculated for all individuals in both groups. Analysis of variance (ANOVA) was used to compare the mean of caries-related factors between EG and NEG and chi-square test to compare the scores. Intra-group comparison of recurrent caries at the tooth level was performed using pairwise t-tests.

Study II. The means and standard deviations of PI, marginal culture, pH-drop including $AUC_{0\text{–}10}$, and the de novo plaque formation rate were calculated for the 20 individuals. Chi-square tests were used to compare the ETT and NETT for the different scores.

Study III. The association between RFT and recurrent caries was studied by means of logistic regression, with recurrent caries as the dependent variable.

Study IV. Descriptive statistics, including means, and standard deviations of all nine points, were calculated for all teeth. Intra-tooth comparison of the percentage difference at the same point before and after surface treatment was performed using pairwise t-tests. The significance different between materials in the inner surface of the coronal part was analyzed with one-way ANOVA and post hoc pairwise t-tests.

For all studies, $p<0.05$ was considered statistically significant.

Results

Study I

Caries risk profiles in endodontic versus non-endodontic group

Frequency distribution of the caries-related factors is presented in Table 6. There was statistically significant difference ($p<0.05$) only for a number of individuals with high mutans streptococcus counts (i.e. $>10^5$ CFU/ml saliva); 48 in EG (26+22=48) and 30 in NEG (11+19=30). Using the Cariogram, analysis showed that the mean percentage of ''chance of avoiding caries'' was 35% in the EG compared to 37% in the NEG (not significant; Table 7).

Caries profiles in endodontic versus non-endodontic group

Overall, the mean DMFS was significantly higher in the EG compared to the NEG ($p<0.001$; Table 7). Moreover, EG showed a higher mean number of surfaces with recurrent caries (RD) (6.1 vs. 2.4) and fillings (FS) (21.9 vs. 9.7) compared to the NEG ($p<0.001$). However, the mean number of surfaces with primary caries (DS) was lower in the EG group (5.0 vs. 7.5) ($p<0.01$).

In the EG group, 32% of the filled surfaces in the root filled teeth were associated with recurrent caries, versus only 19% of the filled surfaces in the non-root-filled teeth (Table 8) ($p<0.01$).

Table 6. Frequency distribution of caries-related factors according to Cariogram score (Chi-square test was used to calculate the difference).

Factor	Cariogram score	EG (n = 100)	NEG (n = 100)	p-value
Lactobacillus score (CFU/ml)				
$0\text{-}10^3$	0	23	36	
$10^3\text{-}10^4$	1	31	23	
$10^4\text{-}10^5$	2	30	28	
$>10^5$	3	16	13	
Diet (meals/day)				
3	0	73	60	
4-5	1	21	35	<0.001
6-7	2	5	0	
>7	3	1	5	
Plaque index				
< 0.4	0	16	10	
0.4-1.0	1	51	55	
1.1-2.0	2	30	29	
>2.0	3	3	6	
Streptococcus score (CFU/ml)				
$0\text{-}10^3$	0	27	38	
$10^3\text{-}10^4$	1	25	32	<0.05
$10^5\text{-}10^6$	2	26	11	
$>10^6$	3	22	19	
Secretion rate (ml/min)				
≥1.1	0	71	67	
0.9-1.1	1	13	12	
0.5-0.9	2	10	16	
<0.5	3	6	5	
Buffer capacity (pH)				
>5.5 (Blue)	0	58	50	
5.5-4.5 (Green)	1	19	18	
<4.5 (Yellow)	2	23	32	
Fluoride (F) program				
Constant additional F	0	4	2	
Infrequent additional F	1	26	19	
Toothpaste with F only	2	56	70	
No F	3	14	9	

Table 7. Mean values, standard deviation (SD), and range of various parameters in the study groups. The "chance to avoid caries" (%), according to Cariogram, is also shown.

Factor	EG (n = 100)			NEG (n = 100)			p-value
	Mean	SD	Range	Mean	SD	Range	
Age	34.3	12.3	17-66	32.9	12.8	18-66	
Number of teeth	24.8	3.1	15-28	25.2	3.6	8-28	
DMFS	48.7	21.8	6-97	33.6	22.5	2-118	<0.001
Primary caries (DS)	5.0	5.7	0-36	7.5	9.8	0-62	<0.01
Recurrent caries (RD)	6.1	6.7	0-38	2.4	3.2	0-14	<0.001
Missing surfaces (MS)	15.7	15.5	0-65	14	18.5	0-94	
Filled surfaces (FS)	21.9	16.7	0-71	9.7	10	0-62	<0.001
Approximal caries	2.7	2.3	0-10	3.6	2.7	0-12	
Saliva secretion (ml/min)	1.7	0.9	0.3-5.4	1.7	1.2	0.3-8	
Plaque index	0.9	0.6	0-2.2	1.0	0.6	0.1-2.7	
Cariogram (%)	35	21.7	4-80	37	21.5	6-82	

Table 8. Comparison between coronal filled surfaces associated with endodontically and non-endodontically treated teeth in the EG group (n=100).

Factors	Endodontically treated teeth	Restored teeth	p-value
Number of teeth	362	404	
Mean filling	11.4 surfaces	10.5 surfaces	
Mean recurrent caries	3.6 surfaces	2.5 surfaces	
Percentage of recurrent caries in total fillings	32%	19%	<0.05

Study II

The scores of mutans streptococci and de novo plaque formation were higher in ETT compared to NETT ($p<0.001$; Table 9). The initial pH of the dental plaque (0-min value) was significantly lower in the endodontic treated teeth ($p<0.05$; Fig. 9). However, there was no significant difference in the overall pH-drop between the two types of teeth. Clinical evaluation of the tested teeth showed that irrespective of the type of restoration, recurrent decay was significantly higher in endodontically treated teeth (47%) compared to their counterparts (23%) ($p<0.001$; Table 10).

Table 9. Frequency of scores of lactobacilli, mutans streptococci and de novo plaque formation obtained from mesial and distal surfaces of ETT n=20 and NETT n=20. Chi square test was used to calculate the difference in distribution ($p<0.001$).

Factor	Score	ETT (n = 20)	NETT (n = 20)	p-value
Lactobacilli (CFU/ml)				
$0\text{-}10^3$	0	12	18	
$10^3\text{-}10^4$	1	48	44	
$10^4\text{-}10^5$	2	20	18	
$>10^5$	3	0	0	
Mutans streptococci (CFU/ml)				
$0\text{-}10^3$	0	4	8	
$10^3\text{-}10^4$	1	22	58	<0.001
$10^5\text{-}10^6$	2	52	14	<0.001
$>10^6$	3	2	0	
de novo plaque formation				
PI 0	0	0	0	
PI 1	1	4	7	
PI 2	2	12	25	<0.001
PI 3	3	24	8	<0.001

Table 10. Frequency of scores of marginal integrity, anatomic form, surface texture, and recurrent caries. Chi-square test was used to calculate the difference in distribution ($p<0.05$).

Criteria	RFT (n=40)		NRFT (n=40)		p-value
	Satisfactory	Unsatisfactory	Satisfactory	Unsatisfactory	
Marginal Integrity	(21) 53%	(19) 47%	(29) 73%	(11) 27%	<0.05
Anatomic form	(20) 50%	(20) 50%	(22) 55%	(18) 45%	
Surface texture	(19) 48%	(21) 52%	(21) 53%	(19) 47%	
Recurrent caries		(29) 47%		(19) 23%	<0.05

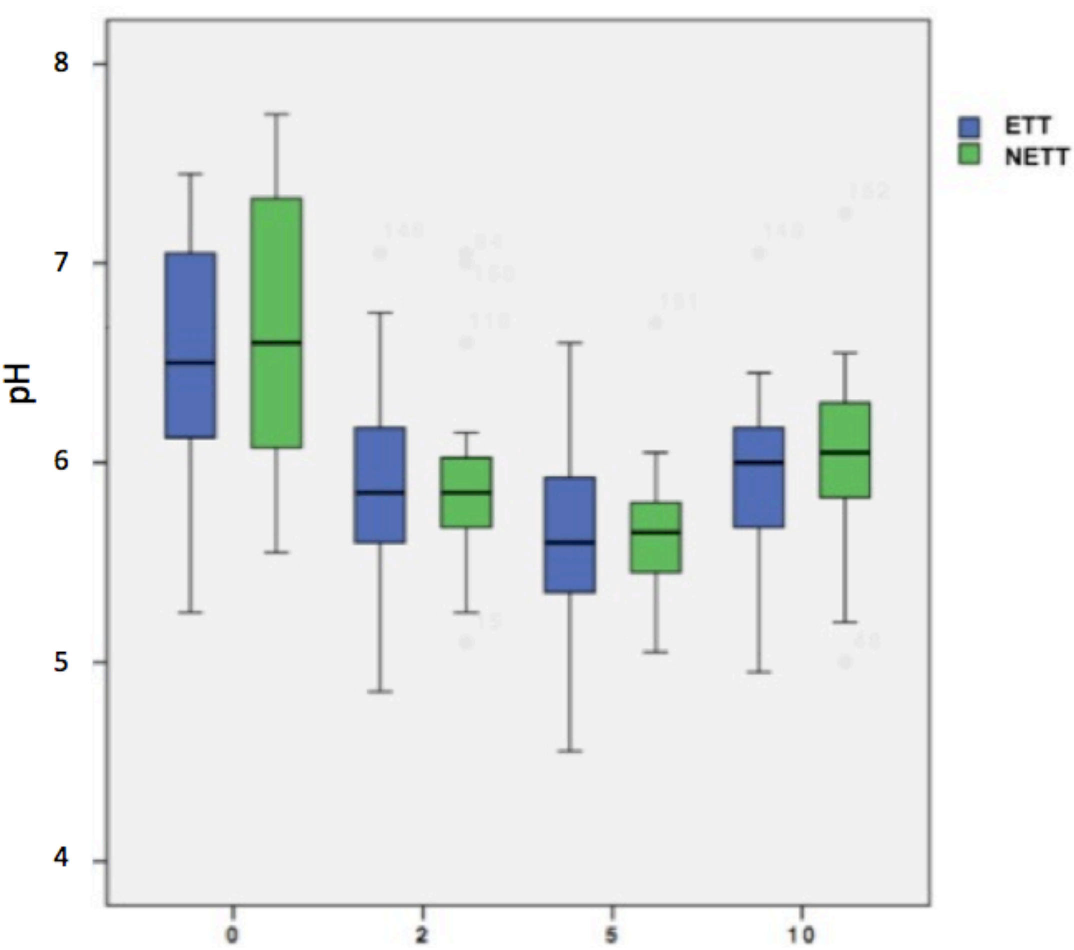


Figure 9. Box plots of pH in the RFT and NRFT. The line within the box indicates the median value; the lower and upper bounds indicate the 25th and 75th percentiles, respectively, the lower and upper whiskers indicate the 10th and 90th percentiles, respectively.

Study III

At tooth level, the multivariate logistic regression analysis revealed root-filled teeth to be predictive of recurrent caries, odds ratio (OR=1.68) (95% confidence interval [CI] 1.41-2.0), when controlling for number of restored surfaces (Table 11). When stratifying the data according to year of examination, the association remained significant. The association between number of restored surfaces and recurrent caries in root-filled teeth was significant for 5 surfaces fillings when compared to one surface fillings (Table 11). For non root-filled teeth, there were significant associations between number of restored surfaces and recurrent caries for teeth with 2-5 restored surfaces and full crowns when compared to teeth with one surface fillings (Table 11).

Table 11. Logistic regression model analyzing the association between
A) endodontic status and recurrent caries, controlled for number of restored surfaces
B) number of restored surfaces in root filled teeth and recurrent caries
C) number of restored surfaces in non-root filled teeth and recurrent caries.

	A	B	C
teeth	All restored teeth (N=9202)	Root filled teeth (N=1196)	Non-root filled (N=8006)
	OR (CI)	OR (CI)	OR (CI)
Endodontic status			
Non-root filled	Reference	N/A	N/A
Root filled	1.68 (1.41-2.0)	N/A	N/A
Number of restored surfaces			
1 surface	Reference	Reference	Reference
2 surfaces	2.80 (2.14-3.67)	5.35 (0.66-43.23)	2.72 (2.07-3.57)
3 surfaces	4.55 (3.51-5.89)	4.84 (0.62-37.70)	4.59 (3.53-5.96)
4 surfaces	5.66 (4.29-7.46)	3.49 (0.45-27.21)	6.50 (4.90-8.61)
5 surfaces	5.34 (3.98-7.16)	7.75 (1.02-59.17)	4.55 (3.27-6.31)
Full crown	2.69 (1.99-3.64)	3.47 (0.46-26.47)	2.42 (1.70-3.43)

To further test the association between root filled teeth and recurrent caries, only individuals with 1 or 2 decayed surfaces were included in a sub analysis "studied sample 2", yielding a sample of 163 individuals with 577 teeth with full crown coverage. This strategy was chosen in order to render two homogenous samples with regard to caries frequency and type of restoration, which were considered to be confounding factors. A bivariate logistic regression analysis resulted in a significant association between root-filled teeth and recurrent caries (OR=2.20; 95% CI 1.07-4.52).

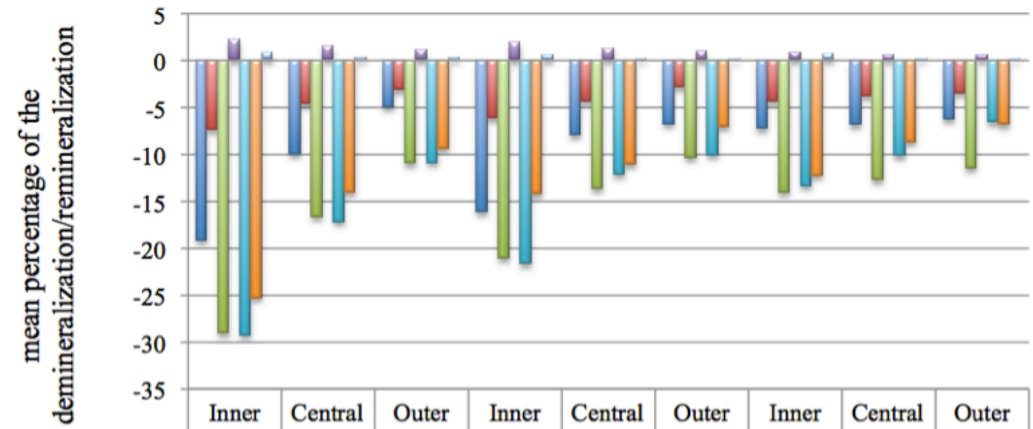
At the individual level, subjects with one root-filled tooth with full crown coverage and recurrent caries had a higher decayed surface (DS) than individuals with a root-filled tooth with full crown coverage without recurrent caries. The two groups also differed with regard to number of remaining teeth and restored surfaces (RS) (Table 12).

Table 12. Age, number of teeth, frequency of decayed surfaces (DS), frequency of restored surfaces (RS) in individuals with root filled teeth with full crown coverage with and without recurrent caries. Mean values and standard deviation (independent t-test, CI 95% (except for decayed surface; Mann-Whitney U-test)).

	With recurrent caries (n=63)	Without recurent caries (n=170)	p-value
Age	57.3 (11)	55.7 (11.2)	NS
Number of teeth	23.3 (2.9)	24.5 (2.9)	p= 0.005
Decayed surfaces	7% (8.4)	3% (2.6)	p<0.001
Restored surfaces	42% (11)	39% (10)	p= 0.021

Study IV

The results showed that, the inner ROI of the coronal section was most affected by the demineralization effect of the irrigation solutions, while the outer ROI of the apical section was least affected (Table 13). Groups 3 and 5 irrigants elicited the most pronounced demineralization effect, and was statistically significant in inner and middle ROI of all parts of the root ($p<0.05$). Group 1 and 6 irrigants showed a statistically significant demineralization effect only in the inner ROI of the upper part of the root ($p<0.05$). Group 4 showed the least effect that was not statistically different from the negative control.



	Upper			Middle			Lower		
	Inner	Central	Outer	Inner	Central	Outer	Inner	Central	Outer
1: NaOCl 5%	-19.2 *	-10	-5	-16.2 *	-8	-6.8	-7.3	-6.9	-6.3
2: NaOCl 2.5%	-7.4	-4.7	-3.2	-6.2	-4.4	-2.9	-4.4	-3.8	-3.5
3: EDTA	-29.1 *	-16.7 *	-11	-21.1 *	-13.7 *	-10.4	-14.1 *	-12.8 *	-11.5
4: CHX	2.2	1.5	1.1	1.9	1.3	1	0.9	0.6	0.6
5: Mix 1	-29.3	-17.2	-10.9	-21.7	-12.2	-10.1	-13.4	-10.2	-6.6
6: Mix 2	-25.3 *	-14.1 *	-9.4	-14.3 *	-11.1 *	-7.1	-12.3 *	-8.7 *	-6.9
7: Saline	0.8	0.3	0.3	0.6	0.2	0.1	0.7	0.2	0.1

* Statistically significant

Table 13. The mean percentage of the demineralization/remineralization for each point for each group.

Discussion

The main finding in the present thesis suggests that dental caries could be considered as a potential risk factor following root canal treatment and that clinicians should be aware of this risk. The research concept came from an observation of a common clinical problem related to the longevity of root-filled teeth, in which non-restorable carious destructions were reported as the main reason for their extraction (5). Despite the importance of the problem, only few studies have been aimed to explore the relationship of caries and root-filled teeth (17-19).

The present thesis contains different research designs, i.e., retrospective, prospective, clinical, and in vitro experiments. The diversity in the research designs was aimed to answer the main question of this thesis, which was: “are the endodontically treated teeth more prone to develop caries than vital teeth”

Initially, the aim was to explore if this problem exists on a population level (Paper I). The caries risk profile of individuals using the Cariogram and the frequency of recurrent caries with a RFT were evaluated on a Saudi population. The conclusion was that there is no difference in risk profile, but the proportion of recurrent caries was higher in the RFT (Paper II). However, the results may be considered as representative for a Saudi population, with several confounding factors, such as treatment quality, individual variation, and caries risk. The second clinical study (Paper III) was conducted on a Swedish population and results showed a significant association between RFT and recurrent caries, which is in agreement with the first study (Paper I).

As mentioned above, root canal treatment may cause some changes in tooth structure. These changes could be due to both intrinsic and extrinsic factors. Extrinsic factors are related to the surrounding environment, such as saliva, plaque, and microorganisms. Intrinsic factors are related to the tooth itself and include both the physiologic role of the pulp and the root canal treatment procedures. Therefore, Paper II addressed external factors that influence caries risk at tooth level, while Paper IV evaluated the effect of endodontic materials on dentin quality using micro-CT.

Assessment of caries prevalence (DMFS or DMFT) provides a general description of the extent of the disease and sheds light on related risk factors. In countries such as Saudi Arabia, where the prevalence is high, caries risk assessment is considered a necessity. This has recently been addressed in patients with many dental restorations (30). Moreover, it is important to evaluate the various caries-related risk factors, as well as to investigate the possibility of other mitigating factors.

A caries risk assessment may aid in the identification of etiological factors, so that suitable preventive treatment may be rendered for that particular individual (48). The Cariogram is regarded as a useful tool for caries risk assessment and prediction and has been used and validated for both children and elderly individuals (28, 29).

Several factors can influence the microbial metabolic activity in the dental biofilm. These include plaque composition and thickness, cariogenic bacteria, diet content, and frequency of food intake. The flow rate, buffer capacity of saliva, and presence of fluoride are risk inhibitors, providing protection against caries. In addition, previous caries experience, as well as social and behavioural factors, are also risk indicators that could indicate the probability of developing caries, but they are not be directly involved in the causal chain (49, 50). In the present thesis, all these factors are collectively referred to as “caries-related factors”.

In Paper I, the idea of an existing relationship between endodontic treatment and caries risk was proposed, and the hypothesis was tested among a group of Saudi adult citizens. As an exploratory step, the DMFS figures between EG and NEG were compared. The results showed that the mean DMFS was high, both in EG (mean 48.7) and NEG (mean 33.6). These results were consistent with previous studies in Saudi Arabia (30, 37). The mean DMFS value was about 50 in patients between 18 and 56 years old. When the DMFS was divided into its basic components (D, M and F), the results showed a higher statistically significant mean number of filled surfaces (FS) in the EG (21.9) compared to the NEG (9.7). On the other hand, the NEG had higher DS (7.5) compared with the EG (5.0) (Table 9). A possible explanation could be that carious teeth in the EG were treated and filled more frequently than in the NEG.

To further investigate the relationship between endodontic treatment and caries risk, the Cariogram profiles of patients with ≥2 endodontically treated teeth (EG) were compared with an aged-matched group, without any endodontically treated teeth (NEG), with the same number of teeth (mean=25 teeth) (Paper II). There was no overall difference between the two groups regarding “chance of avoiding caries;”

both groups showed low mean values (<40%), i.e., high caries risk. The range was, however, large in both groups, from 4 to 82%.

One can argue that, if there is a relationship between endodontic treatment and increased caries risk, why was this not confirmed by the statistical analyses? Cariogram evaluates the individual as a unit, with multiple confounding factors influencing the total score. Further studies are needed in order to draw any conclusions regarding endodontic treatment as an independent confounding factor; i.e., to assess the risk factor in relation to the endodontic tooth, rather than the individual. Therefore, the aim of the Paper II was to evaluate the susceptibility of RFT versus NRFT to develop caries, by evaluating the quality of the coronal fillings/crowns of the targeted teeth, including recurrent caries in a subsample of Study I.

The main conclusion from Study II is that the mutans streptococcus count and de novo plaque formation are risk factors in endodontically treated teeth. These findings confirm the results from Study I, which showed significantly higher mutans counts in saliva from patients with endodontically compared to non-endodontically treated teeth. One possible explanation could be that a root-filled tooth often has extensive dental restorations, which increase the retention of plaque (51). Another reason may be that the plaque microflora on these surfaces have an altered composition with more acidogenic microorganisms. A third reason could be changes of the outer dentin surface, which may promote plaque accumulation and presence of mutans streptococci.

Study I showed that that patients with at least two endodontically treated teeth differed significantly in their mutans streptococcus count, when compared with patients with no endodontically treated teeth. The microorganisms were isolated from saliva samples. Therefore, a direct relationship between the mutans count and endodontically treated teeth could not be established. In Study II, the mutans strepococci were isolated from plaque samples obtained from surfaces of endodontically treated teeth and non-endodontically treated teeth of the same individual. The data showed that mutans streptococci count and de novo plaque formation were higher in endodontically treated teeth compared to their vital counterparts.

Most endodontically treated teeth have large fillings. Filling surfaces might retain more plaque due to surface roughness and differences in surface tension (51). Additionally, dental plaque deposited on filling material may have an altered composition due to lack of ion exchange (calcium and phosphate) that occurs naturally on the enamel surface during demineralization and remineralization. Thus, the surface area of the filling may increase the risk of caries, not to mention the quality of the filling, which has significant influence on caries risk.

In Study II, the quality of coronal fillings/crowns were examined using modified USPHS-Ryge criteria (45), where evaluation of color match was not performed because it was irrelevant to caries-related factors. The results showed that marginal integrity and recurrent decay were significantly higher in endodontically treated teeth. Furthermore, there was no significant difference in the pH-drop between endodontic and non-endodontic treated teeth. However, the initial (resting) pH was significantly lower in the endodontic treated teeth, which can reaches the critical value of demineralization faster.

The data from Studies I and II showed endodontically treated teeth were more susceptible to caries, this could be attributed to the increase in the mutans counts, and alteration of the biological environment of the tooth. Further studies are needed to explore the effect of loss of physiologic role of the pulp and of root canal treatment procedures on dentin.

The material in the first three publications is based on two populations, one from Jeddah, Saudi Arabia, and the other from Jönköping, Sweden. Epidemiological studies on caries and endodontics are useful in exploring the existent relationship between these two factors. In Sweden, several epidemiological investigations describing caries and oral health have been published (46, 52, 53). The repeated cross-sectional studies carried out in Jönköping over more than three decades are useful for studies on a population level.

The original sample of Jönköping included subjects aged 3 to 80 years. Some of the subjects were not relevant to the study. Therefore, a homogeneous sample of dentate individuals aged 20-70 years with eight or more remaining premolars and molars were selected for analysis. Young patients with deciduous teeth and elderly edentulous patients were eliminated, in order to have similar caries risk group. Regarding type of restoration, it was presumed that teeth with full crown coverage would constitute a more homogenous group compared to all restored teeth with differing numbers of restored surfaces (54). The results of Study II showed that RFT had an increased susceptibility to caries, ascribed either to alteration in their biological environment, or to inadequacy of the marginal fit of the dental restoration. In Study III, the role of fillings was eliminated as confounding factor as only teeth with full crowns were examined.

Restoring endodontically treated tooth with full crowns has been suggested to prevent fractures (55). The data in Study III showed that a full crown has a lower risk to develop caries compared to the 2-surface fillings. The reason could be due to three factors: 1) surface area, only the circumference of the finish line, 2) the accuracy and adaptation of the margin, and 3) the subgingival ecology which is not favourable for acidogenic bacteria such as mutans streptococci. However, one could argue that the result is a false negative because of the difficulties and limitation of caries diagnosis associated with full crowns.

In general, there are certain locations of the tooth that are prone to caries, i.e. the occlusal pit and fissure, the approximal surface cervical to the contact point, buccal or lingual surfaces along the gingival margin, and tooth-restoration interfaces. These areas do not differ from other tooth surfaces with regard to tooth structure, but they are susceptible to caries because the biofilm tends to stagnate and remain for a prolonged period.

With regard to recurrent caries, occlusal fillings had a low risk to develop caries and the risk was directly proportional with the number of filled surfaces. This could be related to individual factors, in other words, the presence of several restored tooth surfaces in a patient may reflect the current or past history of high level of caries activity. In addition to the individual factors, the surface area of the filling may increase the risk of caries, not to mention the quality of the filling, which has a significant influence on caries risk.

The quality of the coronal restoration may also have an impact on the periapical health of root-filled teeth (23). Despite the numerous studies that have evaluated the coronal leakage and recurrent caries in endodontically treated teeth, most of them used radiographic evaluation (23, 56). The main limitation of using only radiographs is that type and density of materials may influence the detection of caries lesions (57, 58). In Paper II and III, the diagnosis was done by using both clinical and radiographic examinations, in order to increase the sensitivity and specifity of caries examination.

Histological sectioning of extracted teeth has been conventionally used as the gold standard to which new diagnostic modalities are compared. In general, sectioning is destructive, with demands on both time and personnel. In Cariology research, there is an increased demand for a non-destructive, fast, easy technique, which will not only simplify the investigative procedure, but also allow for the preservation of sample for longitudinal use.

Micro-CT provides series of cross sectional images are generated and combined to reconstruct an image of the tooth. In addition, it allows sagittal, coronal, and cross sectional evaluation of the same tooth material at one time. However, there are some disadvantages with this method, such as: 1) the long time required for scanning, 2) high cost, 3) learning curve, and 4) it remains a research tool and cannot be employed for human imaging in vivo. The validity of micro-CT has been established and researchers have concluded that the technique might provide a viable alternative to histology in caries diagnosis, (59) as the scan can quantify the volume of caries and other hard and soft tissues (60).

Micro-CT has also been used in endodontics studies (61-64). Comparison of the effects of biomechanical preparation on canal volume on reconstructed root canals in extracted teeth using micro-CT data was shown to assist with characterization of morphological changes associated with these techniques (62). Peters et al. (61) used the micro-CT to evaluate the relative performance of ProTaper NiTi (Dentsply Maillefer, Ballaigues, Switzerland) instruments in shaping root canals of varying preoperative canal geometry. A study to examine the potential and accuracy of micro-CT for imaging filled root canals showed it to be a highly accurate and non-destructive method for the evaluation of root canal fillings and their constituents. Qualitative and quantitative correlation between histological and micro-CT examination of root canal fillings was found to be high (63, 64).

One of the objectives of cleaning and shaping is to eliminate the smear layer that contains remnant of bacteria, pulp debris, and toxins (65-67). The effect of different irrigation solutions on the quality of dentin has been addressed in several studies (68-72). In Paper IV, efficacy of irrigation solutions used in clinical practice, alone or in combination and at different concentrations, was evaluated.

The sequential application of NaOCl and EDTA has been recommended as an effective irrigation regimen (73, 74). It is well known that NaOCl is a non-specific proteolytic agent that is capable of removing organic material, as well as magnesium and carbonate ions and denatures the collagen components of the smear layer (75). While EDTA demineralizes the inorganic components of dentin via calcium chelation.

Sim et al. (76) showed that irrigation with 5.25% NaOCl, as compared to saline solution, reduces the flexural strength and elastic modulus of dentin. In addition, it has been shown to adversely affect the sealing ability and adhesion of dental materials to dentin (77). The application of 10% NaOCl for two minutes on human root dentin showed under microradiography a subsequent mineral loss ranged between 15% and 42%, (78). A similar effect was observed in Paper IV. Mineral loss was dependent on the NaOCl concentration. Thus, NaOCl of 5.5 % showed significant demineralization of the inner ROI of the coronal section of the treated root dentin, compared to the 2.25%, which did not show any significant demineralization. Similarly, mineral loss associated with the use of mixes of NaOCl and EDTA was also concentration-dependent, but the demineralization was more dramatic (Table 13). EDTA alone showed a statistically significant demineralization of the inner and middle dentin of all parts. The use of EDTA, in addition to an increased concentration of NaOCl, seems to increase the extent of the demineralization effect.

Chlorhexidine gluconate, on the other hand, is recognized as being an effective oral antimicrobial agent. It is routinely used in endodontic therapy and for caries prevention (79). Zaura-Arite and ten Cate (80) showed that CHX-containing varnishes have an inhibitory effect on demineralization, as well as a caries preventive effect. In tandem with that study, Paper IV showed that 2% CHX gluconate did not have any demineralization effect on dentin.

Demineralization is the beginning of dental caries, dentin and cementum are more susceptible to caries than enamel because they have lower mineral content (81). Thus, when root surfaces are exposed due to gingival recession or periodontal disease, caries can develop more readily. Even in a healthy oral environment, however, the tooth is susceptible to dental caries. Pascoe and Seow (82) showed a strong association between enamel hypoplasia and dental caries, suggesting that enamel hypoplasia might be a significant caries risk factor. Using the same concept, demineralization effect of EDTA and NaOCl can either directly accelerate the caries process by minimizing the demineralization required for cavitations, or indirectly through increasing the patency of the dentinal tubules and permitting the penetration of microorganisms (70, 71).

Cumulatively, these factors may interact to contribute to the development of recurrent caries in RFT. Therefore, it is important to identify the possible effects of the different materials used during endodontic treatment on the structure and physical properties of RFT. Equally important is to understand the relationship between these effect and caries in RFT. To reduce the deminralization effect of EDTA and NaOCl, it may be suitable to use flouride to protect the dentin surface. Inaba et al. (83) showed that use of 10% NaOCl, for two minutes increases remineralization by 27% if followed by a remineralization solution containing fluoride.

Conclusions

The main conclusion from the present thesis suggests that dental caries could be considered as a risk factor following root canal treatment.

- Paper I did not show any significant difference in risk profile at the individual level, except for the mutans streptococcus count. A significant difference was detected in the proportion of recurrent caries, which was higher in RFT compared to vital teeth.

- Paper II showed that endodontically treated teeth had an increased susceptibility to caries, ascribed either to alteration in their biological environment or to inadequacy of the marginal fit of the dental restoration.

- Paper III showed significant association between endodontically treated teeth and recurrent caries.

- Paper IV showed that NaOCl and EDTA irrigation solutions changed the quality of dentin, in a way that might increase caries susceptibility of endodontically treated teeth.

Overall, endodontically treated teeth were more susceptible to caries. This finding should raise the awareness among dental clinicians regarding this risk and encouraged the use of topical application of fluoride-containing products, such as use of fluoride varnish and proper tooth cleaning by the patient and the use of fluoride tooth paste and rinsing solution on a daily bases at home. Further studies are needed to explore the effect of loss of physiologic role of the pulp and the effect of root canal treatment procedures on dentin.

Acknowledgements

In the name of Allah, the Beneficent, the Merciful. Praise and gratitude be to Allah for giving the strength and guidance and all what I wished to have in my life.

First and foremost, I offer my sincerest gratitude to my supervisors, Dowen Birkhed and Claes Reit, who have supported me throughout my thesis with their patience and knowledge, whilst allowing me the room to work in my own way. I attribute the level of my PhD degree to their encouragement and effort. Without them this, thesis would not have been completed or written. One simply could not wish for better or friendlier supervisors.

In my daily work, I have been blessed with a friendly and cheerful group of friends, especially Sahar Boukhary, Hilal Sonbul, and Fredrik Frisk. I would like to thank them for their valuable suggestions, contributions, and advice. As well, I would like to thank Ann-Britt Lundberg and Ann-Charlott Börjesson for their help and support in the establishing the laboratory work.

As well, I would like to thank The Ministry of Higher Education in Saudi Arabia, along with the King Abdulaziz University, Saudi Arabia, for fully funding this project.

Finally, I am heartily thankful to my mother (Rokia), my wife (Rana), my two boys (Abdulaziz and Abdulrahman) for their continuous support, prayers, patience, and motivation during the research and writing of this thesis.

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