Trends in Dutch Dentistry; The Need for Prevention

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The Netherlands, with a population of approximately 16 million people in an area of 41.500 km^2 with an average population density of 380 persons/km^2, in size and population roughly comparable to Kyushu, has seen a dramatic improvement in the dental health of its population since the introduction of fluorides (mainly by the use of fluoride dentifrices and topical fluoride application through dental practices). The mean DMFS for 12 year old children in the region of Friesland for example decreased from roughly 14 in 1973 to around 2 in 1988[1].

The dental practice has reacted to this change in the incidence of caries by raising the standard of care for natural teeth, more emphasis on aesthetics and consequently specialization. At the same time, the dentist has also remained responsible for the by now more mundane tasks of educating patients in oral health, preventive treatment and the minimally invasive procedures. In this process, the dental practice has evolved from the original 'single chair' dentist through the dentist with an assistant to a dental practice with multiple chairs that employs assistants, oral hygienists and more than one dentist, each with his or her own specialty (approximately 1/3 of all dentists[2]) . In the process, maybe also because of the relative absence of new means and methods for prevention as compared to the restorative aspects of dentistry, prevention has lost most of its original urgency as is reflected in the most recent change to the reimbursement system in the Netherlands where specific codes for preventive measures where abandoned and presumed to be included in the standard inspection.

However, with all the overall improvement of dental health, still 20% of the patients experience 80% of the caries (DMFS decrease occurred mainly in middle- and high-income children). Then there are indications that caries incidence may be rising again. Also, as a result of the improved dental health, the care for the elderly is now being confronted with an increased number of patients that enter the institutions while still in the possession of their natural denture which demands a different approach towards the oral care provided by these institutions. Last not but least, the population has emancipated and is demanding for more involvement in the management of their own health.

All of these factors have caused the expenditure on dental care to grow. The Dutch government has reacted to this challenge by actively supporting specialization in dental care and at the same time encouraging dental professionals to form multidisciplinary teams to provide all inclusive dental care and providing pressure to re-allocate tasks to improve efficiency and the quality of care and to reduce the costs. To this end it is in the process of differentiating the dental profession: where originally the dentist had the monopoly on oral care, the

government has now introduced a new degree: bachelor of Health. The dental bachelor of Health is an independent professional who can provide non- and minimal invasive oral care and ideally is part of a multidisciplinary team. Prevention and non-invasive oral care are intended be an important part of the bachelors task.

This new approach has met with some resistance. Apart from a reluctance of the traditional dentists to relinquish some of their hold on dental care, there has been concern that by allowing the bachelor of Health to perform invasive dentistry, considering the current restorative paradigm of dental care, the quality of preventive care will deteriorate even further, which is not the effect that the government has in mind.

This is where new diagnostic methods do play a crucial role by providing a quantitative measurement of the caries risk of the individual patient before restorative procedures are inevitable and a quantitative assessment of the effectiveness of preventive therapy. Quantitative Light-induced Fluorescence (QLF) is the first of these new diagnostic methods that has been successfully introduced into the standard clinical care. This is exemplified by case studies of two practices in the Netherlands. In 2004, QLF was introduced into these practices. Each practice reported a marked increase in patient-compliance to (preventive) therapy, an increase in the quality of the therapy and an increase in patient- as well as worker-satisfaction.

With its quick visual feedback, QLF was found to be very good instrument to involve the patient in treatment decisions and to empower the patient to take the responsibility for his or her own oral health. The improved detection of early lesions and bacterial activity and the quantitative monitoring of these factors helped the professional to more accurately assess risk, evaluate the results of their efforts and hence improve the quality of their work.

By providing a much needed evidence based platform for early detection of caries, gingivitis and other oral health threats, prevention can be boosted to recapture the place that it should have in the dental care as an independent and invaluable specialization.

References

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The longitudinal development of caries lesions after orthodontic treatment evaluated by quantitative light-induced fluorescence.

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Abstract

Decalcifications or white spot lesions are one of the risks of an orthodontic treatment [1-3]. White spots during orthodontic treatment are formed more rapidly than normally, due to prolonged accumulation and retention of plaque. It is generally believed that once the appliances are removed and oral hygiene is restored that these lesions regress. Existing studies report an improvement of lesions after debonding [4, 5] but the long term implications of white spot lesions occurring during orthodontic treatment are unknown. To date there exists only one study where the natural behavior of white spot lesions resulting from orthodontic treatment with fixed appliances was studied quantitatively by following these lesions longitudinally in time after removal of the appliances [5]. The size of this study was limited, but the behavior of the lesions was well documented using Quantitative light induced fluorescence (QLF). In the present investigation (MEC01/099#01.17.594) we studied the natural behavior of white spot lesions in a larger group of orthodontic patients developed during treatment with fixed orthodontic appliances, after the removal of those appliances using quantitative light-induced fluorescence (QLF). A total of 64 subjects were enrolled in the study of which 58 subjects, 29 males and 29 females were still included at the 6 month retention visit. Eligible participants were at least 12 years of age and had received treatment with fixed orthodontic appliances at the Academic Centre for Dentistry Amsterdam, The Netherlands (ACTA) for a period of at least one year. Subjects were examined with QLF for presence and extent of caries on their buccal surfaces directly after debonding and 6 weeks and 6 months thereafter. A number of 51 subjects, 24 males and 27 females, were also examined for presence of caries 2 years after debonding. The fluorescence loss (ΔF [%]) and area of lesions [mm2] were determined for all lesions found. A total of 421 carious lesions were recorded at debracketing with an average fluorescence loss (ΔF_0) of 10.3% (SD 5.4%). 97% of all subjects and on average 30% of the buccal surfaces in a person were affected. On average, in males 40% of surfaces and in females 22% showed white spots ($p<0.01$). Caries prevalence was lower ($p<0.01$) in incisors and cuspids than in molars and premolars [6]. During the first 6 months a total of 15 lesions were lost from QLF analysis: 11 lesions, all with a maximum fluorescence loss at baseline ΔF_{MAX}, 0>25%, in two subjects were restored and 4 were not analyzed because they were not imaged properly. Lesions varied from incipient, i.e. white spot, ($\Delta F_0<10\%$, N=257) to advanced, i.e. dentinal ($\Delta F_0>25\%$, N=12).

Using the subjects as unit of research we observed a small lesion improvement 6 weeks after debracketing ($p<0.01$) and a further lesion improvement was seen after 6 months ($p<0.01$). Using the lesions as unit of research we noticed that incipient lesions on average showed a smaller improvement (relative decrease 2%, SD 20%) than lesions with $\Delta F_0>10\%$ (relative decrease 12%, SD 20%, $p<0.01$). The 51 participants seen also 2 years after debonding had a total of 370 lesions at baseline. Of these, 5 lesions in 1 subject were restored by the 6 month visit and another 14 lesions in 5 other subjects were restored by the 2 year visit. In these 51 participants we lost significance for lesion improvement 6 weeks after debracketing but lesion improvement was still seen after 6 months ($p=0.02$), from 6 months to 2 years after debonding no further improvement ($p=0.6$) was seen. This implicates that lesions formed during orthodontic treatment, even when they remineralize to some extent, remain as permanent scars. Furthermore, in the 51 participants followed for 2 years, we found a number of 35 lesions that had significantly progressed on top of the 19 lesions mentioned earlier that were restored. The longitudinal follow-up of lesions with QLF in this study has shown that lesions developed during orthodontic treatment had the ability to improve once the fixed appliances were removed even when they were advanced, but the overall regression was small and one may debate the clinical relevance of it. Furthermore, 6 months after debonding no further significant changes were seen and about 15% of lesions worsened to an extent that restorative treatment was necessary or will become necessary in the near future. Given the amount and extent of lesions, research to investigate the potential of preventive measures to enhance lesion improvement is necessary. The use of QLF to monitor lesion development can provide useful information about efficacy of a treatment strategy in the individual patient and thus aid in controlling the caries process.

Introduction

It is well known that decalcifications are one of the risks of an orthodontic treatment [1, 3]. These incipient lesions, commonly known as white spot lesions, are situated on the buccal surfaces of teeth that normally show a low prevalence of caries [2]. White spot formation during orthodontic treatment has been attributed to the effect of prolonged accumulation and retention of the bacterial plaque. The fixed appliances make conventional oral hygiene for plaque removal more difficult and adjacent to the brackets the clearance of plaque by saliva is also reduced. There seems to be a difference in progression rate between traditional caries formation and white spot lesions induced by deficient oral hygiene combined with fixed orthodontic appliances. The latter has a rather superficial and "speedy" character and can become apparent within one month after placement of fixed appliances [2]. The formation of a 'normal' caries lesion is usually a slower process which takes at least six months [7]. It is generally believed that once the appliances are removed and oral hygiene is restored that these lesions regress. Nevertheless, the use of extra preventive measures is frequently promoted, as in many cases the white spot lesions remain visible as a permanent scar.

Epidemiological investigations of a disease commonly start with studies of prevalence and incidence of the disease. After the extent and the distribution of the disease are investigated, the available information is then utilized in search for etiological factors and the nature of the disease. The reported prevalence of white spot lesions among orthodontic treated patients varies widely from 2 to 96% [3, 8-14]. This large variation could be attributed

to the variety of methods and the difficulty in standardizing clinical examinations. A more objective and reproducible method to investigate the caries prevalence is required.

In the early 1980's, researchers started to investigate more sensitive methods, such as (laser)-light induced fluorescence, to enable early detection and quantification of caries lesions [15, 16]. Caries can be detected by light induced fluorescence, because the fluorescence radiance at the site of a caries lesion is decreased. With the introduction of cameras together with image analysis software, it became possible to monitor changes in the enamel over time [17]. The fluorescence image of enamel with incipient lesions can be digitized and then the loss of fluorescence in the lesion can be quantified in comparison to the fluorescence radiance level of sound enamel. The amount of fluorescence radiance loss has been validated by the use of transversal and longitudinal micro-radiography and is closely correlated (r=0.97) to the mineral loss in the lesion [18-21]. Recent studies indicated that quantitative light-induced fluorescence is suitable for in vivo monitoring of mineral changes in incipient enamel lesions [22, 23]. Furthermore the use of QLF to follow caries development during orthodontic treatment was suggested in studies performed in vitro [24, 25].

To date there exists only one study where the natural behavior of white spot lesions resulting from orthodontic treatment with fixed appliances was studied quantitatively by following these lesions longitudinally in time after removal of the appliances [22]. The size of this study was limited, but the behavior of the lesions was well documented using Quantitative light induced fluorescence (QLF). At the orthodontic department of ACTA we have used QLF to investigate the occurrence of white-spot lesions at debracketing and followed the natural behaviour of these white-spot lesions during the first 2 years of the retention phase.

The aims of the present investigation were to study the prevalence and severity of white spot lesions in orthodontic patients after treatment with fixed orthodontic appliances, and the natural behavior of these white spot lesions after the removal of those appliances for a period up to 2 years. A further aim was to investigate the effect of caries risk factors, such as oral hygiene, socio-economic status, gender, and duration of the treatment on the prevalence of initial dental caries at the time of debonding of the fixed appliances in orthodontic patients [26].

Materials and Methods

Approval of the Medical Ethical Committee of the Academic Medical Center of the University of Amsterdam was obtained (MEC01/099#01.17.594) for this study.

The participants were recruited consecutively among patients who were being treated with a fixed appliance in patients from the department of orthodontics at the Academic Center for Dentistry Amsterdam, the Netherlands. The inclusion criteria were: age 12 years or older, good general health, a treatment period with fixed appliances of at least one year at the debonding appointment and an informed consent signed by the participant and for those under 18 also signed by their parents or guardians. The participants' dentists were informed that their patients were participating in the study and asked not to administer extra fluoride to these patients while they were participating and to contact the study investigator in case restorations were required.

Presence or absence and extent of lesions on the

buccal surfaces of all teeth except second and third molars was determined at the debracketing visit and at three retention visits, 6 weeks, 6 months and two years after debracketing, with QLF (Inspektor Research Systems B.V., Amsterdam, Netherlands). Thus, a maximum of 24 surfaces per subject were judged for caries. No special measures were taken to remove plaque from the surfaces, apart from normal cleaning as part of the debonding procedure. The examination interval of 6 weeks and 6 months was according to the treatment protocol of the department.

QLF images at T0 were captured immediately after debonding. QLF-examinations were repeated at the next visit since it was expected that the gingival swelling at T0 would obscure part of the buccal tooth surfaces, but would have receded at T1. Lesion presence or absence was also determined by conventional visual examination at the debonding visit and 6 weeks thereafter. Digital white light photographs taken immediately after debonding, as part the normal treatment protocol of the department, were used to limit the number of false positives called during QLF analysis caused by stains on the surface or remnants of bonding material.

The socio-economic status, gender, number of sugar intakes per day, level of oral hygiene and oral soft tissue health were recorded by means of a questionnaire. Bacteriological tests, bleeding and plaque score examinations were also performed.

QLF

QLF- images of the buccal surfaces of all teeth except the second and third molars were captured at T0 and T1 for each patient. Images were captured using an intra-oral fluorescence camera on a PC using the image capturing software (QLF Patient version 3.0.0.4) delivered with the system. To ensure that images of tooth surfaces are always captured with the same camera position and from the same angle, the software uses video-repositioning techniques. The video-repositioning technique displays baseline and live image simultaneously and computes their correlation based on similar geometry of the fluorescence intensities [27]. Images are stored in a list when the correlation is higher than 0.90 and the system automatically stops grabbing when the correlation has reached 0.98. In this way images captured at T0 and T1 should show the tooth surface from the same angle and with the same size, except for changes due to e.g. differences in gingival swelling.

QLF images were judged visually for signs of decalcification, which appear as dark areas surrounded by bright green fluorescing sound tooth tissue [17]. Presence or absence of lesions was scored on a per-surface base in each patient. If lesions were detected the fluorescence loss (ΔF [%]) and lesion area [mm^2] were determined using the system's analysis software to determine lesion severity (Fig. 1). To determine the reliability of lesion detection by the investigator (JGB), lesion detection on QLF images was performed twice both by the investigator and a researcher experienced with QLF (MHV). In this way the inter- as well as intra-examiner correlations were determined.

Visual examination

At T0 and T1 all visible lesions on the buccal sites of the teeth were recorded for all teeth except the 2nd and 3rd molars. A surface was considered carious when it had a white spot, a brown discolored lesion or was cavitated. Hypoplastic or fluorotic enamel was not scored as caries. The number of filled buccal surfaces (FS) were also recorded.

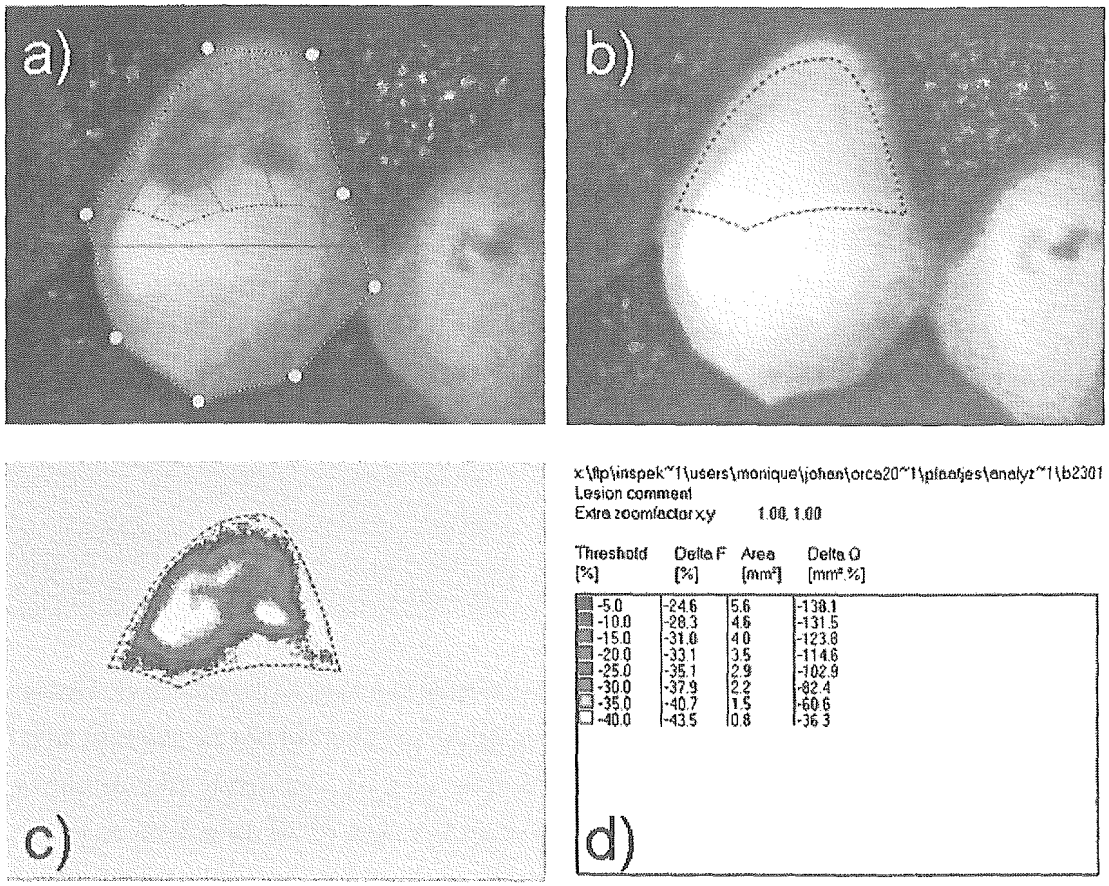

Figure 1. Example of image analysis of a lesion resulting from orthodontic treatment. On the original image (a) a patch is created with borders (light and dark-blue dashed lines) on sound enamel, from which sound enamel green fluorescence radiance values are calculated for each pixel inside the patch, resulting in the reconstructed image (b). From original and reconstructed pixel values the lesion fluorescence loss is derived and displayed in pseudo-colors (c). Fluorescence losses (-Delta F) exceeding different thresholds corresponding to the pseudo-colors from (c) for the lesion in this example are given in (d). Contour points (solid blue circles) are used to outline the tooth surface (a) and copied together with the patch from one image to the next, allowing the patch to be positioned and aligned on each image with respect to the tooth-surface in the same way.

White light photographs

White light photographs (Fig. 2) were taken to prevent stains on the enamel surface to be interpreted as caries on the QLF images. In this way, areas of fluorescence loss associated with stain rather than caries could be omitted from QLF analysis. Photographs were captured using a Nikon (Haarlem, the Netherlands) camera body with CCD, DCS from Kodak (Odijk, the Netherlands) with an image size of 1012 by 1524 pixels or 4.5 Mb. The camera has a 2.8/60mm macro-lens that, combined with the CCD surface size, resulted in images comparable to those made with a 160-mm lens. The camera was equipped with a Nikon SB 21 macro flasher. Photographs were stored on a PC. Photographs were captured under an angle of 0° and 45° with the front from both left and right side of the face, in general resulting in images from all buccal surfaces except the 2nd and 3rd molars.

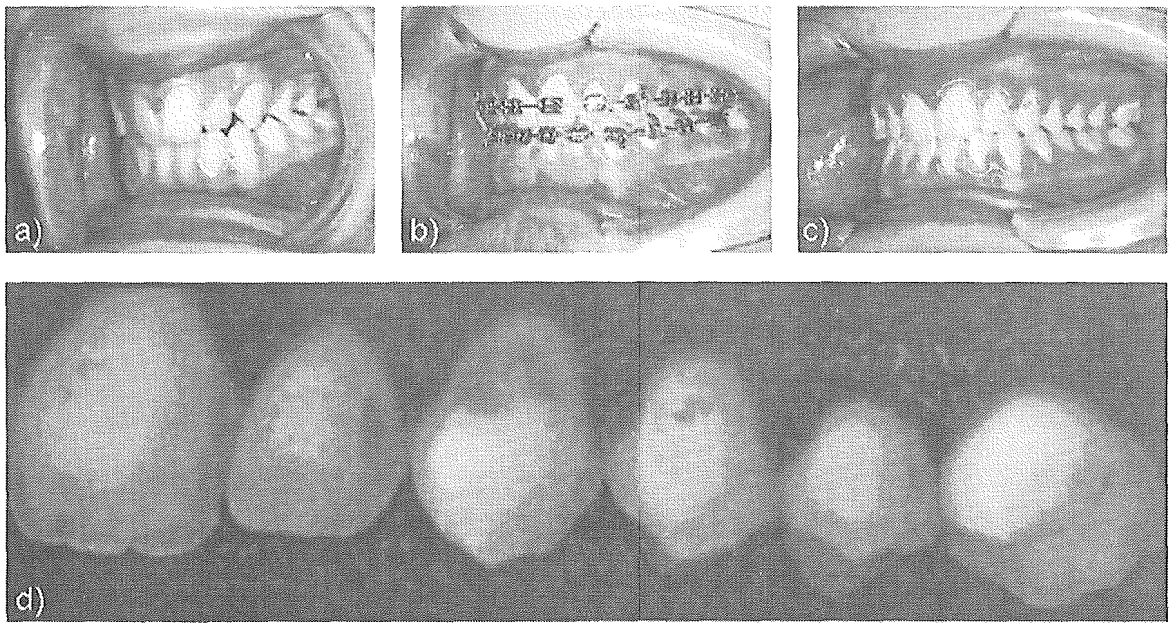

Figure 2. Images of an orthodontic patient, showing caries incidence as a result of the orthodontic treatment. In a) the situation before the treatment with fixed appliances is started. In b) the orthodontic treatment with fixed appliances is shown just prior to debonding and in c) the same situation immediately after debonding and cleaning of the teeth. The image in d) is a composition of QLF images of the same teeth as in a-c. The decalcifications are quite apparent.

Participant history and social economic status

Gender, age and duration of the orthodontic treatment with fixed appliances were recorded for each participant. At T1, the socio-economic status of the participants was established using the level of education and occupation of both parents. The highest level of education attained by the participant was categorized into four ordinal levels: 1) primary school or lower vocational training only, 2) lower secondary school or middle vocational training, 3) higher secondary school and 4) university or higher professional education. The level of occupation was measured using a modified four steps ordinal scale. The mean of the scores of both parents was used and translated to a 3 level scale of low middle and high socio-economic status [28].

Diet

Each participant was given a diet-diary to assess the dietary habits, and was requested to fill it out at home on a Friday and a Saturday. The participants were asked to return this booklet by mail in a pre-stamped and addressed envelope. From the diary the frequency of sugar intakes per day was extracted. Any sugar containing consumption taken during a 30 minutes period were counted as one sugar exposure.

Oral soft tissue examinations

At T1, the health status of the oral soft-tissue was examined by measuring the gingival bleeding index (GBI). The oral soft-tissue was only recorded, at teeth 16, 21, 24, 36, 41, and 44 [29]. A 0.5 mm diameter periodontal probe was gently inserted into the gingival crevice parallel to the long axis of the tooth until a slight pressure was felt, avoiding undue

penetration of the tissue [30]. Bleeding was recorded as 1 and no bleeding as 0. Bleeding was recorded at six places per tooth, the buccal and the palatal/lingual side mesially, distally, and in the middle. The sum of elicited bleeding points gave the GBI.

Saliva test

Salivary levels of cariogenic bacteria were assessed at T0, just before debonding, and at the six week recall T1 using dip slide tests for mutans streptococci and lactobacillus casei (® Ivoclar Vivadent ag, Schaan, Liechtenstein). Participants were not asked to refrain from eating or tooth brushing prior to the appointment [31].

Participants were given paraffin to collect 2-ml of stimulated whole saliva. A sodium hydrogen carbonate ($NaHCO_3$) tablet was added to the saliva to stimulate bacterial growth. The saliva sample was pipetted onto both agars of the dip slide to ensure they were entirely covered. The samples were incubated at 37° C for two days.

Scores 1 and 2 indicated low counts of a magnitude of 10^3 and 10^4 CFU/ml. Findings of scores 3 and 4 corresponded to more than 10^5 respectively 10^6 CFU of mutans streptococci or lactobacilli per ml of saliva. Scores 1 and 2 were considered to indicate low to moderate risk and scores 3 and 4 were considered to indicate high to extreme caries risk [32, 33].

Statistics

The prevalence and number of decalcifications were calculated. Multivariate analysis and t-tests were performed to determine the influence of factors such as diet, oral soft tissue health and salivary bacterial counts. Correlation between oral soft tissue health and salivary bacterial counts and the caries prevalence were determined using Spearman's Rho. Student t-tests were used to determine the influence of the dichotomous factors, gender and socio-economic status.

Inter- and intra-examiner correlation (Cohen-Kappa) for QLF detection of white-spots was determined on 12 randomly selected participants with a total of 288 teeth by repeated analysis of the QLF images obtained at T1. The images were examined for signs of decalcification by two examiners on two separate occasions, at least a week apart.

Results

Caries prevalence and influencing factors:

A total of 64 patients were recruited for the study. Two participants dropped out between the debonding and the 6-week recall visit. 62 participants remained of which 29 were male and 33 were female. Almost all participants (97%) had one or more decalcifications (visual- or QLF-examination). The prevalence of the affected teeth as recorded by QLF was 30 % at T0 and 31% at T1. One person had 94 % of the buccal surfaces affected and only two persons had no signs of decalcifications. Bar graphs, showing the caries distribution for the participants scored with QLF and visual-examination at T1, are given in Figure 3.

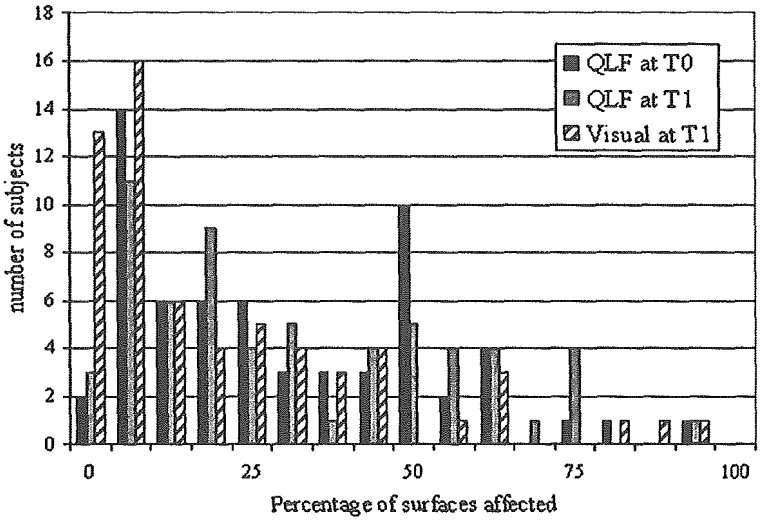

Figure 3. Frequency distribution for the study participants of the percentage of caries affected surfaces as scored by QLF at T0 and T1 and visually at T1.

Using QLF, 406 and 427 carious surfaces were recorded at T0 and T1 respectively, after correction for stains found on the white-light photographs. Average fluorescence loss for lesions scored at T0 was 10.7% (SD 5.8%) and at T1, 10.3 % (SD 5.8%). Of the initially recorded lesions at T0, 39 were no longer observed at T1, whereas 58 lesions not observed at T0, due to swelling of the gingiva, were observed at T1.The numbers of carious surfaces found visually at T0 respectively T1 were 284 and 285. The average fluorescence loss of the visually detected lesions as determined by QLF was 12.6%. Lesions visually detected had an average fluorescence loss in a part of the lesion of more than 15%.

Bar graphs show the caries distribution at T1 for the different tooth surfaces using QLF and visual examination (Fig. 4). A significant difference in number of lesions was found between incisors and canines on the one hand and molars and premolars on the other (t-test, $p<0.01$) indicating more carious lesions in the molar-premolar region. This effect was mainly due to the distribution in the lower jaw (Fig. 4). No significant differences were found between the left and right side of the mouth or upper and lower jaw. The distribution patterns found with visual and QLF examinations were similar. The inter-examiner Cohen-Kappa scores for QLF were determined to be 0.99 and 0.85 for each measurement cycle and the intra-examiner Cohen-Kappa was 0.77 and 0.78 for each examiner.

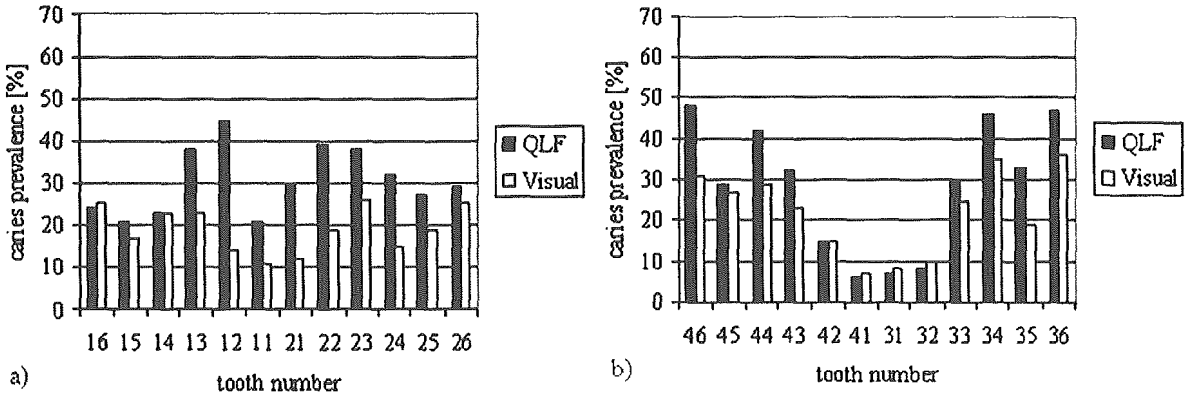

Figure 4. Numbers of lesions found with QLF and Visually at T1 per tooth element.

The participants were found to be evenly distributed over low, middle and high socioeconomic status (20 low, 25 middle and 17 high), but differences in socioeconomic status could not explain differences in caries prevalence. We did find a gender-specific difference in caries prevalence; in male patients 40% of the buccal surfaces were affected, whereas in female patients only 22% of buccal surfaces were affected by caries ($p<0.01$).
The age distribution was skewed due to a few outliers. The majority of participants, 79%, were between 12 and 18 years old with only 11% of subjects above 30 years old (median 16 yr, mean 19.5 yr, SD 9.6 yr). The average treatment period with fixed appliances was 25.4 months (SD 8.9 months) for the upper jaw and 23.9 months (SD 9.2 months) for the lower jaw. The eating frequency, with a mean of 7.7 sugar exposures (SD 2.3) was very similar for all participants. None of these aspects explained differences in caries prevalence.

A modest but statistically significant positive correlation of the gingival bleeding index at T1 with numbers of decalcified surfaces per person was found ($r=0.47$, $p=0.002$). Also, the lactobacilli scores at T0, just prior to debonding, were modestly correlated with the numbers of decalcified surfaces ($r=0.42$, $p=0.001$). In contrast, no correlation was found between mutans streptococci scores and the number of decalcifications. At T1, the correlation between lactobacilli and the number of decalcifications disappeared, but we did find a weak correlation for mutans streptococci ($r=0.27$, $p=0.037$). Lactobacilli scores and mutans streptococci scores just prior to debonding were higher than at the recall visit 6 weeks afterwards. At T0 82% of subjects were considered to be at high to extreme caries risk according to both the lactobacilli and the mutans streptococci counts. According to the mutans streptococci counts at T0 as many as 62% of the participants would be considered to be at extreme risk. At T1 50 % and 76 % of subjects were considered to be at high to extreme caries risk, based on lactobacilli or mutans streptococci scores, respectively. The effect of removal of the appliances was greater for lactobacilli scores than for mutans streptococci scores.

Longitudinal behavior of lesions:

At the six month retention visit fifty-eight participants were still included in the study, of which 29 were male and 29 were female. Using QLF, three subjects, all females, were considered caries free on their buccal surfaces. In the remaining 55 subjects, 421 carious surfaces were recorded immediately after

debracketing of which 15 lesions were lost from QLF analysis: 11 lesions (maximum fluorescence loss at debracketing >25%) in two subjects, 1 male and 1 female, were restored and 4 lesions were not analyzed because they were not imaged properly. Thus, a total of 406 lesions were followed the first 6 months. These lesions had an average fluorescence loss at debracketing (ΔF_0) of 10.3% (SD 5.4%, threshold 5%), varying from incipient (ΔF_0<10%, N=257) to advanced (ΔF_0>25%, N=12).

Overall, on a subject level, we found lesion regression during the first 6 weeks after debracketing and a further, yet smaller, lesion regression was found after 6 months ($p<0.01$). Despite the statistically significant difference between number of lesions in males (259) and females (158) found immediately after debracketing with QLF, no difference between males and females was observed in the lesion behavior during the first 6 weeks or after 6 months retention phase. The total fluorescence loss per subject corrected for the number of missing and filled surfaces was on average 83.6% at debracketing and changed to 79.0% after 6 weeks and 76.7% after 6 months ($p<0.01$).

ANOVA for repeated measures with lesions as entity, again showed a significant lesion regression during the first 6 weeks ($p<0.01$) and a further significant but smaller lesion regression after 6 months ($p<0.01$). When we divided the lesions into categories with different levels of fluorescence loss (See Table 1) we noticed that the incipient lesions with a fluorescence loss immediately after debracketing <10% on average showed a relative decrease in fluorescence loss of only 2% (SD 20%) after 6 months. This change was significantly ($p<0.01$) smaller than the relative change in fluorescence loss found for somewhat more advanced lesions with a fluorescence loss at debracketing >10%. For this category of lesions the relative change found was 12% (SD 20%). No influence of lesion area at debracketing on lesion behavior was found.

Table 1. The behavior of lesions during the first six months of retention after orthodontic treatment with fixed appliances categorized by fluorescence loss immediately after debracketing (ΔF_0).

ΔF_0 [%]	Number of lesions	Relative change in fluorescence loss during 6 months retention (SD) [%]
<10	251	2 (22) [a]
>10	149	12 (23) [b]
10-15	84	9 (26) [b]
15-20	42	15 (18) [b]
20-25	11	17 (16) [b]
>25	12	9 (13) [b]

a, b: Characters indicate significant differences between the categories of fluorescence loss at debracketing.

A small difference in lesion severity at debracketing was seen between upper (ΔF_0=9.8%, SD 4.5%) and lower jaw (ΔF_0=10.6%, SD 5.3%) ($p<0.05$), but this did not affect the regression of the lesions. The number of lesions on cuspids and molars was significantly higher than that on anterior teeth at the debracketing visit but no differences in lesion severity and lesion regression during the 6 month

observation period were found.

When we examined the data from the individual lesions over time with respect to fluorescence loss, we found 40 lesions that showed significant progression (Figure 5). These progressing lesions had fluorescence loss levels at debracketing between 5.1% and 14.8% and they were found in 14 males and 12 females predominantly on first molars (12) second pre-molars (9) and cuspids (11).

The majority of lesions (N=249, $\Delta F_0 \in$ (5.2%, 39.2%)) were considered to be stable in time and 117 lesions ($\Delta F_0 \in$ (5.9%, 35.0%)) regressed significantly (Figure 5). Regressing and progressing lesions were found in the same subjects.

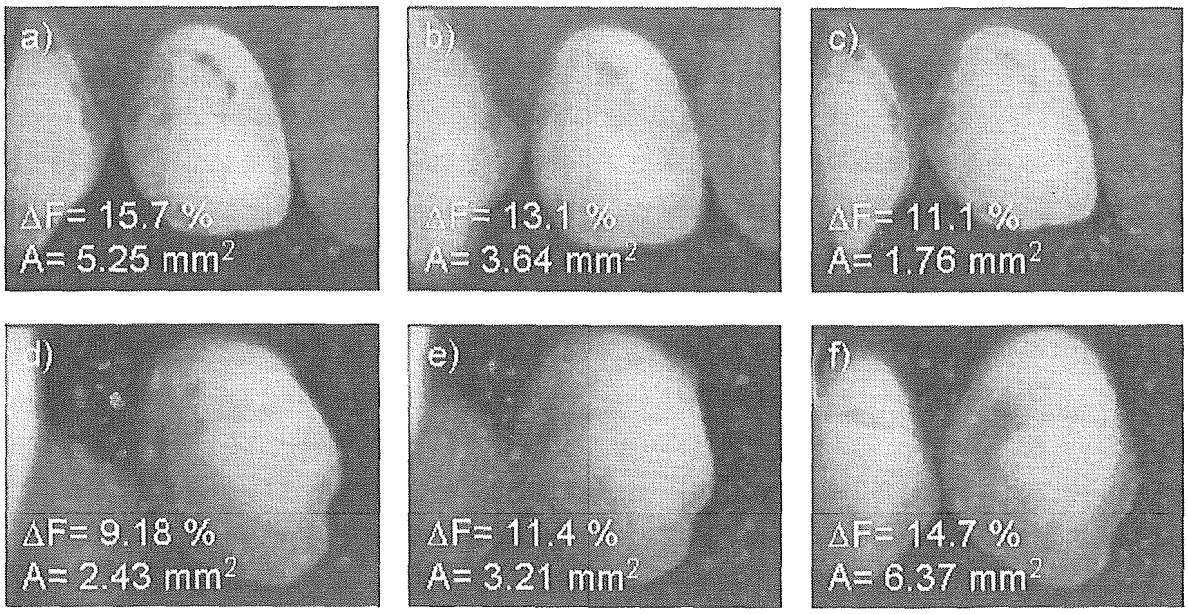

Figure 5. Typical examples of a regressing lesion (a-c) and a progressing lesion (d-f) imaged immediately after debracketing (a and d) and 6 weeks (b and e) and 6 months into the retention phase (c and f). The lesions were analyzed at each time point using the same 'patch' and surface contour to correct for differences in the visible lesion area, e.g. due to swollen gingiva covering part of the lesion at the debracketing visit. Thus it was ensured that at each time-point the same area of the tooth is analyzed for fluorescence loss and corresponding lesion area.

The two year data that was obtained in 51 participants, of which 24 were male and 27 were female. Of these, two subjects, both female, were considered caries free on their buccal surfaces. In the remaining 49 subjects, 370 carious surfaces were recorded immediately after debracketing. Of these, 5 lesions in 1 subject were restored by the 6 month visit and another 14 lesions in 5 other subjects were restored by the 2 year visit. Thus, a total of 351 lesions were followed over time (see Table 2). These lesions had an average fluorescence loss at debracketing (ΔF_0) of 10.0% (SD 4.8%, threshold 5%), varying from incipient (ΔF_0<10%, N= 227) to advanced (ΔF_0>25%, N= 6).

Table 2. Mean sum of Fluorescence loss (ΔF) per subject and the mean ΔF per lesion

	T0 = debracketing	T1 = 6 weeks retention	T2 = 6 months retention	T3 = 2 years retention
	Δ F [%] (s.d.)	Δ F [%] (s.d.)	Δ F [%] (s.d.)	Δ F [%] (s.d.)
51 subjects	75.2[a] (67.9)	71.6[a] (66.1)	69.0[b] (63.4)	66.3[b] (62.3)
351 lesions	10.0 (4.8)	9.5 (4.6)	9.2 (4.5)	8.8 (5.1)

a, b: Characters indicate the statistical differences between the times. Equal characters mean that there is no significant difference. P<0.05 was considered statistically significant

Overall, on a subject level, we found lesion improvement became significant from 6 months after debracketing (see Table 2). No differences were found between the debracketing and the 6 weeks retention visits or between the 6 month and two year retention visits. The total fluorescence loss per subject corrected for the number of missing and filled surfaces was on average 75.2% (SD 67.9%) at debracketing and changed to 71.6% (SD 66.1%) after 6 weeks, 69.0% (SD 63.4%) after 6 months and 66.3% (SD 62.3%) after two years. The lesion areas showed a similar pattern but less pronounced (see Table 3). A significant difference was found between 6 months and baseline visits, as well as between the two year and baseline or 6 weeks visits, yet no differences were found between any of the consecutive visits. The total lesion area per subject corrected for the number of missing and filled surfaces was on average 6.7 mm^2 (SD 10.9 mm^2) at debracketing and changed to 6.5 mm^2 (SD 11.5 mm^2) after 6 weeks, 6.1 mm^2 (SD 10.9 mm^2) after 6 months and 5.4 mm^2 (SD 9.7 mm^2) after two years.

Table 3. Mean sum of lesion area (A) per subject and mean area per lesion

	T0 = debracketing	T1 = 6 weeks retention	T2 = 6 months retention	T3 = 2 years retention
	A [mm^2] (s.d.)	A [mm^2] (s.d.)	A [mm^2] (s.d.)	A [mm^2] (s.d.)
51 subjects	6.7[a] (10.9)	6.5[a,b] (11.5)	6.1[b,c] (10.9)	5.4[c] (9.7)
351 lesions	0.9 (1.4)	0.86 (1.6)	0.82 (1.5)	0.71 (1.4)

a, b, c: Characters indicate the statistical differences between the times. Equal characters mean that there is no significant difference. P<0.05 was considered statistically significant.

Despite the statistically significant difference between numbers of lesions in males (24) and females (27) found with QLF at all four time points, no difference between males and females was observed in the lesion behaviour during the two year retention phase. When we examined the data from the individual lesions over time with respect to fluorescence loss (see Table 4), we found 35 lesions which became significantly worse after two years. The majority of lesions (n=171) were considered to be stable and 145 lesions improved significantly of which only 10 lesions improved to such an extent that the disappeared. Regressing and progressing lesions were found in the same subjects.

Table 4. The behavior of lesions categorized by fluorescence loss (ΔF) and area (A)

Lesions*	ΔF0→ΔF3	ΔF0→ΔF1	ΔF2→ΔF3	A0→A3	A0→A1	A2→A3
Worsened	35	47	39	27	21	19
Improved	145	75	88	71	33	40
Stable	171	229	224	250	297	292
Restored	19	3	11	19	3	11

* Four measure points: 0 = at debonding, 1 = 6 wks –, 2 = 6 months – and 3 = 2 yrs of retention

Discussion

Similar to earlier studies we found many lesions on the buccal surfaces as a result of orthodontic treatment with fixed appliances. These lesions varied from very initial to very advanced and some needed restorative treatment. In our study we examined the natural ability of such lesions to remineralize after removal of the fixed appliances.

In this study the caries distribution in the mouth found with QLF shows the same pattern as with visual examination, yet more lesions were found with QLF than with visual examination. This can be explained by our finding that all lesions detected by visual examination had a QLF determined fluorescence loss in part of the lesion of >15%, and thus using QLF lesions can be detected earlier. Caries detection with QLF seemed reliable and consistent over time. Quantification of lesion severity by determining fluorescence loss and lesion area is a great benefit compared to the qualitative and subjective data obtained by conventional visual examination. Quantification allows for monitoring of lesion progression or regression over time. The potential for QLF to be used, not only after orthodontic treatment, but also during this treatment, to follow caries development was shown in two studies, where de- and remineralization around orthodontic brackets were investigated in vitro [24, 25].

When we look at the results from our study we note that despite the overall lesion regression seen, there are lesions that progress. In fact nearly 15% of the lesions followed longitudinally showed significant progression, i.e. the fluorescence loss from the lesion was higher 2 years after debracketing than at the debonding visit. These results are comparable to the finding of an earlier QLF study on white spot lesions in 7 orthodontic patients were 15 lesions were followed [22]. That study reported an overall regression of 14 lesions over a period of 12 months, while one lesion progressed.

This study showed that the general expected improvement of white spot lesions after the appliances are removed and oral hygiene is restored, does not apply. 19 lesions were that severe, that the dentist found it necessary to restore these. Only 10 of the 370 lesions remineralized complete after two years, 145 white spots showed an improvement and 35 a worsening while a 171 lesions found after orthodontic treatment were considered stable after two years of retention. These findings are in contrast with the general belief that lesions regress once the appliance is removed and oral hygiene is restored. The overall lesion improvement seen in the current study is accounted for by only two fifth of the lesions. That and the loss of 16 lesions from the study due to restorative treatment make clear that removal of plaque stagnation sites by removing the fixed appliances alone is not enough to induce adequate remineralization of lesions. Furthermore, most changes in mineralization happened in the first six months after debonding. White spots still visible after this period of time did not disappear in the remaining retention period.

The patients in this study did not receive extra prevention measures, before during and after orthodontic treatment, except oral hygiene instructions and toothbrushing with fluoride toothpaste. Despite the presence of white spot lesions at debonding, the subjects were not given extra fluoride as it has been suggested that high doses of fluoride used on porous white spot lesions would affect only the outermost surface, thereby inhibiting the complete remineralization of lesions [2, 34]. Nowadays there is some evidence that the use of extra fluorides during fixed orthodontic treatment reduces the occurrence and severity of white spot lesions [2, 35, 36]. Still prevention of white-spot lesions is better than repairing lesions once they exist. Therefore research and orthodontic treatment has focussed mainly on prevention of white spots, for example by starting fixed appliance treatment only in plaque-free patients, providing a strict oral hygiene protocol during treatment and debonding when incipient white spot lesions become visible. Despite these prevention measures there will be patients that show un-aesthetic white spot lesions after debonding. How to treat these lesions best still remains a question. It seems that low doses of fluoride in mouth rinse after debonding does not improve these lesions [34]. Some authors argued to allow remineralization by saliva and if necessary to use acid micro abrasion 10 weeks after debonding [37]. The approach of natural lesion improvement in our study proved unsuccessful. Furthermore, given the high amount of lesions found at debracketing in our study population, research focussing on remineralizing strategies for these types of lesions is necessary next to ongoing research in finding more efficacious prevention treatments that can be used during the treatment phase with fixed appliances.

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Effects of Phosphoryl Oligosaccharide Calcium (POs-Ca) on Enamel Remineralization as measured by QLF™

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Abstract

It was previously suggested that phosphoryl oligosaccharides calcium (POs-Ca) is quite soluble as a Ca supply source and enhance remineralization of enamel. The aim of this study was to examine the combined effects of POs-Ca and fluoride on remineralization of enamel *in vitro* using Quantitative Light-induced Fluorescence (QLF™). The enamel slabs were prepared from bovine incisors and demineralized by immersion in a 0.1 M lactic acid gel containing 6 wt% carboxymethylcellulose (pH 4.5) at 37°C for 2 w. Demineralized enamel samples (ΔF=-36.7±3.1%, ΔZ=5,011±1,175 vol%•μm) were exposed to one of the following solutions up to 7 d: A: mineral solution containing 1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4 and 20 mM Hepes (pH 7), B: mineral solution containing 2-ppm F^- as NaF, C: mineral solution containing 0.1% POs-Ca and D: mineral solution containing 0.1% POs-Ca and 2-ppm F^- (n=6 per group). Finally, the samples were assessed by the QLF™ to quantify mineral changes. The ΔF value in the group D (-15.5±7.2%) was greater by 29% than that of the group C (-21.8±8.6%; p=0.099 by unpaired t-test) and significantly greater (p<0.01 by Tukey-Kramer multiple comparisons test) by about 50% compared to the groups A (-31.4 ±8.0%) and B (-31.1±4.0%). The ΔF values in this study significantly correlated (r=0.980; p<0.01) with the mineral loss values (ΔZ, vol%•μm). In conclusion, it was suggested that the QLF and TMR parameter values corresponded well and that POs-Ca may influence the potential of fluoride enhancing remineralization.

Introduction

Recently remineralization of enamel has been emphasized in dental health and the effects of functional foods and fluorides on remineralization have been assessed in governmental research projects by the Ministry of Health, Labor and Welfare of Japan since 2001[1]. In relation to this trend, it was previously suggested that phosphoryl oligo-saccharides calcium (POs-Ca) is highly soluble Ca source[2-5] and can enhance remineralization of enamel in vitro and *in situ*[6, 7].

One of the most important issues in the governmental research on remineralization is standardization of procedures to evaluate tooth mineral. Nowadays, tooth mineral can be assessed by various quantitative methods such as transversal microradiography as gold standard, Electrical Caries Monitor (ECM, Lode BV, The Netherlands), laser fluorescence (DIAGNOdent®, KaVo, Germany), DIFOTI and Quantitative Light-induced

Fluorescence (QLF™, Inspektor Dental Care BV, The Netherlands) . Of all the devices, QLF™ can be regarded as the most suitable system for standardized mineral assessment because of its various advantages as:

QLF™

1. allows non-destructive evaluation and longitudinal/ sequential assessments,
2. can be used in both of *in vitro* and *in vivo* investigations,
3. visualizes invisible de- and remineralization,
4. can store quantitative digital images and values for further analysis, and
5. can visualize pathogenic biofilm, calculus, sealant leakage, etc.

The aim of this study was to examine the combined effects of POs-Ca and fluoride on remineralization of enamel in vitro using QLF™.

Material and Methods

The enamel slabs were prepared from bovine incisors and demineralized by immersion in a 0.1 M lactic acid gel containing 6 wt% carboxy-methylcellulose (pH 4.5) at 37°C for 2 w. Demineralized enamel samples (ΔF=-36.7±3.1%, ΔZ=5,011±1,175 vol%•μm) were exposed to one of the following solutions (n=6 per group) up to 7 d:

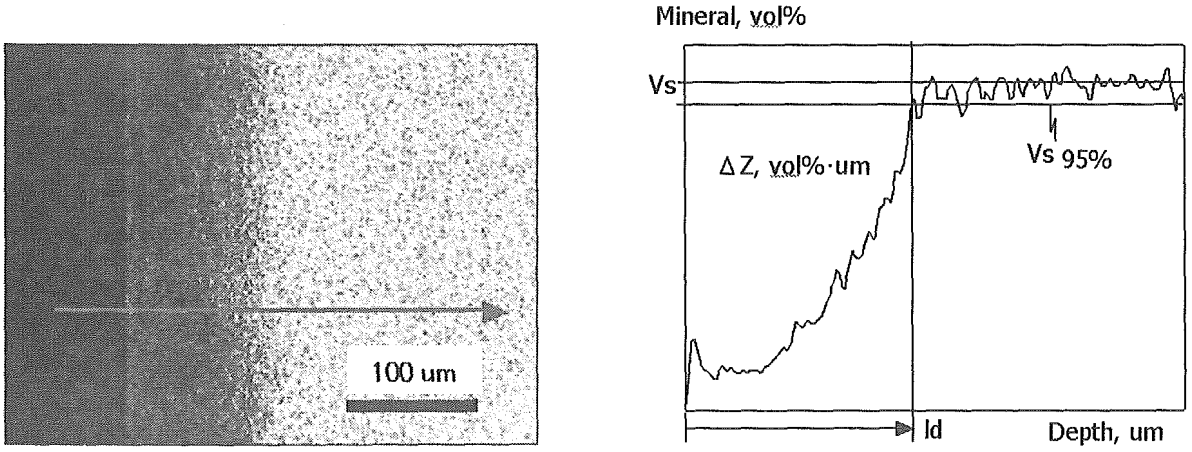
A: mineral solution (1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4 and 20 mM Hepes; pH 7)

B: mineral solution containing 2-ppm F^- as NaF

C: mineral solution containing 0.1% POs-Ca, and

D: mineral solution containing 0.1% POs-Ca and 2-ppm F^-

Finally, the samples were assessed by TMR and QLF™ to quantify mineral changes. TMR and videodensitometric analysis were described in detail by Inaba and Arends *et al.* previously[8, 9]. The mineral parameters of interests were shown in Fig. 1.



a. b.

Fig.1. Typical microradiographic image (a) and schematic mineral profile with mineral parameters; lesion depth ld (μm) and mineral loss value ΔZ (vol%•μm).