NICOLINE C.W. VAN DER KAAIJ

PREVENTION OF WHITE SPOT LESION FORMATION DURING TREATMENT WITH FIXED ORTHODONTIC APPLIANCES

The efficacy of using a fluoride rinse and repeated oral hygiene instructions

Prevention of white spot lesion formation during treatment with fixed orthodontic appliances:

The efficacy of using a fluoride rinse and repeated oral hygiene instructions.

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CHAPTER 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Orthodontics is the area of dentistry concerned with the growth of the craniofacial complex, the development of occlusion and the treatment of dentofacial abnormalities. Orthodontic treatment involves three aspects of the craniofacial complex, namely the dentition, the craniofacial bones- mainly the jaws- and the muscles of face and jaws. Attempts to correct malocclusion go back to the times of the Greek and Etruscans. It was not until around 1900 that Edward Angle developed the concept of occlusion and the classification of malocclusion (Angle, 1899). As time passed by it became clear that an excellent occlusion alone was unsatisfactory, because of aesthetic and stability problems. Thus, in orthodontic treatment the dental and facial aesthetics are nowadays more important than the details of orthodontic occlusion alone. Orthodontic treatment is common amongst both juveniles and adults. Most patients are treated for aesthetic reasons, only a small number of patients receive treatment because of medical or dental indications (Ackerman *et al.*, 2007). Treatment usually occurs with removable or fixed appliances.

White spot lesions in orthodontics

Before start of treatment, potential risk factors such as increased cariogenic challenge, must be considered. Removable appliances, such as a functional activator or an expansion plate, do not directly affect oral hygiene. Fixed orthodontic appliances can impair oral hygiene, and thus unwanted side effects as caries can occur. The first signs of decay around the brackets are called White Spot Lesions (WSL) and are subsurface enamel porosities (fig. 1). WSL are not only aesthetically unfavourable but may progress into cavitated lesions and are therefore an unwanted side effect of clinical relevance. The overall prevalence of WSL after treatment with fixed appliances ranges from 50%-97% (Gorelick *et al.*, 1982, Boersma *et al.*, 2005, Julien *et al.*, 2013). This number varies depending on the examination technique used, the length of the study and also the length of the orthodontic treatment plays a role. The highest incidence of WSL is found on the maxillary lateral incisors and mandibular molars, followed by the maxillary canines, premolars and central incisors (Chapman *et al.*, 2010, Lucchese and Gherlone, 2013).

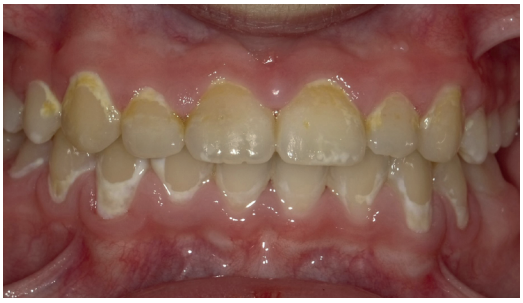


Figure 1. Image of the teeth of a patient with several WSL one week after removal of the fixed orthodontic appliances.

WSL can develop within four weeks after placement of fixed appliances (Øgaard *et al.*, 1988). The environment of the oral cavity changes after placing the fixed appliances. There is an increased number of plaque retention sites and thus more plaque accumulation (Naranjo *et al.*, 2006). This is due to the fact that the appliances hamper mechanical cleaning on surfaces normally showing low caries experience. There is also a shift in the plaque to a more periopathogenic population next to more accumulation (Naranjo *et al.*, 2006).

Cariogenic species such as *Streptococcus mutans* and *Lactobacillus* species and the subsequent decalcification of enamel were the main fields of interest around the 1980's (Mattingly *et al.*, 1983, Forsberg *et al.*, 1991, Rosenbloom and Tinanoff, 1991). Later on the complex system of periopathogenic microbes, more prominent after placing fixed appliances, became the main topic of interest. Naranjo *et al.* (Naranjo *et al.*, 2006) observed a transition in subgingival dental plaque after placement of fixed appliances. The plaque index and the gingivitis index increased significantly and *Porphyromonas gingivalis, Prevotella intermedia, Prevotella nigrescens, Tannerella forsythia*, and *Fusobacterium* species were significantly elevated in the experimental group after placement of brackets compared to a non-bracketed control group (Liu *et al.*, 2004, Naranjo *et al.*, 2006, Lucchese *et al.*, 2018). These modifications are also enhanced by the intake of carbohydrates; frequently used by adolescents (Jepsen *et al.*, 2017). The changes in oral microbiology are clinically expressed in most patients with an increase in gingival inflammation and, regardless of the level of oral hygiene, a gingival enlargement (Zachrisson and Zachrisson, 1972, Boyd and Baumrind, 1992, Gastel J *et al.*, 2008, Pinto *et al.*, 2017). Increased signs of inflammation, gingival swelling and pseudo pocket formation, particularly at the proximal areas are seen as a reaction. Overall the changes in gingival conditions produced by fixed appliances are transient with no permanent effect on periodontal parameters (Zachrisson and Zachrisson, 1972, Alstad and Zachrisson, 1979).

In contrast, the WSL developed during treatment with fixed appliances, were shown to have limited ability to regress after removal of the appliances. WSL are still visible over one year after debonding, with a small number that progress into cavitated lesions (Mattousch *et al.*, 2007, Beerens *et al.*, 2015, Beerens *et al.*, 2018). Orthodontically treated patients had a significantly higher prevalence of WSL than a control group of non-treated subjects even five years after treatment (Øgaard, 1989). Reducing the formation of WSL is, therefore, essential and this is called primary prevention. Orthodontists recommend their patients to brush their teeth at least twice daily with fluoridated toothpaste and to use additional dental aids, such as a proxy brush. Next to this, extra products are prescribed, such as rinses or varnishes containing fluoride or chlorhexidine. Orthodontists also recommend diets that avoids foods that may accidentally debond the brackets or increase the risk of dental caries or erosion (Oosterkamp *et al.*, 2016). When a WSL is detected, secondary prevention is needed to avoid or reduce further demineralization and increase remineralization of the enamel. A novel method to longitudinally follow WSL and to make plaque visible is the use of Quantitative Light-induced Fluorescence (QLF).

WSL & plaque assessment: Quantitative Light-induced Fluorescence

The QLF technique is based on the property of tooth-tissue to autofluoresce when illuminated by visible light. Changes in mineral content of tooth-tissue can be made visible because of an altered fluorescence radiance resulting in a reduced green fluorescence (fig. 2) (de Josselin de Jong *et al.*, 2009). Concentrations of porphyrins in bacterial plaque show an enhanced red fluorescence (fig. 3). One of the main advantages of QLF is that lesions may be detected earlier than through conventional visual inspection (Heinrich-Weltzien *et al.*, 2005). QLF can be used for longitudinal assessment of WSL, and it has been shown to detect and quantify early demineralization of enamel (Hafstrom-Bjorkman *et al.*, 1992, Boersma *et al.*, 2005, Mattousch *et al.*, 2007). In non-bracketed population QLF can be used for monitoring lesions over time (Tranaeus *et al.*, 2002). Orthodontic studies showed that QLF images captured under the same circumstances, that is using the same camera angle, can be reproducibly quantified in vitro (Benson *et al.*, 2003, Pretty *et al.*, 2003, Aljehani *et al.*, 2004).

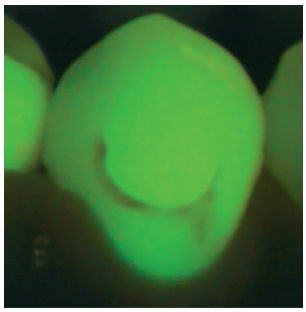
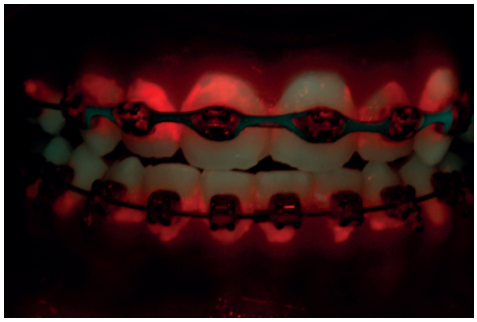


Figure 2. A QLF image using an intra-oral QLF camera (QLF/Clin; Inspektor Research Systems, Amsterdam, the Netherlands). A WSL is seen as reduced green fluorescence just cervical to the place where the bracket had been situated. This camera was used for the study presented in chapters 2 and 3.

Figure 3. A QLF image using a QLF-digital Biluminator camera (QLF-D Biluminator™ 2; Inspektor Research Systems, Amsterdam, the Netherlands). The mature plaque is seen as red fluorescence around the brackets. This camera was used for the studies presented in chapters 4 and 5.



Studies also demonstrate that by sharing visual QLF images with patients, and pointing out lesions, patients are motivated to improve oral hygiene (Tranaeus *et al.*, 2001). In research environment QLF has proven to be a reliable tool for assessing plaque accumulation in vivo on non-bracketed teeth (Tranaeus *et al.*, 2001, Pretty *et al.*, 2005, de Josselin de Jong *et al.*, 2009). For measuring WSL it is known that lesions adjacent to the gingiva or affected by a swollen gingiva are more difficult to analyze. This may also be a problem in orthodontics for the plaque assessment.

Primary prevention of WSL: fluoride

Fluoride is important in the prevention of dental decay in the general population (ten Cate, 2013) and should be used in high caries risk patients. In the Netherlands, many orthodontists recommend the use of a daily fluoride mouthrinse throughout treatment with fixed appliances (Kerbusch *et al.*, 2010). Various forms of fluoride administration may be prescribed during orthodontic treatment. These include topical fluorides as mouthrinses, gel or varnishes or fluoride-releasing materials as glass ionomer for bonding brackets.

A Cochrane review concluded that the use of a topical fluoride varnish applied professionally every six-weeks during orthodontic treatment reduces the incidence of WSL formation (Benson *et al.*, 2013). Also, after using topical fluoride there is a reduction in lesion severity (Stecksen-Blicks *et al.*, 2007). Furthermore, the use of a high-fluoride toothpaste instead of regular fluoride toothpaste resulted in fewer WSL (Sonesson *et al.*, 2014). Research concerning the daily use of a fluoride mouthrinse is less convincing and well conducted evidenced based research is lacking, as concluded by the authors of the Cochrane review. In general dentistry the use of a fluoride mouthrinse is advocated for high caries risk patients (Marinho *et al.*, 2003). An often-mentioned problem of this daily use of a rinse is the compliance, especially for adolescents. In orthodontics it is shown that more compliant patients have fewer WSL, but also that the compliance of fluoride rinse usage was about 50% (Geiger *et al.*, 1988).

Primary prevention of WSL: oral hygiene instructions

Assessing a patient's oral hygiene at each visit is part of the routine oral examination for the dentist and orthodontist. Regular oral hygiene reinforcement can be used to prevent the formation of WSL during fixed appliances. For the improvement of oral hygiene several techniques are used. Clinical investigations have shown that repeated oral hygiene instructions reduce the plaque accumulation (Acharya *et al.*, 2011, Lalic *et al.*, 2012). Likewise, the use of visual aids, such as an image of the severe consequences of biofilm accumulation (Peng *et al.*, 2014) or showing photographs taken intra-orally during treatment (Miller *et al.*, 2016), can decrease the plaque accumulation. Methods used for this chairside motivation and feedback also include the use of plaque disclosing methods or Quantitative Light-induced Fluorescence (QLF).

Since plaque is generally colorless, it can be stained for a better assessment and visibility. Common disclosing agents used are erythrosine, a pink-dye (E127), sometimes combined with a blue-dye (E133). These disclosing agents adhere to the plaque and which remains visible for the patient after water rinsing. Studies have shown that oral hygiene instructions together with plaque self-visualization through disclosing agents and a mirror resulted in an improvement of oral hygiene and gingivitis in non-orthodontically treated children (Bellini *et al.*, 1974, Telford and Murray, 1974).

Outline of this thesis

The overall topic of this PhD thesis was the prevention of White Spot Lesions formation during orthodontic treatment. The first chapters describe a Randomised Controlled Clinical Trial about the effects of the use of a fluoride rinse on the formation of white spot lesions and the microbiome during treatment with fixed appliances.

In **chapter 2** the RCT is outlined. This study aimed to compare daily uses of a placebo rinse versus a fluoride rinse during treatment with fixed appliances. To measure the WSL a QLF device was used. QLF images of buccal surfaces of all teeth in upper and lower jaw from second premolar to second premolar were captured before, during and after treatment with fixed appliances. Besides to the formation of WSL bleeding scores were assessed.

Chapter 3 focusses on the changes in the microbiome of same sample presented in chapter 2. The microbial changes were measured using next-generation sequencing of the bacterial 16S rRNA gene at different moments: before treatment, the first three months into treatment with fixed appliances, immediately before removal of the appliances and until three months after debonding.

Since the use of QLF during treatment with fixed appliances appeared to be difficult, because of movement of the teeth and the presence of a bracket, wire and other accessories, **chapter 4** describes an in vitro study about the reproducibility of QLF measurements and orthodontics. QLF images of WSL were captured directly cervical of a bracket on extracted incisors and canines. Different angles of rotation towards mesiodistal and buccolingual were simulated, and images with bracket, with a wire and elastic ligature and without a bracket were made to test the reproducibility of the WSL measurement.

Besides a fluoride-rinse, proper oral hygiene during treatment is very important to prevent the formation of WSL. Therefore, in **chapter 5** a study is presented on the effect of three different repeated oral hygiene instructions using 3 different feedback methods. The feedback methods used were 1; showing the plaque on QLF images of the teeth of the patient, 2; using erythrosine as disclosing agent to make the plaque present visible and 3; showing the patient in a mirror using a probe to point out the presence of plaque.

In **chapter 6** a general discussion is presented and advices are given on how to prevent the formation of WSL during orthodontic treatment with fixed appliances.

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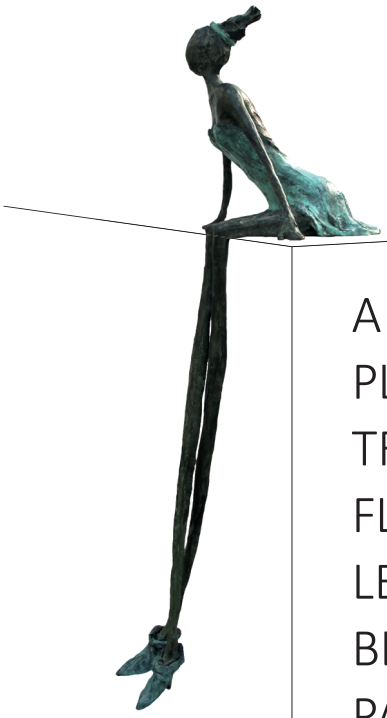

CHAPTER 2

A PROSPECTIVE, RANDOMIZED PLACEBO-CONTROLLED CLINICAL TRIAL ON THE EFFECTS OF A FLUORIDE RINSE ON WHITE SPOT LESION DEVELOPMENT AND BLEEDING IN ORTHODONTIC PATIENTS

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ABSTRACT

Demineralizations around orthodontic brackets are a main disadvantage of orthodontic treatment. To prevent their development several methods have been advocated, such as fluoride rinses or varnishes. In this randomized clinical trial a fluoride rinse (combination of sodium-fluoride and amine-fluoride) was compared with a placebo rinse, to be used every evening after tooth brushing. A total of 81 participants (mean age 13.3 years) completed the study (mean treatment period 24.5 months). Demineralizations, measured using Quantitative Light-Induced Fluorescence, and Decayed, Missing and Filled Surfaces (DMFS) were assessed before treatment (baseline) and around six weeks after debonding (post treatment). Bleeding scores were measured at baseline, during and post treatment. The Incidence Rate Ratio for demineralizations was 2.6 (95% CI 1.1-6.3) in the placebo group versus the fluoride group. In the fluoride group 31% of the participants developed at least one demineralization, compared to 47% in the placebo group. Relative to baseline, gingival bleeding increased significantly in the placebo group one year after start of treatment and onwards. For the fluoride group bleeding scores during treatment were not different from those at baseline. In conclusion, using a fluoride rinse helps to maintain better oral health during fixed appliance treatment, resulting in fewer demineralizations.

INTRODUCTION

Most orthodontic patients are treated because of aesthetic reasons with only a minor part of patients receiving orthodontic treatment due to medical or dental indications (Ackerman, 2010). Any potential disadvantages, such as demineralizations, must therefore be taken into account before a treatment starts. The environment in the adolescent oral cavity will be affected by the placement of fixed orthodontic appliances, changing the microbial composition and increasing the number of retention sites and thus plaque formation (Naranjo *et al.*, 2006, Gastel J *et al.*, 2008). This disturbance of a balanced microbial ecology may in turn contribute to oral diseases such as caries (Crielaard *et al.*, 2011) and periodontitis (Socransky, 1977, Loesche, 1996). Because clinical investigations have shown that generalized gingivitis develops within one or two months of placement of fixed appliances (Zachrisson and Zachrisson, 1972), good oral hygiene is an important prerequisite for sustaining oral health during orthodontic treatment (Kloehn and Pfeifer, 1974, Atack *et al.*, 1996) and also for preventing the formation of white spot lesions (WSL) in enamel (Øgaard *et al.*, 1988). According to the literature the prevalence of WSL ranges between 50-97% (Gorelick *et al.*, 1982, Boersma *et al.*, 2005, Al Maaitah *et al.*, 2011, Julien *et al.*, 2013), depending on the examination technique used and the duration of treatment.

Clinical studies have used several methods to detect and measure WSL, based either on clinical indices (Gorelick *et al.*, 1982), on photographic examinations (Millett *et al.*, 1999), or on other optical methods such as quantitative light-induced fluorescence (QLF) (Boersma *et al.*, 2005, Mattousch *et al.*, 2007). QLF is an optical, visible light-based system that can be used to detect and quantify early demineralization of enamel.

Various methods of reducing the formation of WSL have been described, including the improvement of oral hygiene and the use of additional fluoride such as in varnishes or rinses. The most common oral hygiene protocol recommended by orthodontists is probably a daily 0.05% sodium-fluoride rinse in conjunction with fluoridated toothpaste (Derks *et al.*, 2007). But although this recommendation is based on research showing that the use of sodium-fluoride rinse significantly reduces caries rates in non-orthodontic patients, the evidence with regard to its efficacy in preventing WSL in orthodontic patients is inconclusive (Benson *et al.*, 2013). Some moderate evidence is found that fluoride varnish applied every six weeks during orthodontic treatment is effective (Stecksen-Blicks *et al.*, 2007, Benson *et al.*, 2013).

In this randomized clinical trial (RCT), we compared a fluoride rinse (combination of sodium-fluoride and amine-fluoride) with a placebo rinse in preventing WSL in patients undergoing orthodontic treatment with fixed appliances. WSL incidence was assessed by QLF.

METHODS

Study population and clinical procedures

An RCT was performed, under normal practice settings, on the efficacy of a fluoride rinse during orthodontic treatment with fixed appliances. Approval of the Medical Ethical Committee of the VU Medical Centre of the VU University of Amsterdam was obtained for this study (VU-METc 2009/026 and Dutch trial register: NTR1817).

The study was conducted at the Department of Orthodontics of the Academic Centre for Dentistry Amsterdam (ACTA). Patients who were scheduled for full fixed orthodontic appliances at ACTA were eligible to participate after written informed consent. Patients needed to fulfill the following criteria: (1) age between 10-18 years, (2) good general health, (3) no use of medication, and (4) no demineralizations in need of restauration present at a buccal surface. All patients selected for this study received fixed appliances in both jaws. The brackets used were Roth Ovation Brackets (Dentsply GAC international, Bohemia, New York, USA). After placement of the fixed appliances the participants were randomly assigned to rinse A or B, which contained either 250 ppm fluoride (100 ppm amine-fluoride and 150 ppm sodium-fluoride) (Elmex caries protection, Colgate-Palmolive Europe, Therwil, Switzerland) or was a fluoride-free placebo rinse (also provided by Colgate-Palmolive Europe), further mentioned as fluoride respectively placebo. The bottles with rinse were identical in appearance, consistency, taste and smell. This was tested and regulated by Colgate-Palmolive Europe. Allocation of study id was determined by order of inclusion and appointment scheduled by reception persons. Assignment occurred at the first appointment by using a pre-defined randomization list (made in Microsoft Office Excel 2003). Participants were informed that they could receive either a rinse containing fluoride or a placebo rinse. Participants, examiners, statistician and treating orthodontic postgraduates were blinded for test and placebo product type. During the study period participants were instructed not to use any other fluoride containing products other than fluoride toothpaste. The participants' dentist was informed about the ongoing study and instructed not to apply extra fluoride during the study period. All examinations were done, mainly by the researcher (NK) and by trained dental students. Approximately one week before the placement of the fixed appliances (T0) QLF images were made and an intra-oral examination was performed. QLF images of buccal surfaces of all teeth in upper and lower jaw from second premolar to second premolar were captured. Participants were clinically examined using the Decayed, Missing, and Filled Surface (DMFS) Index (World Health Organisation, 1997) and the International Caries Detection and Assessment System (ICDAS) (Pitts, 2004, Ismail *et al.*, 2007, Topping and Pitts, 2009) followed by assessment of gingival bleeding. Participants were assessed, at regular intervals (approximately 6 weeks (T1), 3 months (T2) and every 6th month after the placement (T3 and further)) during the orthodontic treatment to stimulate optimal oral hygiene, to supply the rinse and to look for unwanted signs of developing

caries. At these intervals bleeding was also recorded. At the day of debonding (TD) and around 6 (TD1) and 12 (TD2) weeks after debonding DMFS, ICDAS and bleeding scores were assessed and QLF images were made to quantify the WSL. The caries assessments of TD1 were used for data analyses. Figure 1 shows a flowchart with the different time points and measurements. The end of data-collection was set at January 2013. After analysing all data obtained the code regarding the rinse was broken.

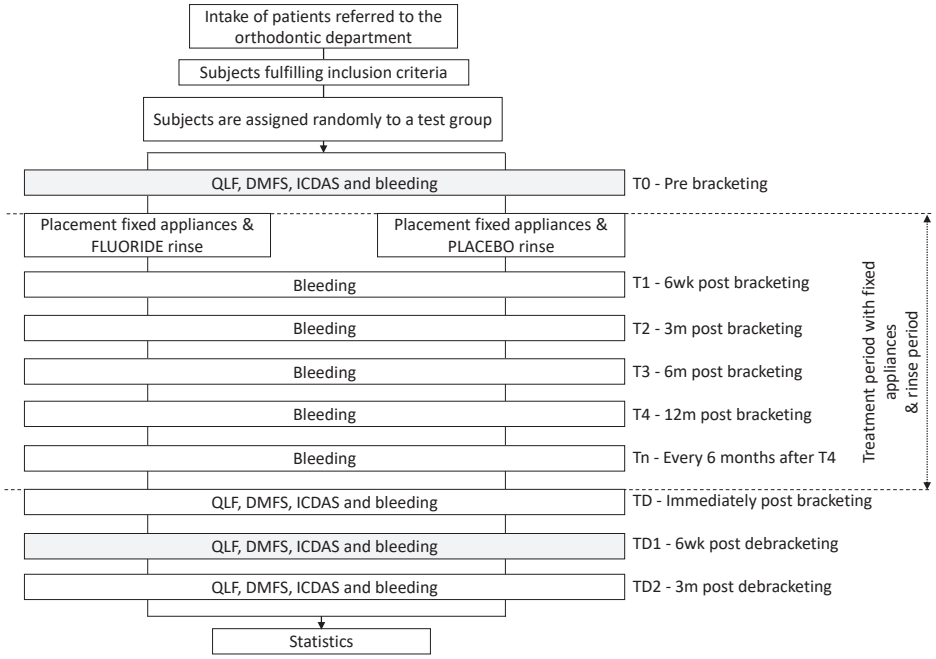

Figure 1. Flowchart showing the different time points and corresponding assessments. The caries assessments of TD1 were used for the analyses.

Primary study parameter

The main study parameter was the number of caries white spot lesions as found by QLF, developed during the treatment with fixed orthodontic treatment.

Secondary study parameters

Secondary study parameters were the ICDAS-score and DMFS measured before and after fixed appliance treatment; the bleeding scores per participant measured at different time points during the fixed orthodontic appliance treatment; and the lesion extent of the WSL as determined by QLF (fluorescence loss and lesion area) after debonding.

Power analysis

No earlier performed studies are known. A power analysis was performed for an effect of 0.25 (with a power of 0.8 and a significance level of 0.05). This results in a total of 94 participants or 47 participants per study group. To compensate for participant attrition, we aimed to include 120 patients in total.

QLF imaging; WSL measurements

Fluorescence images of the (to be) bonded buccal surfaces were captured using an intra-oral fluorescence camera (QLF/Clin; Inspektor Research Systems, Amsterdam, the Netherlands) (Angmar-Mansson and ten Bosch, 2001, de Josselin de Jong *et al.*, 2009). Dedicated software (Inspector-Pro version 2.0.0.48; Inspektor Research Systems) was used to assess the QLF images after debonding (fluorescence loss i.e. white spots). QLF images were analysed for fluorescence loss (ΔF) and size of lesion area (A) using a threshold of 5%, at TD1 in comparison with the QLF images made at T0. If caries was present at T0, the results were subtracted from TD1, using the method described by Mattousch (Mattousch *et al.*, 2007). The number of lesions per participant was calculated and for every participant having at least one lesion mean ΔF and area were calculated.

The measurements were done by the same examiner (NK). The examiner (NK) was trained and calibrated for QLF assessments against experienced examiner (MV) prior to study start. Inter- and intra-observer reliability were established at a random sample of 10% of the participants with an interval of two weeks. The inter-examiner ICC scores for QLF were 0.92 for the ΔF and 0.96 for the lesion area. The intra-examiner ICC was 0.94 for ΔF and 0.98 for the lesion area.

DMFS

DMFS of all participants was scored by examining all teeth with the use of a mouth mirror, an explorer and optimal light (World Health Organisation, 1997). Also, radiographs (ortho-pantomographs and when available bitewings or solo-images) were checked carefully. The D-portion comprised all surfaces with signs of decay diagnosed clinically as caries lesion with enamel breakdown. To determine the M-portion of the DMFS, only surfaces missing due to caries were counted. Teeth extracted for orthodontic purposes were not included. Restorations made because of trauma were excluded from the F-portion.

ICDAS

Before placement (T0) and after removal of the appliances (TD, TD1 and TD2) the buccal surfaces from all bonded teeth were examined using the ICDAS assessment system (Pitts, 2004, Ismail *et al.*, 2007, Topping and Pitts, 2009). Each buccal surface received a code from 0 to 6 to express the degree of caries.

Code 0 depicts sound surface without change after air-drying except for stain, hypoplasia, wear, erosion and other non-caries phenomena.

Code 1 is given for first visual change in enamel, seen after air-drying.

Code 2 is given if a distinct visual change (white or colored) is seen on wet enamel surface.

Code 3 is given when local enamel breakdown is present, but without visible dentine.

Codes 4 to 6 are given to cavitated lesions with increasing severity.

Average ICDAS-scores were calculated for each participant.

Bleeding score

During each visit, a gingival bleeding score was determined by probing each (to be bonded or bonded) tooth mesiobuccal and distobuccal with a periodontal probe. Based on the percentage of the bleeding sites, bleeding scores 1 to 5 were given for the whole mouth (thus per participant).

Score 1 (good)- if none to 5% of the sites were bleeding.

Score 2 (medium/good) – if 6 to 10% of the sites were bleeding.

Score 3 (medium) – 11 to 20% of the sites bleeding.

Score 4 (medium/poor) – 21 to 35% of the sites bleeding.

Score 5 (poor) – if more than 35% of the sites were bleeding.

Statistical analyses

The statistical analyses were performed using IBM SPSS 20.0 and Stata (Intercooled Stata 10.0; Stata Corporation, College Station TX, USA).

We estimated the difference in number of WSL (primary end point) and DMFS score between participants with fluoride and placebo rinses, using a regression model. Because both number of WSL and DMFS are count variables and our data were over-dispersed (variance much greater than mean), we used negative binomial regression (Grainger and Reid, 1954, Bohning *et al.*, 1999). Likelihood ratio tests comparing our negative binomial models to Poisson regression models were used to evaluate our decision. Because caries lesion data often exhibit an excess number of zeroes (Preisser *et al.*, 2012), we also compared our negative binomial models to zero inflated negative binomial regression models using the Vuong test. Both tests indicated a negative binomial regression model offered the best possible fit. Results were expressed using the estimated Incidence Rate Ratio (IRR) and 95% Confidence Intervals (95% CI) were constructed.

To investigate possible confounding by treatment duration, bleeding, DFMS or ICDAS scores at T0 we added these parameters to the model. None of them induced a change of more than 10% in the estimated IRR, so no confounding was present.

Differences in ICDAS, fluorescence loss and lesion area were tested by means of a Mann-Whitney U test. A Wilcoxon signed ranks test was used to compare the bleeding score at different time points, with a Bonferroni correction for multiple comparisons.

RESULTS

Descriptive results

A total of 120 participants were entered into the study between April 2009 and January 2011. Nine participants declined further participation immediately after T0 (approximately one week before bonding), at placement of the fixed appliances, thus not receiving the allocated rinse. Eleven further participants declined to participate later during the study, in addition to one participant who moved away and one who failed to show up for appointments. Eighty-one of the 98 remaining participants were debonded before January 2013. This point was chosen to end the study according to protocol. At the study end point 17 participants were expected to continue treatment with fixed appliances for more than three months, exceeding the study period due to unforeseen treatment complications or non-compliance. The mean treatment time was 24.5 (SD 5.5) months. A flowchart for all different analyses is shown in Figure 2.

Table 1. Characteristics of the study group at baseline (T0).

Characteristic	Fluoride (*n* = 36)	Placebo (*n* = 45)	All (*n* = 81
Age (yr)*	13.1 (10.0-16.6)	13.6 (11.7-16.5)	13.3 (10.0-16.6)
Male gender, *n* (%)	14 (38.9)	21 (46.7)	35 (43.2)
Treatment duration (months)*	25.0 (12.0-36.3)	24.1 (13.3-37.6)	24.5 (12.0-37.6)
DMFS score ***			
0	67	58	62
1-2	11	24	19
3-4	19	7	12
≥ 5	3	4	7
DMFS score overall**	0 (14)	0 (13)	0 (14)
ICDAS			
0.00	47	53	51
0.01-0.05	17	11	14
0.06-0.15	17	18	17
≥ 0.16	20	18	18
ICDAS score overall**	0.05 (0.8)	0 (0.7)	0 (0.8)
Bleeding*	2 (1-4)	1 (1-3)	1 (1-4)

Data are expressed as *mean (range), **median (maximum), or ***percentage.

Table 1 contains baseline data; there were no significant differences between groups. There were no WSL present at baseline. Caries data of 6 weeks after debonding (TD1) were used for 77 participants. For three, who missed appointment TD1, the WSL assessments (QLF, DMFS and ICDAS) from immediately post-debond (TD) were used, and for one the QLF pictures, due to malfunction of the QLF-device were only made at TD2. The WSL assessments were made at an average of 52 days after debonding (with a range from 0-156 days).

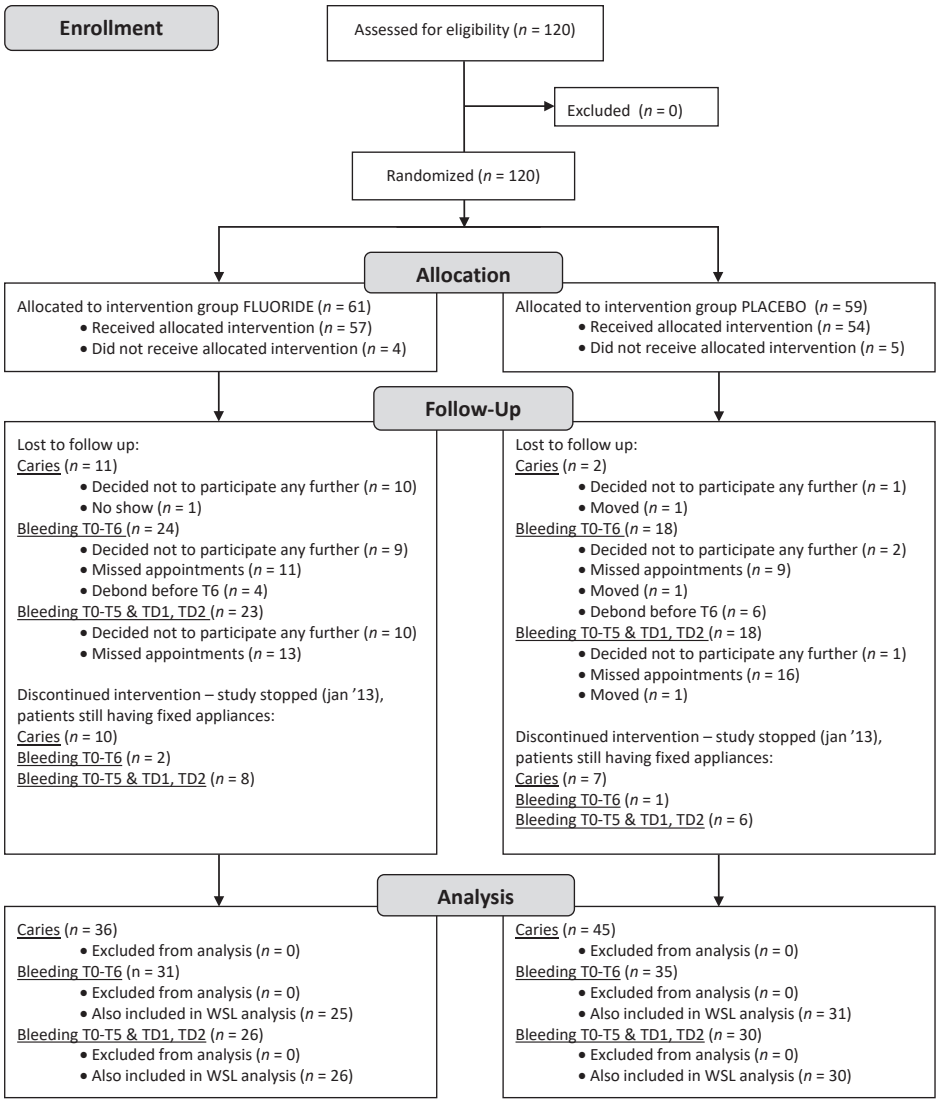

Figure 2. Flowchart showing the participant follow up during the study and the number of participants available for each analysis.

Given the difference in treatment duration between participants and taking account of the loss to follow-up, the bleeding data were assessed in two separate steps: complete dataset from baseline until T6 and complete dataset from baseline until T5, TD1 and TD2. A total of 66 participants (31 fluoride group, 35 placebo) were analysed who had a complete dataset regarding bleeding from start (T0) until two years after placement of fixed appliances (T6; mean 727 days). Of these participants, 25 fluoride and 31 placebo participants were also analysed for WSL after debonding. 56 participants (26 fluoride group, 30 placebo) were analysed with a complete dataset regarding bleeding from start (T0) until one year and six months after placement of fixed appliances (T5; mean 555 days) and TD1, 6 weeks after debonding (mean 50 days) and TD2 3 months after debonding (mean 99 days). Since many appliances were removed between T5 and T6, T6 was excluded from this analysis.

QLF-results

WSL counts

Out of 81 participants, 32 participants had developed at least one WSL (39.5%). In the fluoride group, 11 out of 36 participants developed at least one WSL (30.6%), ranging from one to five WSL per participant (fig. 3). In the placebo group, 21 out of 45 participants had at least one WSL (46.7%), with a range of one to 15 WSL per participant. Participants in the placebo group had an IRR of 2.6 (95% CI 1.1-6.3) compared to fluoride rinse (*P*=0.038). DMFS, ICDAS, and bleeding at T0 and treatment duration were no confounders.

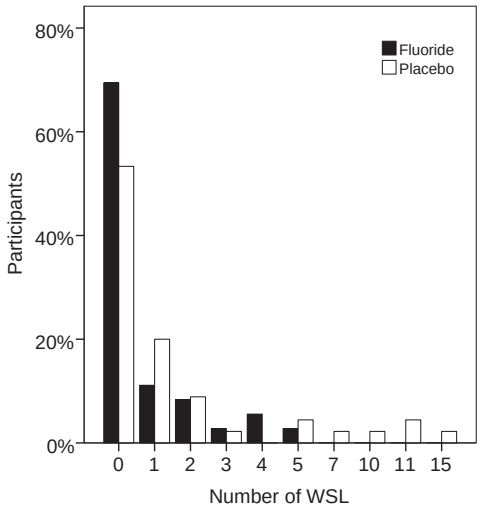

Figure 3. Percentage of total participants and their white spot lesion counts, 52 days after debonding. In the fluoride group 69.4% of the participants were WSL-free, compared to 53.3% in the placebo group after treatment with fixed orthodontic appliances.

Fluorescence loss; ΔF [%] and lesion area [mm^2]

The mean ΔF and mean area was calculated for each participant with at least one WSL (fluoride group n= 11 and placebo group n =21). The mean ΔF was 10.3% (SD 3.0) for placebo

Table 2. Results, 6 weeks post debonding (TD1), according to study group and for the total group.

Variable	Fluoride		Placebo		All	
	n	TD1	*n*	TD1	*n*	TD1
WSL						
Number	36	0 (5)*	45	0 (15)*	81	0 (15)
Mean ΔF (%)	11	11.6 ± 5.0	21	10.3 ± 3.0	32	10.7 ± 3.8
Mean A (mm^2)	11	0.9 ± 0.6	21	1.3 ± 1.6	32	1.15 ± 1.4
DMFS	36	0 (26)	45	1 (13)	81	1 (26)
ICDAS	36	0.05 (0.6)	45	0.05 (1.7)	81	0.05 (1.7)

Data are expressed as the median (maximum), or as mean ± SD.
* Significant difference ($P < 0.05$) between the fluoride and placebo groups.

participants and 11.6% (SD 5.0) for fluoride participants. Mean area was 1.3mm^2 (SD 1.6) for placebo participants and 0.9mm^2 (SD 0.6) for fluoride participants. At a mean of 52 days after debonding there were no statistically significant differences in mean ΔF and mean area of the lesions per participant between both groups (table 2).

DMFS-results

DFMS scores at TD1 ranged from 0 to 13 in the placebo group and from 0 to 26 in the fluoride group (table 2). In the fluoride group there was one outlier, this subject started with a DMFS of 14 and after debonding had a DMFS of 26. Both groups showed a significant increase in DMFS between T0 and TD1. A negative binominal regression analysis showed that there were no differences in the DMFS between both groups.

ICDAS-results

There were no significant differences between the placebo and fluoride rinse regarding the ICDAS scores after debonding. Also no significant difference between T0 and TD1 were found regarding the ICDAS scores (fluoride: *P*=0.88 and placebo: *P*=0.06) (table 2).

Bleeding

Bleeding T0-T6

Gingival bleeding scores of the individuals receiving the placebo rinse were significantly higher at three points: visit T4, one year since start of treatment (mean 373 days) *(P*=0.02); visit T5, one year and six months (mean 546 days) since start of the treatment (*P*=0.00); and visit T6, 2 years (mean 718 days) since the start of the treatment (*P*=0.00) compared with the respective baseline visit scores. For the group receiving the fluoride rinse, this difference did not reach statistical significance (fig. 4). A Mann-Whitney U test showed that there were no differences between the groups at the different time points.

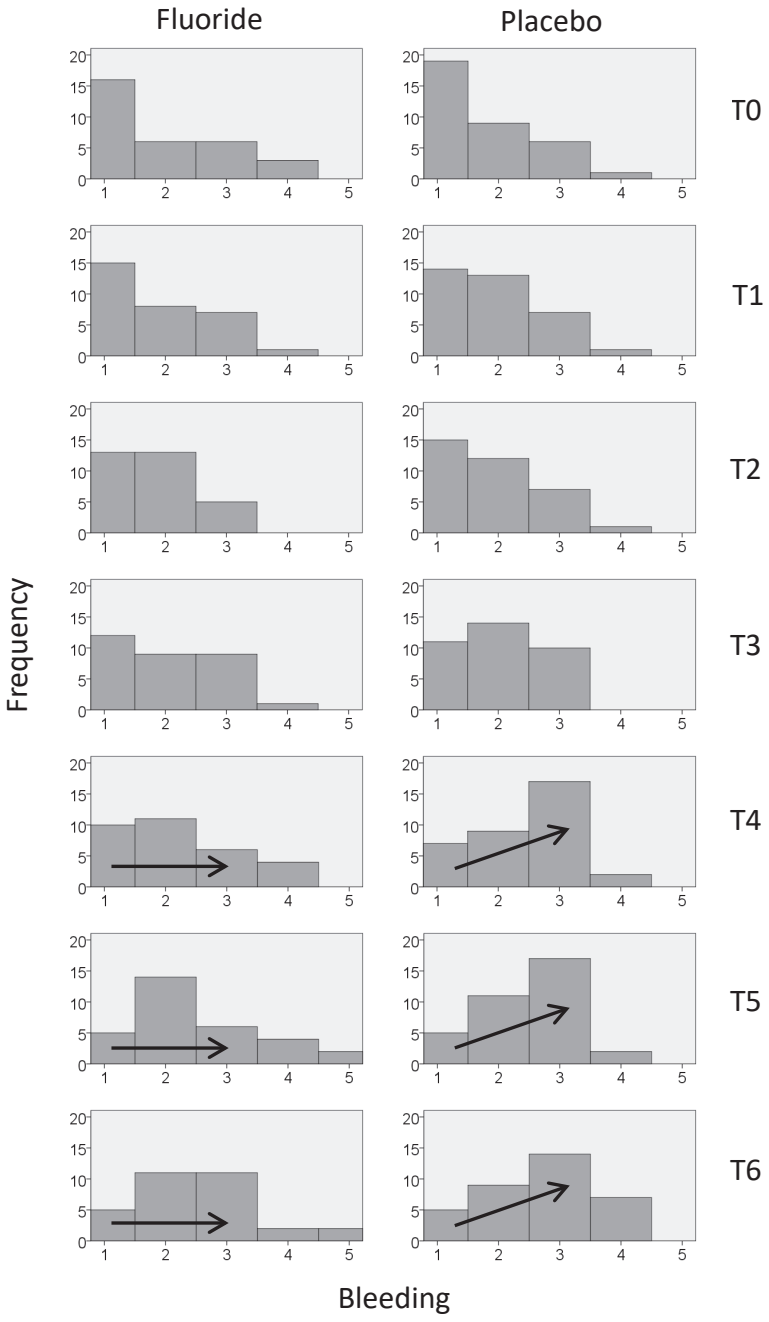

Figure 4. Bleeding scores during visit T0- T6, for the fluoride (left panel) and placebo rinse (right panel). At visit T4 and at subsequent visits the placebo group differs significantly compared to baseline. The fluoride group did not show a difference.

Bleeding T0-T5 and TD1, TD2

Comparison of participants in the two groups indicated that there were no differences after removal of the fixed appliances.

DISCUSSION

This is the first randomized, triple-blind, placebo-controlled study showing that a fluoride rinse reduces the formation of WSL during fixed orthodontic appliance treatments. Participants using placebo developed 2.6 times more WSL during the study period than participants using a daily fluoride rinse containing 100 ppm amine-fluoride and 150 ppm sodium-fluoride. In the fluoride group 31% of participants developed at least one WSL. A previous study showed that 33.5% of the patients developed WSL after using a 0.05% sodium-fluoride rinse, no placebo was used in that study (Geiger *et al.*, 1992). In our study 47% of the participants developed at least one WSL, while using a placebo rinse. This figure is comparable with the literature, showing around 50% of WSL development without a preventive method or after using a placebo foam (Gorelick *et al.*, 1982, Jiang *et al.*, 2013). We could not demonstrate a reduction in overall lesion size and depth using a fluoride rinse if WSL developed. Our finding of considerably fewer WSL than observed in the QLF study of Boersma (Boersma *et al.*, 2005) might be due to the fact that the ACTA orthodontic department has introduced a stringent oral hygiene protocol since Boersma's study.

In this study we measured WSL (QLF, DMFS and ICDAS) at 6 weeks after debonding, since it is known that the gingival swelling immediately after debonding obscures part of the buccal surfaces, but recedes six weeks later and thus showing a higher number of WSL at TD1 (Boersma *et al.*, 2005).

WSL are not only an aesthetic problem, after debond an overall improvement is seen in only 16% of the lesions, a large portion (49%) of the caries lesions remains stable over time and 15% of lesions are in need of or received restorative care two years after debonding (Mattousch *et al.*, 2007).

Compliance is often mentioned as a shortcoming of prescribing a rinse. It has been reported that the more compliant a patient is, the fewer WSL are formed (Geiger *et al.*, 1992). The latter colleagues also found that those patients who exhibited poor oral hygiene, but were strict in their rinsing, showed a reduction in the incidence of WSL. Since both groups in our study used a rinse, we assume compliance was similar in both groups. Compliance was not checked, thus mimicking normal practice settings. Thus, we demonstrated that a rinse is an effective method for WSL prevention during treatment with fixed orthodontic appliances, even if participants may be non- or partially compliant.

Even though we showed a difference in WSL development between groups, we did not meet the goal of our power analysis. This was firstly due to the overall load of the appoint-

ments, although mostly scheduled in combination with regular visits, resulting in a higher attrition. Secondly, extensions of treatment duration in combination with the end-point of January 2013 resulted in fewer participants in our analyses than planned.

Our study showed that rinsing with fluoride helped to maintain good oral health during fixed appliance treatment, as evidenced by a lower bleeding score over time. For non-orthodontic populations it is known that use of a Meridol (amine/stannous fluoride) rinse retards the development of gingivitis, resulting in a lower bleeding score as well as a lower plaque gingival bleeding indices (Brecx *et al.*, 1993, Madléna *et al.*, 2012). In an orthodontic population one study reported the effect of Meridol on bleeding (Øgaard *et al.*, 2006), indicating that bleeding increased significantly between bonding and debonding for the group only using a fluoride toothpaste, whereas in the group using Meridol bleeding did not change between bonding and debonding. No other studies are known that show a positive effect on bleeding using a fluoride rinse during fixed appliance treatment.

Based on this study, we conclude that the prescription of a fluoride rinse, to be used at home, has a measurable positive preventive effect on the overall oral health. It helps to prevent WSL formation and to slow down the number of WSL and to maintain a better gingival health (measured as bleeding).

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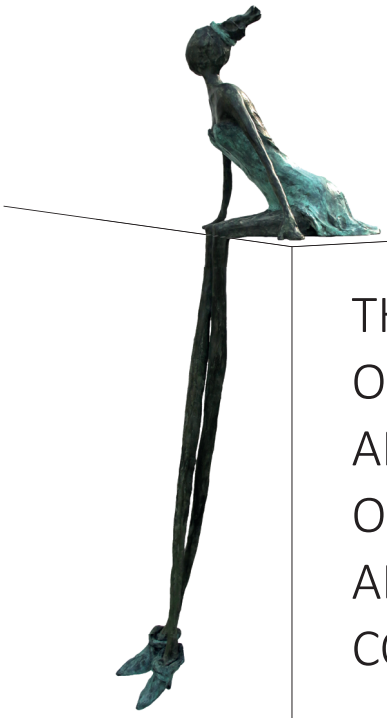

CHAPTER 3

THE EFFECT OF FIXED ORTHODONTIC APPLIANCES AND FLUORIDE MOUTHWASH ON THE ORAL MICROBIOME OF ADOLESCENTS – A RANDOMIZED CONTROLLED CLINICAL TRIAL

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ABSTRACT

While the aesthetic effect of orthodontic treatment is clear, the knowledge on how it influences the oral microbiota and the consequential effects on oral health are limited.

In this randomized controlled clinical trial we investigated the changes introduced in the oral ecosystem, during and after orthodontic treatment with fixed appliances in combination with or without a fluoride mouthwash, of 10-16.8 year old individuals (N = 91). We followed several clinical parameters in time, in combination with microbiome changes using next-generation sequencing of the bacterial 16S rRNA gene.

During the course of our study, the oral microbial community displayed remarkable resilience towards the disturbances it was presented with. The effects of the fluoride mouthwash on the microbial composition were trivial. More pronounced microbial changes were related to gingival health status, orthodontic treatment and time. Periodontal pathogens (*e.g*. *Selenomonas* and *Porphyromonas*) were highest in abundance during the orthodontic treatment, while the health associated *Streptococcus*, *Rothia* and *Haemophilus* gained abundance towards the end and after the orthodontic treatment. Only minor compositional changes remained in the oral microbiome after the end of treatment.

We conclude that, provided proper oral hygiene is maintained, changes in the oral microbiome composition resulting from orthodontic treatment are minimal and do not negatively affect oral health.

INTRODUCTION

The aesthetic effects of orthodontic treatment are often readily visible; in contrast to the effect orthodontic treatment might have on the non-visible part of the oral cavity - the microbiome.

The possible changes in the oral microbiome during orthodontic treatment are likely to be related to, the more easy observable, clinical parameters. For instance, the impaired gingival health status (van Gastel *et al.*, 2008, Gong *et al.*, 2011) and increased plaque formation (Sukontapatipark *et al.*, 2001, Demling *et al.*, 2009) that are associated with the placement of fixed orthodontic appliances. Besides, the latter could lead to the formation of white spot lesions, creating an undesirable aesthetic effect and possibly resulting in a cavity in need of restauration (Mattousch *et al.*, 2007, Ren *et al.*, 2014).

So far, studies aimed to investigate the changes in bacterial taxa during orthodontic treatment, used culturing or targeted molecular approaches, allowing for a limited number of opportunistic pathogenic species to be observed (Choi *et al.*, 2009, Thornberg *et al.*, 2009, Kim *et al.*, 2012, Torlakovic *et al.*, 2013). This implies that the response of the entire microbiome to orthodontic treatment is unclear, as are the possible long-term changes in bacterial composition.

A full understanding of the effects of fixed orthodontic appliances on the oral microbiome and the consequences on clinical parameters, should allow for the preservation of a healthy oral cavity during and after orthodontic treatment, justifying orthodontic treatment.

Our aim was to investigate changes introduced in the oral ecosystem during and after orthodontic treatment in combination with a fluoride mouthwash. To our knowledge, this is the first study to investigate the dynamics of the oral microbiome of adolescents during orthodontic treatment, and the use of a fluoride mouthwash using an open-ended molecular approach.

MATERIALS AND METHODS

Sampling and treatment

A randomized placebo-controlled parallel clinical trial was performed as described by van der Kaaij *et al*. (van der Kaaij *et al.*, 2015). The study was approved by the Medical Ethical Committee of the VU Medical Centre of the VU University of Amsterdam (VU-METc 2009/026 and Dutch trial register: NTR1817). The randomization allocation list was made in Microsoft Office Excel 2003 (Microsoft, Redmond, WA, USA) using the random number generation function in the analysis toolpack for one variable with a discrete distribution, allocating 50% of the 120 subjects to the test and 50% to the control group. The study was powered on the basis of the primary outcome; the data presented here were secondary outcomes.

All subjects participating in this study were scheduled to receive full fixed orthodontic appliances. Subjects could only be scheduled to receive full fixed orthodontic appliances if they maintained a proper oral hygiene and had no severe gingivitis. The guidelines at the Orthodontic Department at ACTA state that orthodontic appliances will not be placed when the bleeding by probing score is above 2 (1: 0-5% of the sites are bleeding, 2: 6-10% of the sites are bleeding, 3: 11-20% of the sites are bleeding, 4: 21-35% of the sites are bleeding, 5: > 35% of the sites are bleeding), except if immediate orthodontic treatment is indicated, for example, in case of traumatic occlusion.

The inclusion criteria for the study were: 10-18 years of age, good general health, no use of medication and no demineralization in need of restauration present at a buccal surface, in addition to providing their written informed consent. A total of 120 subjects set to receive fixed orthodontic appliances in both jaws were to participate in the study. Roth Ovation Brackets (Dentsply, GAC International, Bohemia, NY, USA) were used and all were bonded following the same procedure and methods, using Transbond XT primer and adhesive (3M unitek, Monrovia, USA).

In this triple-blind study, the subjects received a randomly assigned mouthwash containing 100 ppm amine fluoride (AmF) and 150 ppm sodium fluoride (SnF_2) (Elmex caries protection, Colgate-Palmolive Europe, Therwil, Switzerland) or a placebo, also provided by Colgate-Palmolive Europe. The mouthwash was used from the time of bonding until debonding. The subjects were instructed not to use (extra) fluoride containing products, other than toothpaste, during the course of the study. Their dentist was informed about the study and was asked not to apply extra fluoride during the study period. Furthermore, the subjects received oral hygiene instructions after placement of the fixed appliances and were advised to use interproximal brushes to clean the areas of the tooth adjacent to the bracket underneath the orthodontic wire.

The subjects were instructed not to clean their teeth 24 hr before supragingival plaque samples for microbiome analysis were taken. These samples were obtained at six time-points during this study: T0 (approximately one week before placement of the fixed orthodontic appliances), T1 (six weeks after placement), T2 (twelve weeks after placement), TD (debonding, average of 25 months after placement), TD1 (six weeks after debonding) and TD2 (twelve weeks after debonding). Supragingival plaque was collected from the buccal surface of the upper left premolars using a sterile plastic spatula. In presence of the brackets (visits T1, T2 and TD), which were placed on the middle of the tooth, the plaque was collected between the gingiva and the bracket. Gingival swelling often occurs within one or two months after placement of orthodontic appliances (Zachrisson and Zachrisson, 1972, Ristic *et al.*, 2007, van Gastel *et al.*, 2008). Hence, in cases where the gingival margin reached the bracket, the plaque was collected mesially and/or distally from the bracket. The plaque samples were spun down for 30 s at 16.100 x g and stored at -80°C.

The number of white spot lesions of the subjects was recorded at visits T0, TD, TD1 and TD2, and is described in more detail by van der Kaaij *et al*. (van der Kaaij *et al.*, 2015). Additionally, a bleeding by probing score was recorded at each visit for each patient. The percentage-based bleeding score was determined by probing each (bonded or to be bonded) tooth mesiobuccally and distobuccally with a periodontal probe (van der Kaaij *et al.*, 2015). For statistical analysis, the bleeding score was dichotomized into a healthy (score 1) and a gingivitis (score 2-5) group.

DNA isolation and sequencing

DNA was isolated from the supragingival plaque samples as described by Zaura *et al*. (Zaura *et al.*, 2009). The V5-V7 regions of the 16S rDNA were used to prepare barcoded amplicon libraries for each sample (Kraneveld *et al.*, 2012). The equimolar pooled samples were sequenced at the Academic Medical Center (Amsterdam, the Netherlands) and Macrogen Inc. (Seoul, Republic of Korea) using the 454 FLX Titanium chemistry (Roche, Basel, Switzerland). The reads are available at NCBI's Sequence Read Archive under SRP055565.

Sequencing data analysis

Quantitative Insights Into Microbial Ecology (QIIME) v1.5.0 was used to analyze the sequence data (Caporaso *et al.*, 2010). The downstream analyses and clustering into OTUs was done according to Koopman *et al*. (Koopman *et al.*, 2015), with the exception that 1 ambiguous base (N = 1) was allowed. The OTUs were manually aligned against NCBI's nucleotide (nr/nt) collection using Megablast (Zhang *et al.*, 2000, Morgulis *et al.*, 2008) to obtain species level identification (Table S1).

Statistical analysis

The Shannon diversity index and Bray-Curtis similarity index were calculated using PAST v3.0 (Hammer *et al.*, 2001). This program was also used to construct non-metric multidimensional scaling (nmMDS) plots based on the Bray-Curtis coefficient to visualize similarity between the samples. *Stress* < 0.2 (Kruskal's stress formula 1) was used as a threshold (Clarke, 1993).

The statistical significance of individual OTUs in relation to clinical parameters was determined using QIIME's paired t-test and correlation. The OTUs that were significant after FDR correction for multiple comparisons were analyzed further using IBM SPSS Statistics v21 (IBM Corp, Armonk, NY, USA). The Mann-Whitney test was used to determine if there was a statistically significant difference between the mouthwash groups, or gingival health status per visit for the phyla, genera and OTUs. The Wilcoxon Signed Ranks test was used to examine if there was a statistically significant difference between the visits at phylum, genus and OTU level and for the Shannon diversity index.

RESULTS

Study population

A total number of 120 subjects participated in the study. Contribution of 22 subjects to this study was discontinued because they declined further participation, moved or failed to show up. For 7 of the subjects, no supragingival plaque samples could be obtained because they brushed their teeth prior to sampling or the quality of the reads after sequencing was poor. From the 91 remaining subjects, one or more supragingival plaque samples were obtained. The number of microbiological samples obtained per visit was: T0; n = 76, T1; n = 73, T2; n = 68, TD; n = 44, TD1; n = 43 and TD2; n = 45. The number of subjects per mouthwash group per visit and the gender ratio per visit are described in Table S2. At the time of bonding, the average age of the subjects was 13.3 years old (SD 1.4, range 10-16.8). There was no significant difference in gingival bleeding between the group receiving the fluoride mouthwash and the group receiving the placebo at the baseline visit (van der Kaaij *et al.*, 2015).

Sequencing output

Of the processed sequencing reads, 78% passed quality control and 75% (2607737 reads) remained after the removal of chimeric reads. For 31 of the samples the number of reads was too low (8-769 reads per sample, average 227 reads); these were excluded from further analyses. The remaining 349 samples had an average of 7164 reads per sample (SD 5131, range 835-28432). The reads clustered into 461 OTUs. The subsampling threshold was set at 800 reads and the remaining subset, containing an average of 49 OTUs per sample (SD 14, range 11-94), was used for further analysis.

The reads were classified into 15 phyla and, when averaged over all time-points, dominated by Firmicutes (27%), Actinobacteria (22%), Proteobacteria (22%), Bacteroidetes (16%), Fusobacteria (11%) and Candidate division TM7 (1%). At a lower taxonomic level, the reads were classified into 149 genera, dominated by *Streptococcus* (12%), *Neisseria* (11%), *Corynebacterium* (9%), *Veillonella* (7%), *Leptotrichia* (7%) and *Actinomyces* (6%).

Mouthwash effect

Non-metric multidimensional scaling plots were made by mouthwash group per visit. These plots did not show any separation of the microbial profiles based on mouthwash (fig. 1). There were no statistically significant differences in Shannon diversity index at any of the visits. To assess the stability of the microbiome composition in time, the Bray-Curtis similarity index between visit T0 and the subsequent visits was calculated per individual and tested for each mouthwash group. The difference in similarity did not reach statistical significance at any of the time-points.

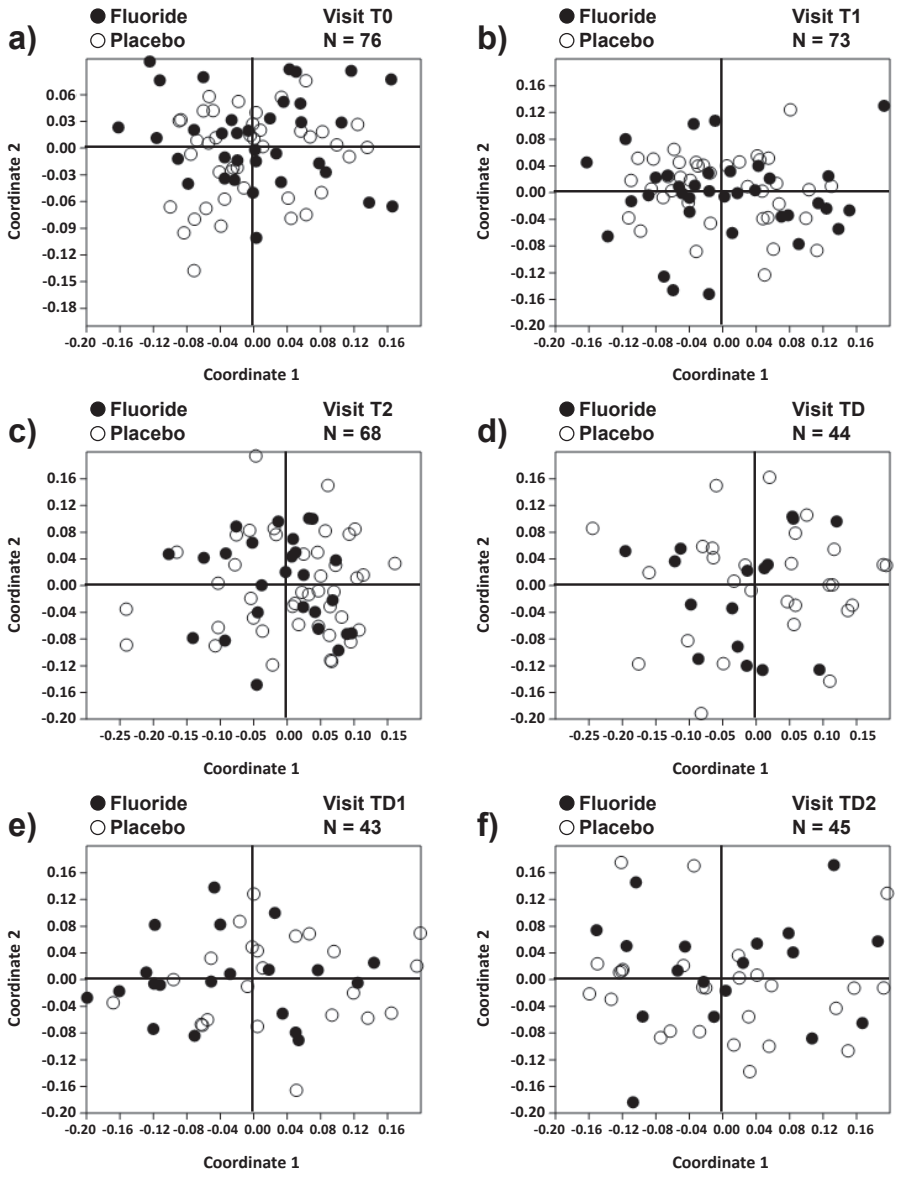

Figure 1. Non-metric multidimensional scaling plots based on the three-dimensional Bray-Curtis similarity index by mouthwash. The plots are constructed per visit. The subjects receiving the fluoride mouthwash are symbolized by ●, the subjects receiving the placebo mouthwash are indicated by ○. Mouthwashes were administered between visits T0 and TD. The *stress* for each individual plot is (a) 0.1543, (b) 0.1465, (c) 0.1256, (d) 0.1402, (e) 0.1495 and (f) 0.1531.

There was no significant difference in relative abundance of any bacterial phylum between the two mouthwash groups at any visit.

At genus level, within the placebo group, *Fusobacterium* decreased significantly in abundance from visit T0 to T1 (p = 0.049) and from T1 to T2 (p = 0.002). Between visits T2 and TD, the level of abundance became significantly higher again (p = 0.038) (fig. S1). In the fluoride mouthwash group, there was no significant difference in abundance of *Fusobacterium* between any of the visits.

At the OTU level, the abundance of OTU381 (*Kingella*) was higher (p = 0.028) in the placebo group compared to the fluoride group at visit T1 (fig. S2).

Gingival health

The gingival health status of the subjects was determined by probing. To assess the relation between gingival health and the supragingival plaque microbiome, we dichotomized the group into subjects with healthy gingiva and with gingivitis. The highest prevalence of gingivitis was recorded at visit TD (fig. 2). Non-metric multidimensional scaling plots based on the OTU profiles of each subject per time-point showed that gingivitis-microbiome profiles were less scattered, especially at visits T0, T1 and T2, in space compared to the healthy-gingiva microbiome profiles (fig. 3).

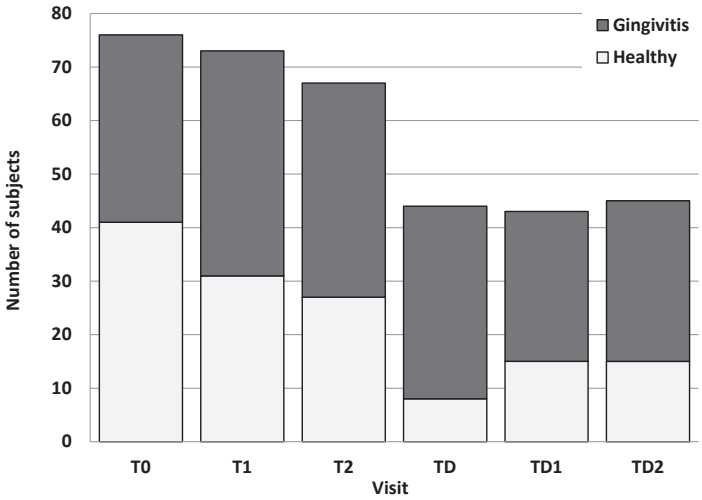

Figure 2. Count of subjects with healthy gingiva and with gingivitis per visit.

At the phylum level, the proportion of Bacteroidetes was higher in the individuals with gingivitis compared to those with healthy gingiva at visits T0 (p = 0.012) and T1 (p = 0.035) (fig. S3a). The abundance of Candidate division TM7 was significantly elevated in individuals with gingivitis at visits T0 (p = 0.001), T1 (p = 0.029), T2 (p = 0.032) and TD2 (p = 0.037) (fig. S3b). The proportion of the phylum Fusobacteria was higher in the subjects with gingivitis at visits T1 (p = 0.031) and TD2 (p = 0.024) (fig. S3c).

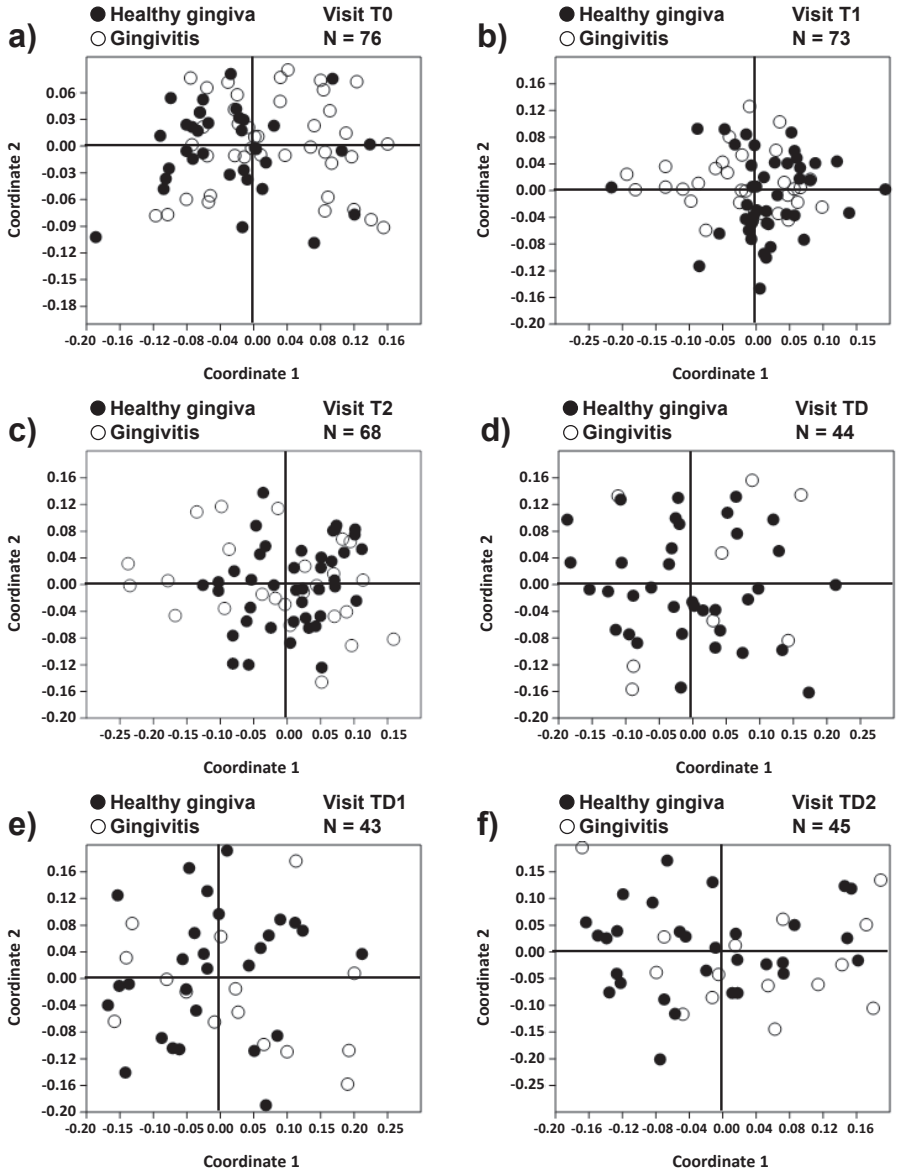

Figure 3. Non-metric multidimensional scaling plots based on the three-dimensional Bray-Curtis similarity index by gingival health status. The plots are constructed per visit. The subjects with healthy gingiva are indicated with ○, the subjects with gingivitis are indicated with ●. The *Stress* for each individual plot is (a) 0.1539, (b) 0.1474, (c) 0.1265, (d) 0.1401, (e) 0.1495 and (f) 0.1531.

At genus level, the relative abundance of the genus *Selenomonas* was significantly higher in the gingivitis group compared to the healthy group at visits T0 (p = 0.022), T1 (p = 0.041) and TD2 (p = 0.012) (fig. S4a). The same applied to *Porphyromonas* at visits T0, T1 and

T2 (p = 0.036, p = 0.010 and p = 0.033, respectively) (fig. S4b) and *Johnsonella* at visits T0 (p = 0.004), T1 (p = 0.013) and TD2 (p = 0.042) (fig. S4c). In contrast, the genus *Derxia* was significantly higher in the healthy group at visits T0 and T1 (p = 0.046 and p = 0.028, respectively) (fig. S4d). The same was observed for the genera *Haemophilus* at visit T0 (p = 0.021) and visit TD2 (p = 0.024) (fig. S4e) and *Rothia* at visit T0 (p = 0.004) (fig. S4f).

In agreement with the genus *Rothia*, OTU65 (*Rothia*) was significantly more abundant in the healthy subjects compared to those with gingivitis at visit T0 (p = 0.011) (fig. S5a). The difference in abundance in OTU351 (*Streptococcus*) between the two groups was significant at visit T1 (p = 0.023) where the OTU was higher in number in the healthy group (fig. S5b). On the other hand, OTU424 (*Johnsonella*) was more abundant in the gingivitis group compared to the healthy group at visits T0 (p = 0.032), T1 (p = 0.039) and TD (p = 0.044) (fig. S5c). The OTUs 55, 171 and 355, all three classified as Candidate division TM7, were higher in the gingivitis group at visit T0 (p = 0.005, 0.006 and 0.005, respectively). OTU355 was also higher at T1 (p = 0.011), while OTU55 was higher at visit T2 (p = 0.011) in the gingivitis group (fig. S5d-f). The OTU302 (*Selenomonas*) was significantly higher in the gingivitis group compared to the healthy group at T0 (p = 0.038), T1 (p = 0.045) and TD2 (p = 0.010) (fig. S5g) as was OTU398 (*Fusobacterium*) at TD2 (p = 0.012) (fig. S5h).

Time

Next, we assessed the changes in microbiome of the study population in time. An nmMDS plot on OTU level was constructed of the individuals (N = 19) whose samples were available from all six time-points. However, no discernable effects of time on the microbiome profiles were found (fig. 4). The microbiome diversity became higher between visit T0 and T1 (p = 0.003) and became lower between visits TD and TD1 (p = 0.003) (fig. 5).

The abundance of the phylum Actinobacteria decreased between visit T0 and T1 (p = 0.043), while the same phylum increased at visits TD1 and TD2 compared to the baseline (p = 0.002, p = 0.006, respectively) (fig. S6a). The phylum Firmicutes had increased in abundance at visits T1 (p = 0.005), TD (p = 0.021) and TD2 (p = 0.035) over visit T0 (fig. S6b). Compared to visit T0, the abundance of Bacteroidetes had decreased in both post-debonding visits: TD1 (p = 0.015) and TD2 (p = 0.025) (fig. S6c). Between visits T0 and TD1, the abundance of Candidate division TM7 decreased (p = 0.031) (fig. S6d), while Fusobacteria decreased from T0 to T2 (p = 0.001) and TD1 (p = 0.001) (fig. S6e). The abundance of Proteobacteria was significantly lower at visit TD compared to the baseline (p = 0.001) (fig. S6f).

Several genera showed significant differences in abundance between the visits (fig. 6). *Streptococcus* became significantly more abundant at visits T1 (p = 0.036), TD (p = 0.025), TD1 (p < 0.001) and TD2 (p = 0.001) compared to the baseline. An increase in abundance from visit TD to TD1 (p = 0.048) was observed as well (fig. S7a). The abundance of *Neisseria* became higher at visit T2 compared to T0 (p = 0.008), while at visits TD and TD1 the abundance became lower compared to visit T0 (p = 0.006, and p = 0.029, respectively).

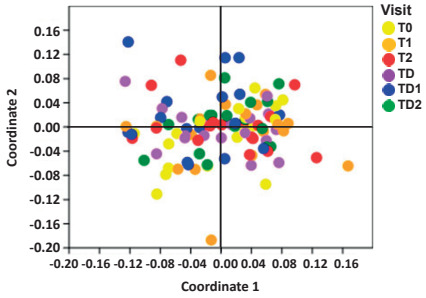

Figure 4. Non-metric multidimensional scaling plot based on the three-dimensional Bray-Curtis similarity index by time. The plot consists of samples of all subjects (N = 19) who were present at all six time points. The *stress* for this plot is 0.1836.

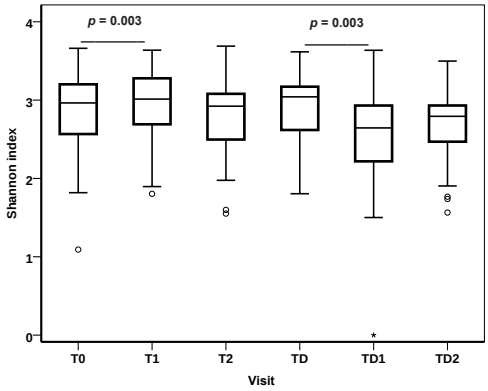

Figure 5. Shannon diversity index for the entire study population per visit. T0; N = 76, T1; N = 73, T2; N = 68, TD; N = 44, TD1; N = 43, TD2; N = 45. Statistical significance ($p < 0.05$) was determined using the Wilcoxon Signed Ranks test.

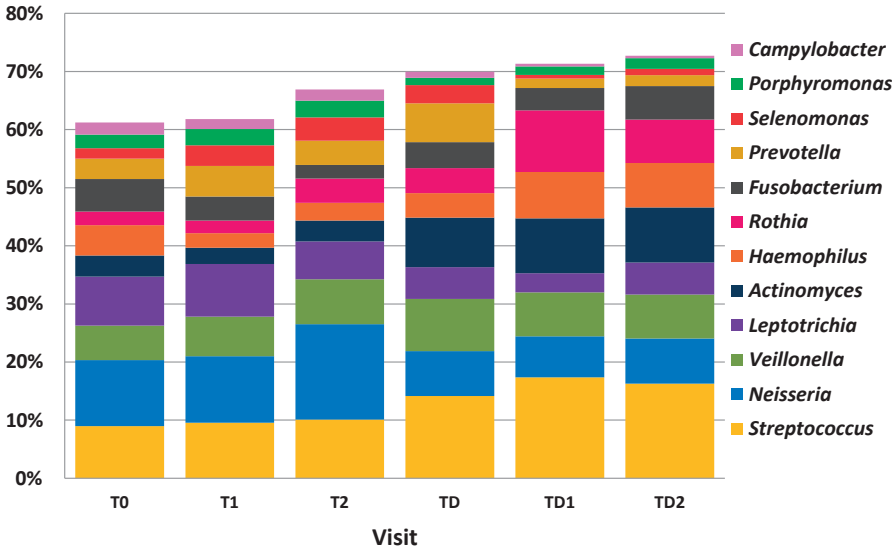

Figure 6. Average proportions of the genera that differed significantly in abundance between one or more of the visits.

Moreover, the abundance of *Neisseria* increased significantly at visit T2 compared to visit T1 ($p = 0.011$), yet it was significantly lower again at visit TD ($p = 0.018$) (fig. S7b). *Actinomyces* had increased significantly at the last three visits when compared to visit T0 (TD: $p = 0.004$, TD1: $p < 0.001$ and TD2: $p < 0.001$) (fig. S7c). Both *Veillonella* (fig. S7d) and *Porphyromonas* (fig. S7e) were only at visit TD significantly more abundant when compared to visit

T0 (p = 0.033 and p = 0.011, respectively). Additionally, the abundance of *Porphyromonas* decreased significantly between T2 and TD (p = 0.017). For *Leptotrichia*, the abundance became significantly lower at TD1 (p < 0.001) and TD2 (p = 0.037) compared to the baseline (fig. S7f). The abundance of *Campylobacter* had decreased at the last three visits compared to visit T0 (TD: p = 0.033, TD1: p < 0.001 and TD2: p < 0.001) (fig. S7g). At both visits T1 and TD, *Prevotella* had increased in abundance compared to visit T0 (p = 0.004 and p = 0.001, respectively), while at TD1 the abundance had become significantly smaller again (p = 0.010) (fig. S7h). For the genus *Haemophilus*, the only significant increase in abundance was between visits TD and TD1 (p = 0.033) (fig. S7i). For the genus *Selenomonas* a decrease in time was observed (fig. S7j). The abundance of the genus *Fusobacterium* was significantly lower at T2 and TD1 compared to the baseline (p < 0.001 and p = 0.043, respectively) (fig. S7k). The abundance of *Rothia* was higher in the last three visits compared to the baseline (TD: p = 0.009, TD1: p < 0.001, TD2: p < 0.001) (fig. S7l).

At the OTU level, the abundance of OTU28 (*Actinomyces*) was higher at TD1 (p < 0.001) and TD2 (p = 0.001) compared to visit T0 (fig. S8a). When compared to visit T0, the abundance of OTU65 (*Rothia*) was higher in the last three visits (TD: p = 0.009, TD1: p < 0.001, and TD2: p < 0.001) (fig. S8b). In addition, both OTU28 and OTU65 were elevated significantly between visits TD and TD1 (p = 0.049 and p = 0.002, respectively). The abundance of OTU351 (*Streptococcus*) became higher between visits TD and TD1 (p = 0.033) and was significantly higher compared to visit T0 at visit TD1 (p < 0.001) and visit TD2 (p = 0.002) (fig. S8c). In comparison to the baseline, the abundance of OTU398 (*Fusobacterium*) was lower at visit T2 (p < 0.001) and at visit TD1 (p = 0.043) (fig. S8d). The abundance of OTU143 (*Leptotrichia*) decreased significantly between visits TD and TD1 (p = 0.003). Moreover, at visit TD1, the abundance of OTU143 was significantly smaller compared to visit T0 (p = 0.007) (fig. S8e). The abundance of OTU151 (*Campylobacter*) was lower at visit TD compared to visit T2 (p = 0.032) and at TD1 the abundance was lower compared to visit TD (p = 0.001). At both visits TD1 and TD2, the abundance of OTU151 was significantly lower compared to visit T0 (p < 0.001 and p < 0.001, respectively) (fig. S8f). When compared to visit T0, the abundance of OTU302 (*Selenomonas*) had increased at visits T1 (p = 0.002), T2 (p < 0.001) and TD (p = 0.029), while the abundance had decreased at visit TD1 (p = 0.003) (fig. S8g).

DISCUSSION

The results of our study indicate that the fluoride mouthwash had little effect on the adolescent oral microbiome composition during fixed orthodontic appliance treatment. More pronounced were the microbial changes observed in relation to gingival health status and orthodontic treatment. Yet, the resilience of these adolescent oral communities was noteworthy in regard to the interference caused by the orthodontic treatment, fluoride

mouthwash and the physiological changes of puberty itself. There was no observable shift in the composition of the total community in time (fig. 4). A remaining change in abundance was observed for a few genera (fig. 6) and, interestingly, most genera that did increase in abundance in time were associated with a healthy oral cavity.

In this study, an amine fluoride (AmF) combined with stannous fluoride (SnF_2) mouthwash was used to reduce the amount of demineralization, since fluoride is a well-established anti-caries agent (van Loveren *et al.*, 2009) and caries is an infectious bacterial disease. Compliance is regarded as a drawback in studies aiming to observe the effect of a mouthwash. Nonetheless, van der Kaaij *et al*. (van der Kaaij *et al.*, 2015) observed that the use of an AmF/SnF_2 mouthwash inhibited formation of white spot lesions during this study. Likewise, Øgaard *et al*. (Øgaard *et al.*, 2006) observed that there was no difference in white spot lesions before and after orthodontic treatment of patients using an AmF/SnF_2 mouthwash. Madléna *et al.* (Madléna *et al.*, 2012) observed a decrease in plaque index, gingival index and bleeding on probing within one month in orthodontic patients using AmF/SnF_2 toothpaste, regardless if the toothpaste was combined with an AmF/SnF_2 mouthrinse. Gerardu *et al*. (Gerardu *et al.*, 2006, van Loveren *et al.*, 2009) did observe dental plaque shifting towards less acidogenic plaque, yet there was no significant difference in bacterial composition after the use of AmF/SnF_2 products compared to fluoride-free periods. This is similar to our findings, as we did not observe a clear effect of the fluoride mouthwash on the microbial composition. Although it is suggested that fluoride has antibacterial properties, its main effect appears to be on the demineralization and remineralization processes in the oral cavity (van Loveren, 2001, ten Cate, 2009, Rosin-Grget *et al.*, 2013, ten Cate, 2013).

We did observe that the abundance of several bacterial taxa was associated with the gingival health status of the subjects. Gingivitis during orthodontic treatment is presumably related to plaque accumulation caused by the newly created retention sites and consequently impaired oral hygiene(Ren *et al.*, 2014). Yet, it is not only the orthodontic treatment that is related to the onset of gingivitis in these subjects, for 'puberty itself' is also associated with increased gingivitis (Morishita *et al.*, 1988, Mombelli *et al.*, 1990, Nakagawa *et al.*, 1994). Generally, orthodontic treatment takes place during adolescence, as was the case in our study. During this period, the human body experiences many (*e.g*. behavioral and hormonal) changes (Vetter-O'Hagen and Spear, 2012).

The exact reason why gingivitis becomes prevalent in this age-group is unclear but hormonal changes are likely to play a part. Our study did not include a control group of adolescents that did not receive orthodontic treatment. Therefore it is difficult to discern which microbial changes are related to the orthodontic treatment, and which ones to the onset of puberty. Thus far, most studies regarding the (changes in the) oral microbiome during adolescence or orthodontic treatment have focused on a limited number of bacteria, due to the nature of their techniques.

The use of an open-ended molecular approach allowed us to detect Candidate division TM7 (and OTUs 55, 171, 355) (fig. S3b and fig. S5d-f). Next-generation sequencing has demonstrated that these bacteria, of which only recently a member was grown as a pure laboratory culture (Soro *et al.*, 2014), are widespread in the human oral cavity (He *et al.*, 2015). Crielaard *et al*. (Crielaard *et al.*, 2011) reported that Candidate division TM7 increased with advancing age, in a study regarding children aged 3-18 years. Duran-Pinedo *et al*. (Duran-Pinedo *et al.*, 2014) presumed a role for Candidate division TM7 in periodontitis. We found Candidate division TM7 to be associated with gingivitis, in accordance with Huang *et al*. (Huang *et al.*, 2011).

Interestingly, we observed the presence of the genus *Derxia* (fig. S4d), although low in abundance in our study population, to be related to a healthy state of the gingiva. Members of this genus are known to fix nitrogen in different environmental habitats (John *et al.*, 2011, Chen *et al.*, 2013). Recently *Derxia* has been observed as a member of the human (and canine) oral cavity (Hu *et al.*, 2013, Sturgeon *et al.*, 2013, Jiang *et al.*, 2014), yet its role in this particular environment remains to be elucidated.

Well-known inhabitants of the oral cavity are members of the genus *Prevotella*; often associated with an unhealthy state of the periodontium (Nadkarni *et al.*, 2012). Moreover, an increase of *Prevotella intermedia* has been associated with orthodontic treatment (Ristic *et al.*, 2007, van Gastel *et al.*, 2011, Kim *et al.*, 2012). In addition, van Gastel *et al*. (van Gastel *et al.*, 2011) observed a decrease of *P. intermedia* after the removal of the orthodontic appliances. This coincides with our finding of the abundance of the genus *Prevotella* (fig. S7h). Hence, there appears to be an association between orthodontic treatment and the prevalence of *Prevotella*, although Choi *et al*. (Choi *et al.*, 2009) did not find a significant decrease of *Prevotella* after orthodontic treatment was ended. This discrepancy might be due to difference in sampling sites or detection techniques.

In this study, we found that the genus *Actinomyces* increased with time (fig. S7c), while OTU28 (*Actinomyces naeslundii*) increased mainly after debonding (fig. S8a). According to Delaney *et al*. (Delaney *et al.*, 1986) the levels of *A. naeslundii* are higher in prepubertal subjects compared to postpubertal subjects. Gusberti *et al*. (Gusberti *et al.*, 1990) observed that the levels of the species *Actinomyces odontolyticus* elevate during puberty. Tanner *et al*. (Tanner *et al.*, 2012) found *Actinomyces* sp. to be associated with gingivitis, whereas Tsuruda *et al.* (Tsuruda *et al.*, 1995) observed a relation between *Actinomyces* species and healthy pubertal children. These diverse findings indicate that the role of *Actinomyces* in the oral microbiome cannot be determined on genus level, yet it does not explain the contradictory findings of the study by Delaney *et al*. (Delaney *et al.*, 1986) and our own results. Although sampling site and used technique might again be of influence.

The genus *Veillonella* had previously been shown to increase during adolescence (Moore *et al.*, 1993, Crielaard *et al.*, 2011). In this study population however, the abundance of *Veillonella* remained stable throughout time (fig. S7d). In addition, the abundance of *Veillonella*

was not significantly different between the two mouthwash groups or between the healthy and gingivitis groups.

Both the genus *Campylobacter* (fig. S7g) and OTU151 (*Campylobacter gracilis*) (fig. S8f) decreased with time. A similar pattern of decrease has been observed for *Campylobacter rectus* (Choi *et al.*, 2009, Thornberg *et al.*, 2009, Kim *et al.*, 2012). This decrease could be explained primarily by the reduction of retention sites due to the alignment of the teeth and secondly by the removal of the orthodontic fixed appliances, causing an additional loss of retention sites.

A similar decrease in time was observed for the genera *Porphyromonas* (fig. S7e) and *Selenomonas* (fig. S7j). Additionally, we found that *Porphyromonas* (fig. S4b), *Selenomonas* (fig. S4a) and OTU302 (*Selenomonas*) (fig. S5g) were associated with gingivitis. Members of both these genera are among the main periodontal pathogens (Huang *et al.*, 2011, Mysak *et al.*, 2014). Therefore their decrease in time might be considered desirable. Why they decrease in time, if it is *e.g*. the reduction in retention sites through alignment of the teeth or hormonal changes in the host, remains unclear.

Neisseria became lower in abundance during the advancement of the visits (fig. S7b), in agreement with Moore *et al*. (Moore *et al.*, 1993), who found this genus to be more associated with prepubertal children than older children. Thus far, most studies investigating the oral microbiome during orthodontic treatment or puberty did not target members of the genus *Neisseria*. Nonetheless, Tanner *et al*. (Tanner *et al.*, 2012) found *Neisseria elongata* to be associated with reduced gingivitis in orthodontic patients. They made the same observation for *Fusobacterium periodonticum*.

Tsuruda *et al.* (Tsuruda *et al.*, 1995) found *Fusobacterium* sp. to be more abundant in pubertal children with gingivitis compared to healthy children. *Fusobacterium nucleatum* is regarded as a bridging organism in the formation of dental biofilms (Wright *et al.*, 2013). This might explain our observation that the genus *Fusobacterium* decreases during the orthodontic treatment, yet increases again in time (fig. S7k). The additional retention sites created by the brackets leave *Fusobacterium* superfluous in the formation of biofilms. On the other hand, Wojcicki *et al*. (Wojcicki *et al.*, 1987) found that *Fusobacterium* sp. was lower in their circumpubertal group compared to a younger and older test group, suggesting that the presence of *Fusobacterium* sp. is influenced by the physiological maturity of the host.

In contrast to *Fusobacterium*, the abundance of the genus *Streptococcus* (fig. S7a) and OTU351 (*Streptococcus*) (fig. S8c) showed an increase in time without decreasing first. Increase in *Streptococcus* abundance in puberty has been observed before (Moore *et al.*, 1993), although we cannot identify this member of the genus *Streptococcus* on species level, we speculate that it is associated with a healthy state of the gingiva.

Haemophilus (fig. S4e), *Rothia* (fig. S4f) and OTU65 (*Rothia*) (fig. S5a) were associated with a healthy state of the gingiva as well. Their increase after debonding appeared to

coincide with the decrease in gingivitis after debonding (fig. 2). Members of these two genera were usually not included as target microorganisms in studies of the oral microbiome during puberty or orthodontic treatment. Although the role of *Haemophilus* in health and disease of the oral cavity remains somewhat ambiguous, *Rothia* is generally associated with health (Zaura *et al.*, 2009, Fujinaka *et al.*, 2013).

In conclusion, the effects of the fluoride mouthwash on the adolescent microbiome were indiscernible and promoted neither health nor disease associated bacterial growth. Yet, van der Kaaij *et al*. (van der Kaaij *et al.*, 2015) did observe fewer demineralizations in subjects using the fluoride mouthwash compared to those using the placebo. Thus, the use of a fluoride mouthwash during orthodontic treatment might be beneficial for the health status of the oral cavity.

Nevertheless, we did observe changes in the abundance of various bacteria. In general, the bacteria that were associated with periodontal pathogenesis decreased in abundance in time, while the abundance of the health related bacteria increased, suggesting that orthodontic treatment during puberty does not have a lasting negative effect on the gingival health status. Still, the lack of an age-related control group not receiving orthodontic treatment precludes us from making a clear distinction between microbial changes instigated by puberty and the effects on the oral ecology caused by orthodontic treatment with fixed appliances. A future study including such a control group would be necessary to determine which microbial changes are truly caused by the presence of orthodontic appliances, allowing for the maintenance of a healthy oral microbiome during orthodontic treatment.

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SUPPLEMENTARY MATERIAL

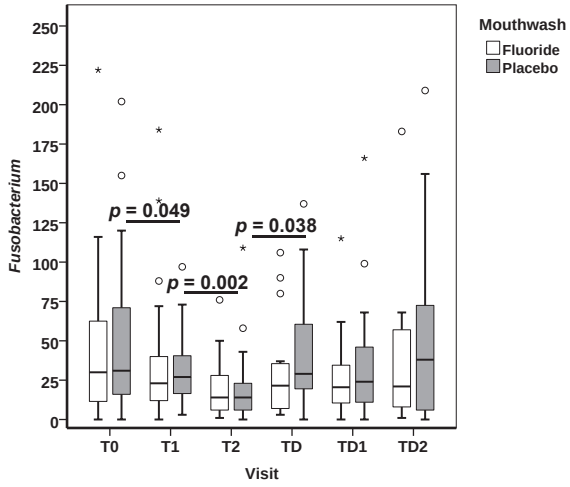


Figure S1. Difference in abundance of the genus *Fusobacterium* between mouthwash groups per visit. The read count is displayed on the *y*-axis. Mouthwashes were administered between visits T0 and TD. Statistical significance ($p < 0.05$) was determined using the Mann-Whitney test between the two groups per visit, or the Wilcoxon Signed Ranks test within the same group between different visits. The boxes represent the median and interquartile range (IQR), the whiskers represent the minimum and maximum values. Outliers more than 1.5x IQR are depicted by ○, and more than 3x IQR by *.

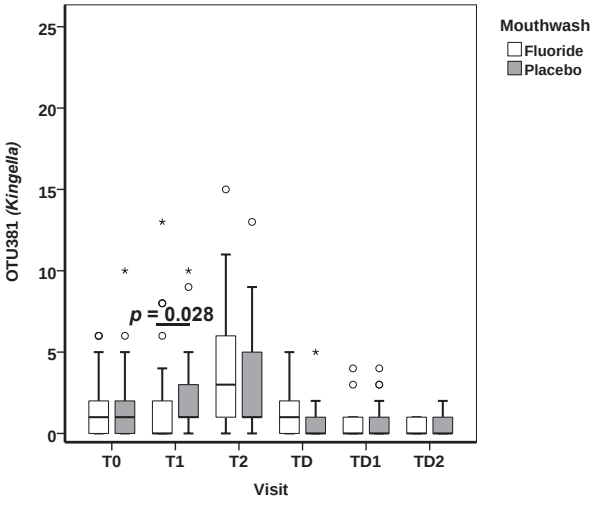


Figure S2. Difference in abundance of OTU381 (*Kingella*) between mouthwash groups per visit. The read count is displayed on the *y*-axis. Mouthwashes were administered between visits T0 and TD. Statistical significance $p < 0.05$) was determined using the Mann-Whitney test between the two groups per visit, or the Wilcoxon Signed Ranks test within the same group between different visits. The boxes represent the median and IQR, the whiskers represent the minimum and maximum values. Outliers more than 1.5x IQR are depicted by ○, and more than 3x IQR by *.

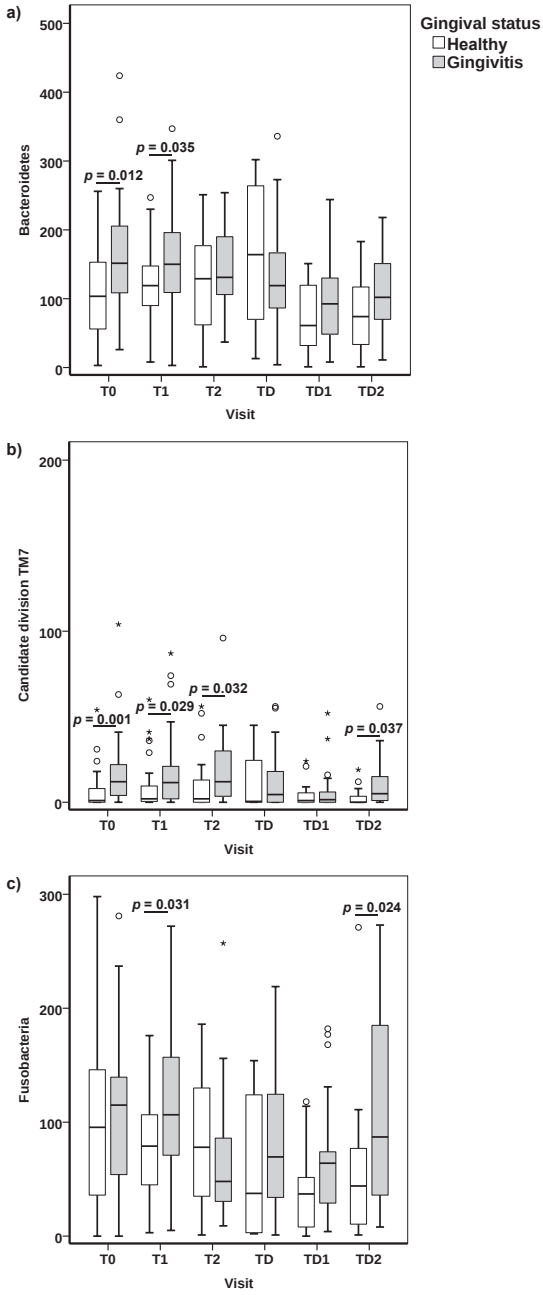


Figure S3. Difference in abundance of the phyla Bacteroidetes (a), TM7 (b) and Fusobacterium (c) based on gingival health status per visit. The read count is displayed on the *y*-axis. Statistical significance ($p < 0.05$) was determined using the Mann-Whitney test. The boxes represent the median and IQR, the whiskers represent the minimum and maximum values. Outliers more than 1.5x IQR are depicted by ○, and more than 3x IQR by *.

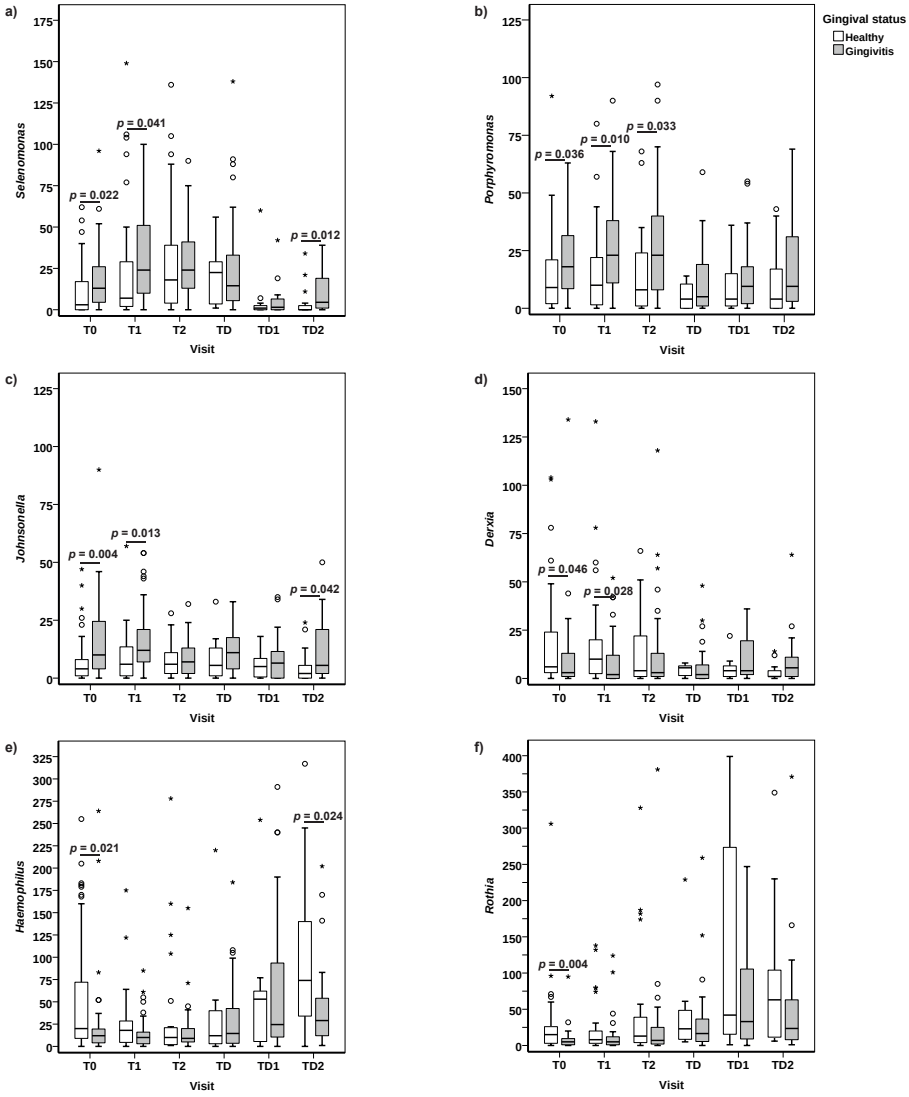


Figure S4. Difference in abundance of the genera *Selenomonas* (a), *Porphyromonas* (b), *Johnsonella* (c), *Dexia* (d), *Haemophilus* (e) and *Rothia* (f) based on gingival health status per visit. The read count is displayed on the *y*-axis. Statistical significance ($p < 0.05$) was determined using the Mann-Whitney test. The boxes represent the median and IQR, the whiskers represent the minimum and maximum values. Outliers more than 1.5x IQR are depicted by ○, and more than 3x IQR by *.

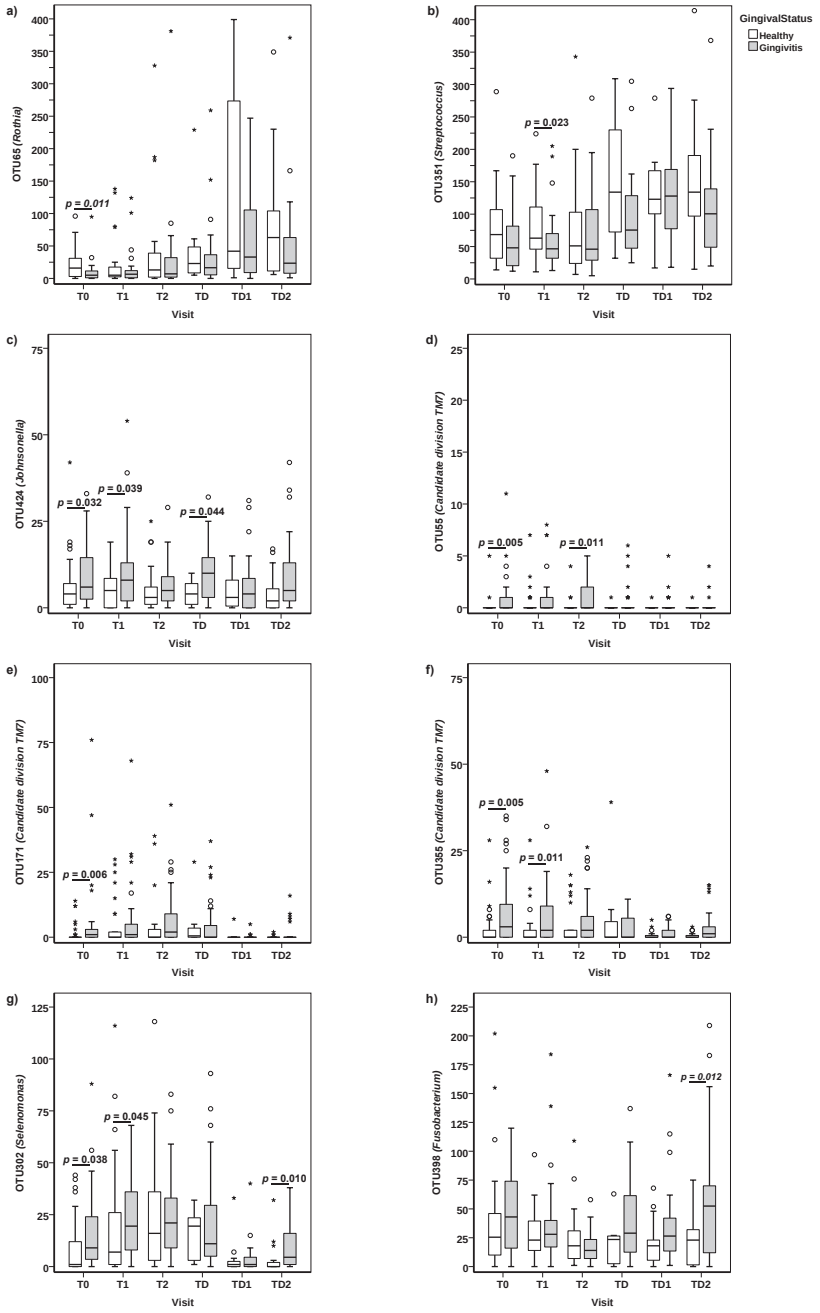


Figure S5. Difference in OTU abundance based on gingival health status per visit. The read count is displayed on the *y*-axis. Statistical significance ($p < 0.05$) was determined using the Mann-Whitney test. The boxes represent the median and IQR, the whiskers represent the minimum and maximum values. Outliers more than 1.5x IQR are depicted by o, and more than 3x IQR by *.

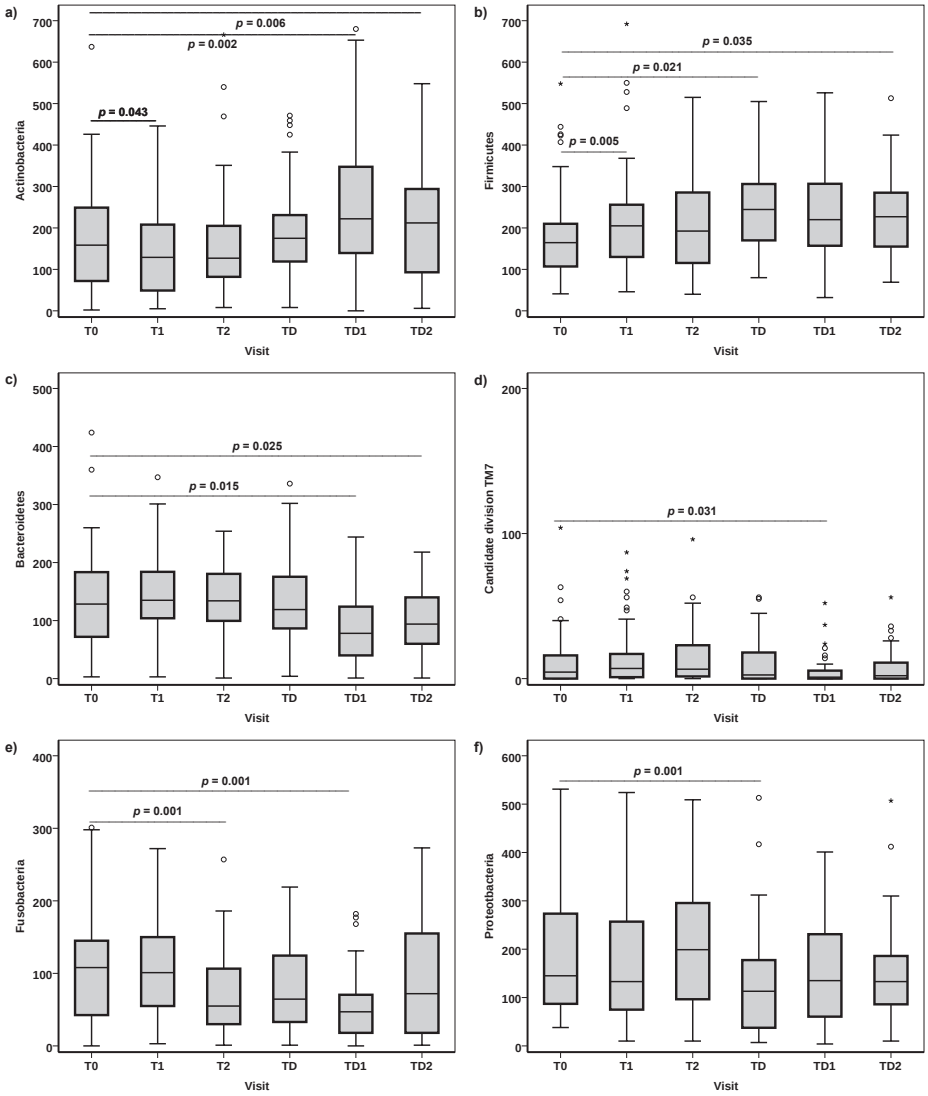


Figure S6. Difference in phylum abundance between visits for Actinobacteria (a), Firmicutes (b), Bacteroidetes (c), TM7 (d), Fusobacteria (e) and Proteobacteria (f). The read count is displayed on the *y*-axis. Statistical significance ($p < 0.05$) was determined using the Wilcoxon Signed Ranks test. The boxes represent the median and IQR, the whiskers represent the minimum and maximum values. Outliers more than 1.5x IQR are depicted by ○, and more than 3x IQR by *.

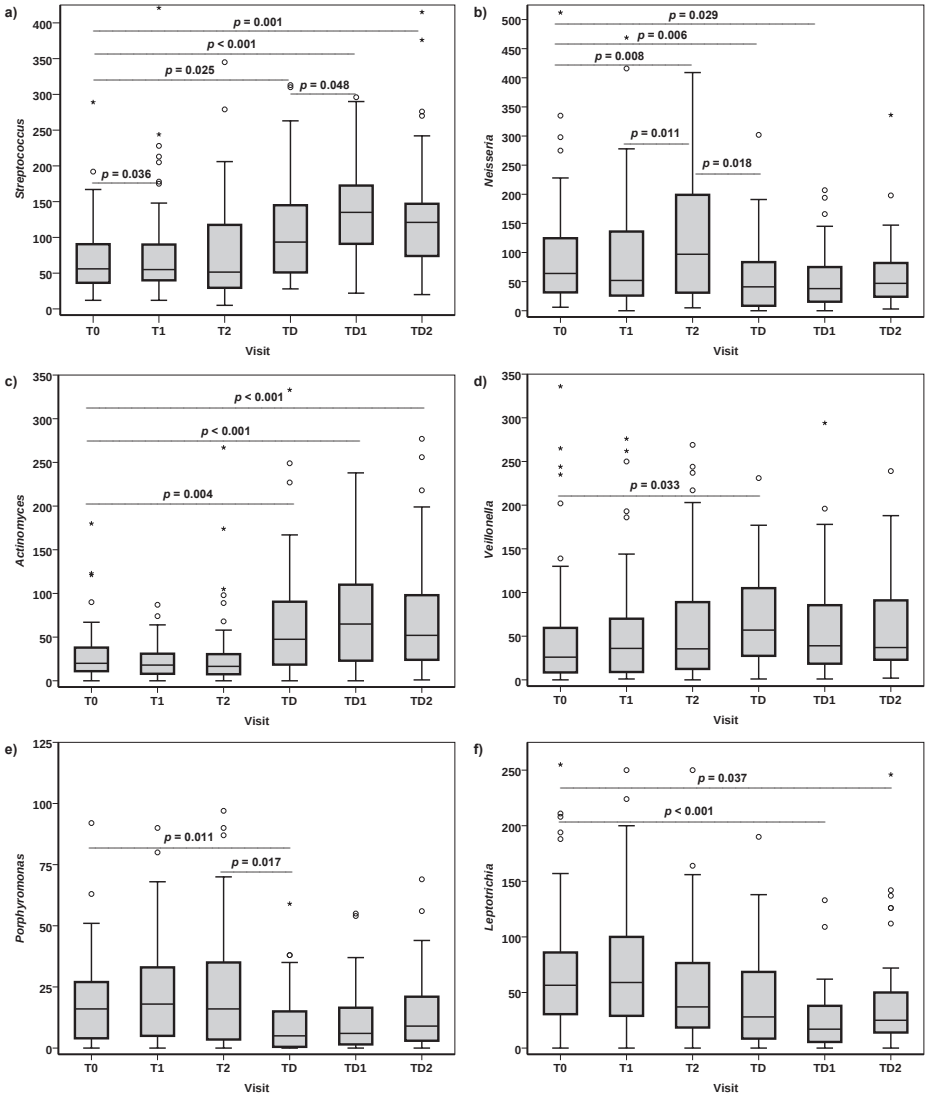


Figure S7. Difference in genus abundance between visits. The read count is displayed on the *y*-axis. Statistical significance ($p < 0.05$) was determined using the Wilcoxon Signed Ranks test. The boxes represent the median and IQR, the whiskers represent the minimum and maximum values. Outliers more than 1.5x IQR are depicted by ○, and more than 3x IQR by *.

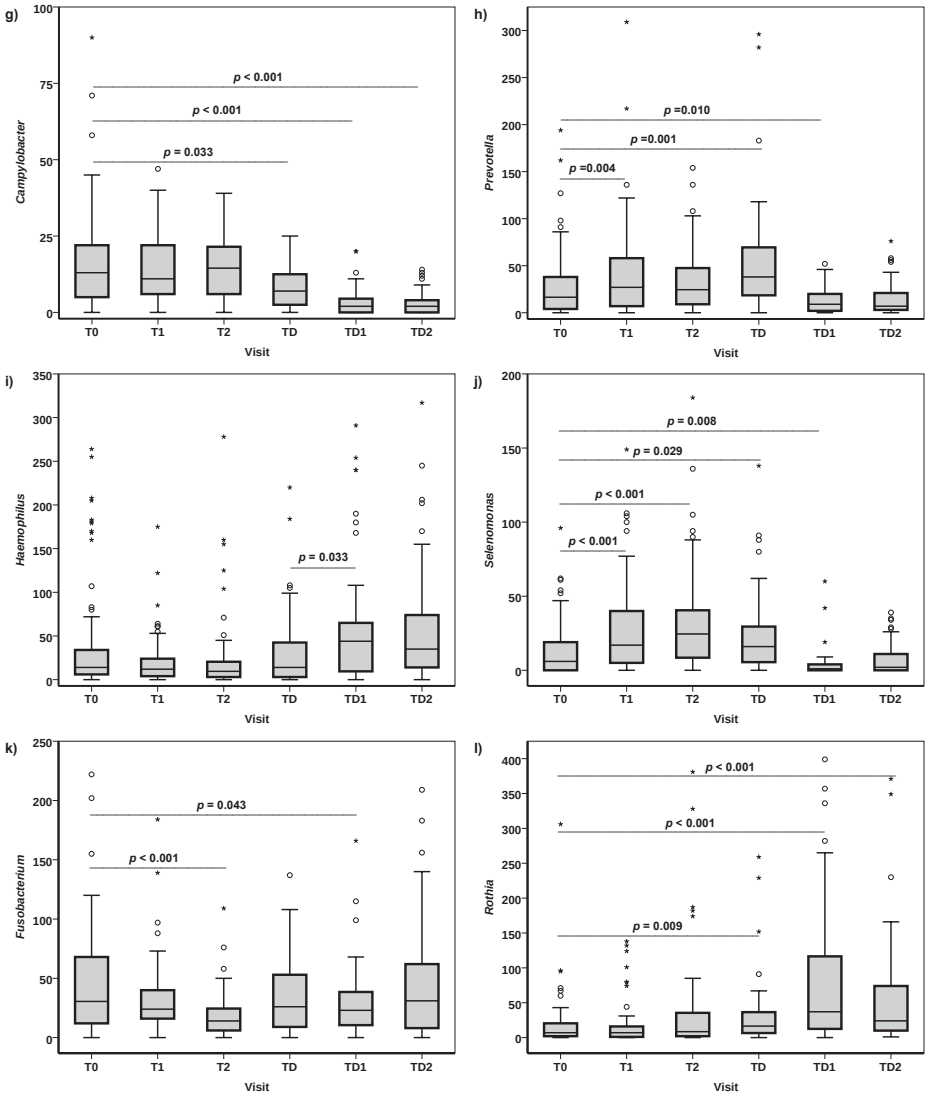


Figure S7. (continued) Difference in genus abundance between visits. The read count is displayed on the *y*-axis. Statistical significance ($p < 0.05$) was determined using the Wilcoxon Signed Ranks test. The boxes represent the median and IQR, the whiskers represent the minimum and maximum values. Outliers more than 1.5x IQR are depicted by ○, and more than 3x IQR by *.

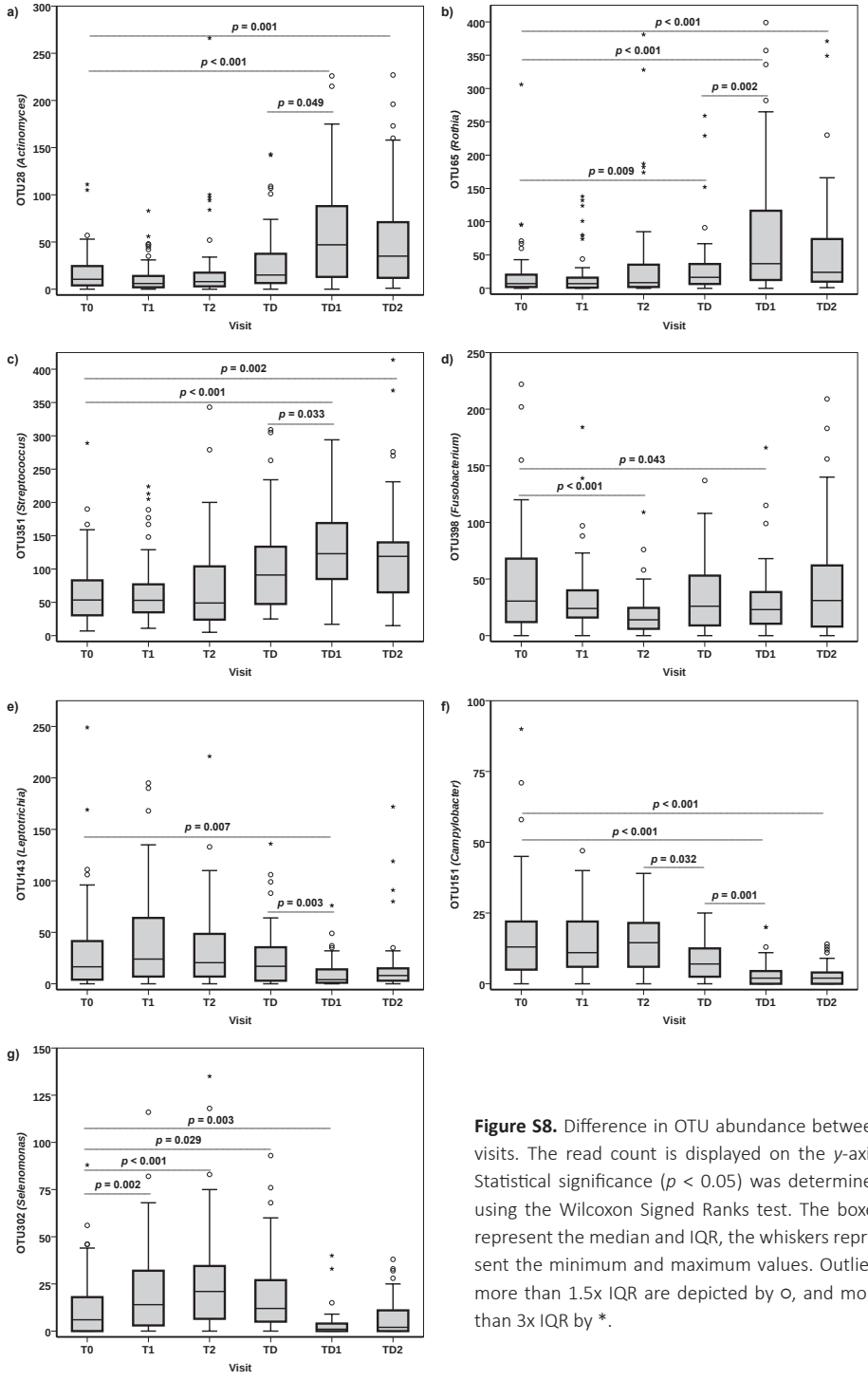


Figure S8. Difference in OTU abundance between visits. The read count is displayed on the *y*-axis. Statistical significance ($p < 0.05$) was determined using the Wilcoxon Signed Ranks test. The boxes represent the median and IQR, the whiskers represent the minimum and maximum values. Outliers more than 1.5x IQR are depicted by o, and more than 3x IQR by *.

Table S1. BLAST results of the OTUs

#OTU	Description	E-value	Accession
0	*Catonella morbi* clone _Z022 16S ribosomal RNA gene, partial sequence	0.0	GU407024
2	*Bacteroides cf. forsythus* oral clone BU063 16S ribosomal RNA gene, partial sequence	0.0k	AY008308
12	*Porphyromonas catoniae* strain JCM 13863 16S ribosomal RNA gene, partial sequence	0.0	NR_113082
28	*Actinomyces naeslundii* 16S rRNA gene, strain CCUG 33914	0.0	AJ234048
40	Bacteroidetes bacterium 'Oral Taxon 274' strain F0058 16S ribosomal RNA gene, partial sequence	0.0	FJ577256
54	*Capnocytophaga ochracea* FDC 7b 16S ribosomal RNA gene, partial sequence	9e-178	U41354
55	TM7 phylum sp. canine oral taxon 322 clone 1C049 16S ribosomal RNA gene, partial sequence	4e-166	JN713492
65	*Rothia dentocariosa* ATCC 17931, complete genome	0.0	CP002280
69	*Capnocytophaga* sp. AHN9576 16S ribosomal RNA gene, partial sequence	0.0	DQ012327
114	*Centipeda periodontii* strain HB-2 16S ribosomal RNA, partial sequence	0.0	AF458222
143	*Leptotrichia wadei* strain AGU20 16S ribosomal RNA gene, partial sequence	0.0	GU561362
151	*Campylobacter gracilis* strain ATCC 33236 16S ribosomal RNA gene, partial sequence	0.0	NR_118516
157	*Neisseria sicca* strain ATCC 29256 16S ribosomal RNA gene, complete sequence	0.0	NR_121688
171	TM7 bacterium human oral taxon HOT-869 clone 4W02 16S ribosomal RNA gene, partial sequence	0.0	KM018321
185	*Actinomyces dentalis* strain R18165 16S ribosomal RNA gene, complete sequence	0.0	NR_025633
218	*Prevotella saccharolytica* strain D080A-01 16S ribosomal RNA gene, partial sequence	0.0	FJ825150
229	*Capnocytophaga sputigena* strain NF10-3338 16S ribosomal RNA gene, partial sequence	0.0	JF422019
246	*Prevotella* sp. canine oral taxon 298 clone ZY032 16S ribosomal RNA gene, partial sequence	6e-150	JN713465
271	*Cardiobacterium hominis* 16S ribosomal RNA gene, partial sequence	0.0	AY360343
289	*Selenomonas sputigena* strain ATCC 35185 16S ribosomal RNA gene, complete sequence	0.0	NR_074905
302	*Selenomonas noxia* strain ATCC 43541 16S ribosomal RNA gene, partial sequence	0.0	NR_028796
303	*Capnocytophaga leadbetteri* strain AHN8730 16S ribosomal RNA gene, partial sequence	0.0	DQ012358
306	TM7 phylum sp. canine oral taxon 363 clone 2A026 16S ribosomal RNA gene, partial sequence	9e-128	JN713533
326	*Leptotrichia* sp. PG10 16S ribosomal RNA gene, partial sequence	0.0	GU561363
332	*Bergeyella* sp. AF14 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal RNA intergenic spacer, complete sequence; and 23S ribosomal RNA gene, partial sequence	0.0	DQ241813

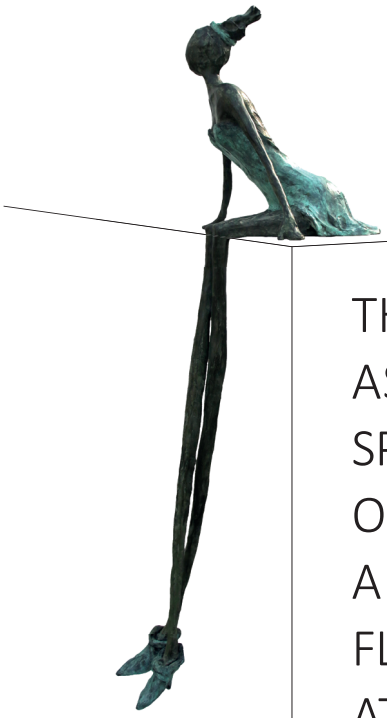
Table S1. BLAST results of the OTUs (continued)

#OTU	Description	E-value	Accession
345	*Lachnospiraceae* bacterium canine oral taxon 346 clone 1O089 16S ribosomal RNA gene, partial sequence	4e-151	JN713515
347	*Prevotella micans* strain F0438 16S ribosomal RNA gene, partial sequence	0.0	HM596284
351	*Streptococcus* sp. VT 162, complete genome	0.0	CP007628
355	TM7 phylum sp. oral taxon 348 clone BN036 16S ribosomal RNA gene, partial sequence	0.0	GQ422738
370	Candidatus *Prevotella* conceptionensis strain 9403948 16S ribosomal RNA gene, partial sequence	0.0	HM587326
381	*Kingella denitrificans* strain ATCC 33394 16S ribosomal RNA gene, complete sequence	0.0	NR_044658
390	*Leptotrichia buccalis* strain GEJ9 16S ribosomal RNA gene, partial sequence	0.0	GU561361
398	*Fusobacterium nucleatum* strain FDC 364 16S ribosomal RNA gene, partial sequence	0.0	KM023647
411	*Leptotrichia buccalis* DSM 1135, complete genome	9e-168	CP001685
424	Human oral bacterium AC32 16S ribosomal RNA gene, partial sequence	0.0	AF201979
435	*Actinomyces* sp. TeJ5 16S ribosomal RNA gene, partial sequence	0.0	GU561315
453	*Peptostreptococcaceae* bacterium OBRC9 16S ribosomal RNA gene, partial sequence	0.0	HQ616354
454	*Porphyromonas catoniae* strain ATCC 51270 16S ribosomal RNA gene, partial sequence	3e-172	NR_026230

The BLAST results were retrieved on 13 November 2014. Sequences were aligned against the Nucleotide collection
(nr/nt) using Megablast. Uncultured or environmental samples were excluded from the search results.

Table S2. Number of subjects per group per visit

		Mouthwash		Gender	
Visit	Total	Fluoride	Placebo	Male	Female
T0	76	34	42	36	40
T1	73	34	39	38	35
T2	68	27	41	32	36
TD	44	16	28	20	24
TD1	43	20	23	20	23
TD2	45	18	27	22	23



CHAPTER 4

THE REPRODUCIBILITY OF ASSESSMENT OF WHITE SPOT LESIONS ADJACENT TO ORTHODONTIC BRACKETS, WITH A QUANTITATIVE LIGHT INDUCED FLUORESCENCE DIGITAL CAMERA AT DIFFERENT ROTATIONS OF TEETH – AN *IN VITRO* STUDY

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ABSTRACT

A quantitative light-induced fluorescence digital (QLF-D) camera is able to assess demineralizations adjacent to orthodontic brackets. Rotations of teeth during and the presence of the brackets may influence the longitudinal follow-up of such lesions over time. In this study brackets were bonded on extracted teeth: 54 incisors and 31 canines. Demineralizations were formed *in vitro* directly cervical of the bracket. Images were captured using a QLF-D camera mounted on an optical bench, equipped with a goniometer on a turntable. The teeth were placed in the goniometer simulating buccolingual rotation (0°, 10°, 20°), the turntable was used for mesiodistal rotations (0°, 10°, 20°). Standardized QLF-D images were made before (with and without a wire) and after debonding at combinations of aforementioned angles of rotation. The image after debonding at 0° buccolingual and 0° mesiodistal rotation served as control. The presence of a bracket resulted in a significantly higher fluorescence loss, yet a smaller lesion area (p <0.05) in comparison to the control. A significant higher fluorescence loss was seen for rotations towards lingual relative to the 0° buccolingual and 0° mesiodistal rotation, while the effect was less explicit towards buccal. It is concluded that fluorescence loss and lesion size are influenced by the angle of rotation under which the demineralization is photographed. The full extent of demineralizations is only apparent after debonding when photographed at rotations of 0° mesiodistal and up to 20° buccal. Precaution must be taken into account assessing demineralizations of patients undergoing fixed appliance treatment when using a QLF-D camera.

BACKGROUND

During orthodontic treatment with fixed appliances, white spot lesions (WSL) are frequently formed around brackets. This serious problem is the result of poor oral hygiene and occurs in 23% to 97% of patients (Boersma *et al.*, 2005, Lovrov *et al.*, 2007, Tufekci *et al.*, 2011, Julien *et al.*, 2013, Lucchese and Gherlone, 2013). Early detection of such lesions is crucial to prevent progression and further decay over time.

Quantitative light-induced fluorescence (QLF) is a method widely used to detect and quantitate WSL. In this procedure, images of teeth are made with a high intensity blue-violet light, showing healthy tooth with green fluorescence. In case of a demineralization, such as a WSL, the natural fluorescence is reduced (van der Veen and de Josselin de Jong, 2000, Angmar-Mansson and ten Bosch, 2001). The light entering such a demineralized area is highly scattered and has a lower chance of being absorbed and reemitted as fluorescence.

One of the main advantages of QLF is that lesions may be detected earlier than by conventional visual inspection (Heinrich-Weltzien *et al.*, 2005). Studies also demonstrate that by sharing visual QLF images with patients, and pointing out lesions, patients can be motivated to improve oral hygiene (Tranaeus *et al.*, 2001). Another suggested advantage of QLF for the practitioner or researcher is the ability to monitor lesions over time in patients with fixed appliances (Livas *et al.*, 2008).

While *in vivo* reproducibility of QLF-assessment has been shown for non-bracketed teeth (Tranaeus *et al.*, 2002), the reproducibility of the assessment of WSL with a QLF digital (QLF-D) camera in patients with fixed orthodontic appliances is not known. Images captured under identical circumstances, that means using the same camera angle, can be reproducibly quantified *in vitro* (Benson *et al.*, 2003, Pretty *et al.*, 2003, Aljehani *et al.*, 2004). However, during orthodontic treatment it is difficult to standardize capturing QLF images because of eruption and orthodontically induced movements of the teeth, specifically rotation and angulation. Moreover, the light intensity of the incident light should be identical for all parts of the lesion and surrounding tooth tissue to get optimal QLF-imaging. When the lesion is adjacent to the bracket, the light path is distorted due to the bracket itself (Aljehani *et al.*, 2004). Similarly, the presence of a wire, ligature or hook attached to the bracket may interfere QLF-imaging, either by covering parts of the lesion or causing reflection of light. An accurate assessment of the lesion is further jeopardized by a lack of sufficient healthy tissue around the lesion, which is required for correct assessment of the lesion. An *in vivo* study performed in non-orthodontic individuals, also revealed that lesions adjacent to the gingiva or affected by a swollen gingiva are more difficultly detected and analysed (Ando *et al.*, 2004). This results in a limited use of QLF for the cervical part of the teeth. Due to the above-mentioned, limiting factors, a QLF-D assessment might be less reliable for use in orthodontic patients (Aljehani *et al.*, 2004).

Furthermore, the camera angle is a crucial aspect in the reproducibility of WSL assessments (Ando *et al.*, 2004, Livas *et al.*, 2008). *In vitro* studies show that, with different camera angles, WSL images can be reproduced correctly, when imaging teeth without brackets, and in turn assessed accurately for lesion area and fluorescence loss with variations in QLF camera angles of up to 20° (Benson *et al.*, 2000, Ando *et al.*, 2004). However, when the angle was increased above 20°, there were significant differences in the outcome of the QLF-parameters (Ando *et al.*, 2004), resulting in a higher percentage of fluorescence loss and a slight reduction of the demineralization area. For teeth with brackets WSL are reproducibly assessed at white light images with lingual angles of rotation up to 20° (Livas *et al.*, 2008).

The objective of this study was to investigate the reproducibility using a QLF-D camera to detect and monitor the area and the loss of fluorescence of WSL adjacent to orthodontic brackets. Reproducibility is assessed for different mesiodistal and buccolingual angles of rotation up to 20° of teeth with or without brackets or in case of brackets with either a hook or elastic ligature and wire. With this data, it can be evaluated whether QLF-D can be used for longitudinal monitoring of WSL in patients with fixed orthodontic appliances.

MATERIALS AND METHODS

Objective

An *in vitro* experiment was performed in which extracted maxillary incisors and canines with an artificial WSL cervical of an orthodontic bracket were assessed with a QLF-D camera. Fluorescence images were captured with bracket (identified as group WB), with bracket, grey elastic ligature and wire (group WE) and after debonding (group AD). The orientation of the buccal surface towards the camera was varied by rotations up to 20° along the tooth's length axis, i.e. mesiodistal rotations, or transverse axis, i.e. tilting the tip of the crown forwards to (simulating rotations around the transverse axis towards buccal) or backwards from (lingual) the camera). These rotations were chosen as being the rotations seen in patients with a near-normal occlusion (Tong *et al.*, 2012).

Sample size and power calculation

To determine the sample size, a power analysis was performed based on a t-test to compare the means of two dependent groups with an alpha of 0.05 (G*Power 3.1.9.2). In comparison to incisors, the surface morphology of canines and presence of a hook on the canine-bracket are expected to have a stronger effect on caries outcomes for different angles of rotation. Thus for the canines the influence of both mesial and distal angles of rotation were assessed, resulting in 75 different angles of rotation. For the incisors only the mesial angles of rotation were assessed, resulting in 45 images per incisor. For the sample size

calculation an effect size of 0.6 was assumed for canines, and an effect size of 0.45 for incisors. This resulted in a minimum sample size of 31 for the canines and 54 for the incisors.

Procedure

Extracted teeth were collected. The selected teeth were all maxillary and had no restorations and showed no caries, discoloration or enamel defects on the surface to be studied. The teeth were polished with a cup and polishing paste (Zircate Prophy Paste, Dentsply International, York, United States). Brackets (APC II Vicory Series Low Profile metal bracket, 3M Unitek, Monrovia, United States) were bonded (Transbond XT Light Cure Adhesive paste, 3M Unitek, Delft, The Netherlands) without using primer or etchant. The brackets were bonded in the middle of the tooth mesiodistally and a periodontal probe was used to bond the brackets 2.5mm from the incisal edge for the incisors and 3mm from the incisal edge for the canines. Primer and etchant were not used to avoid demineralization effects at unwanted places. The brackets bonded to the canines had a soldered hook attached to the cervical part of the bracket. The hooks were all bonded in the same direction. The excess of composite around the bracket was removed with a microbrush and probe. Blue tape (PVC tape, Coroplast, Wuppertal, Germany) measuring two by three millimetres was attached directly cervical of the brackets on the teeth (Torlakovic *et al.*, 2012). The tape covered the area where the WSL was created. The teeth were then covered with fluoride free bonding material (Clearfil SE Bond, Kuraray Dental, New York, United States), thus excluding the places where the bracket and tape were located. Next, the tape was removed and the teeth were placed in a glass container with the bracket facing up. WSL were created according to a demineralization procedure (Lynch, 2006, Lynch and ten Cate, 2006) using methylcellulose gel and lactic acid (pH 4.6) for 17 days. This resulted in less demineralization than anticipated and thus all teeth were etched (Ultra-Etch, Ultradent, South Jordan, United States) for five minutes, on the place where the demineralization was formed in order to obtain homogeneous WSL in the range of ICDAS 1 and 2 for the assessment on QLF images. The teeth were kept in distilled water the refrigerator, except when capturing the images. Prior to capturing the images, the teeth, but not the WSL, were polished to remove the fluoride free bonding.

Standardized set-up and outcome measures

Teeth were captured in a standardized set-up using a QLF-D camera mounted on an optical bench, which further comprised a self-constructed goniometer mounted on a turntable (fig.1). Teeth were placed up to seven millimetres apical of the cement-enamel junction in the goniometer, at 50 millimetres distance measured from the end of the tube, to simulate buccolingual (bl) tooth rotation. The turn-table mounted under the goniometer, was used to simulate mesiodistal (md) tooth rotation.

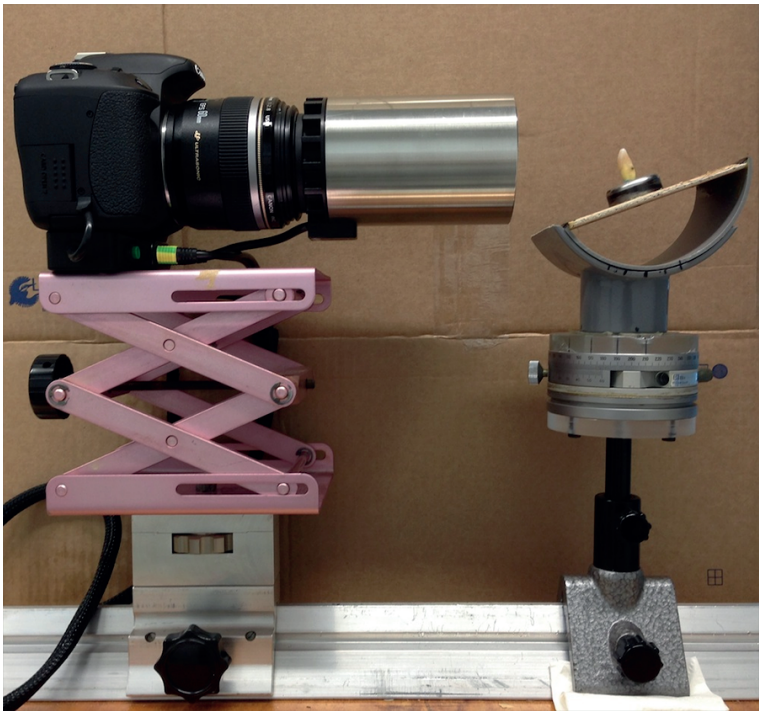

Figure 1. Standardized set-up. Incisor (AD) in goniometer at 0° mesiodistal and 20° buccal.

The camera focal length was fixed for the duration of the experiment. The teeth were rotated along their length axis or transverse axis (tilting the tip of the crown forwards (buccal) to or backwards (lingual) from the camera).

The images were captured in a room with dimmed light. A box was placed over the standardized set-up to create a dark room in order to avoid bias caused by different ambient light conditions. A dark background was used to enlarge the contrast in the images and to mimic the natural situation inside the mouth. QLF-images were captured from the buccal surfaces of all teeth using the QLF-D Biluminator system (Inspektor Research Systems, Amsterdam, The Netherlands). The QLF-D camera consisted of an illumination tube (Biluminator™; Inspektor Research Systems B.V., Amsterdam) fitted on a single-lens reflex camera (Canon model 650-D, fitted with a 60 mm Macro lens; Canon Inc., Tokyo). The illumination tube is composed of a ring with 12 violet-blue LEDs (405 ± 20 nm) with filtering optics in the centre and having transmission peaks of 90% around 640nm, 15% around 500nm and 20% around 440nm. Two images were made of each tooth per position with default settings; one white-light image and one QLF image. Only the QLF images were used for the assessment.

The images were captured at different angles of tooth rotation: for all teeth 0°, 10° and 20° towards mesial (0°md, 10°m, 20°m); towards buccal (0°bl, 10°b, 20°b); and towards

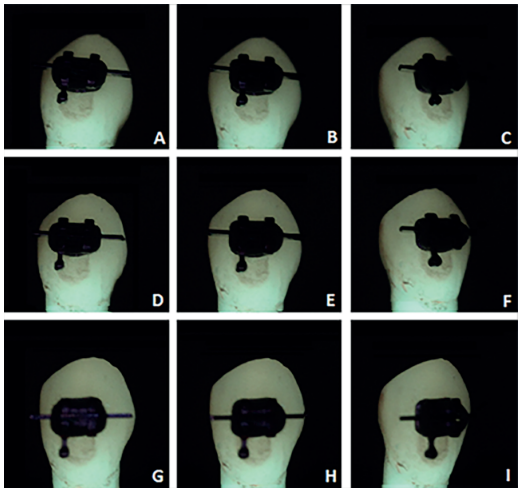

Figure 2. QLF photos of a canine WE at a few different angles.
A) 10°m x 10°l, B) 0°md x 10°l, C) 20°d x 10°l, D) 10°m x 0°bl, E) 0°md x 0°bl, F) 20°d x 0°bl, G) 10°m x 20°b, H) 0°md x 20°b, I) 20°d x 20°b

lingual (0°bl, 10°l, 20°l) and only for the canines, because of the soldered hook on one side of the bracket, also towards distal (0°md, 10°d, 20°d). Images were first captured with just brackets on the teeth (group WB), secondly with brackets and with a wire ligated with a grey elastic (NiTi 16x22, Dentsply Lomberg, Zoetermeer, The Netherlands) in place (group WE) (fig. 2). Finally images were captured after debonding of the brackets (group AD) to obtain a view of the full lesion extent. This led to a total of 45 photographs per tooth for the incisors and 75 images per tooth for the canines.

Data assessment (statistics)

Analysing WSL on the images was done using QA2 Data analysis software (version 1.26, QLF-D Research Suite, Inspektor Research Systems). The program makes a comparison between sound and demineralized enamel (de Josselin de Jong *et al.*, 1995). The comparison is based on a contour, which is drawn around the WSL. A sound patch is reconstructed through a two-dimensional linear interpolation of the sound enamel of the contour. The decrease in fluorescence is determined between the reconstructed sound and demineralized area and the mean percentage fluorescence loss (ΔF[%]) and the lesion area of the WSL are calculated.

A random selection of 10% of the teeth were analysed by two persons (MF and NK) to get inter-examiner reliability. After four weeks, examiner MF analysed the images of six incisors and three canines again to analyse the intra-examiner reliability. For the results, the assessments of examiner MF were used in all cases. Data regarding lesion area and fluorescence loss was presented in figures for incisors and canines separate to visualize changes caused by the rotation of teeth and the direction of such changes. A t-test was used to compare images WB, WE or AD at all angles of rotation to the non- rotated control image after debonding (0°md and 0°bl AD). For the second assessment they were compared to their

corresponding non-rotated control image (*e.g.* 0°md and 0°bl WB) (ANOVA for repeated measures; followed by t-test). For the third assessment the mesiodistal rotations combined without a buccolingual rotation were used as control image in comparison to the images with corresponding mesiodistal rotation and different buccolingual rotations (t-test). A fourth assessment compared the differences between the WB and WE images for the same angle (t-test). Both fluorescence loss ΔF[%] and area were assessed separately. All analyses were carried out using IBM SPSS Statistics 23.

RESULTS

The inter-examiner ICC values for ΔF[%] were 0.75 for the incisors and 0.66 for the canines. For area the ICC values were 0.76 for the incisors and 0.81 for the canines. The respective intra-examiner ICC values (examiner MF) were 0.95 for the incisors and 0.94 for the canines regarding lesion area. Regarding ΔF[%] the intra-examiner ICC values were 0.82 for the incisors and 0.81 for the canines.

Eighty-five extracted teeth were photographed. On one canine the bracket came loose during placement of the elastic ligature and wire. As a result 30 canines were used in the analysis of WE.

The presence of a bracket (both WB and WE) resulted in a significantly higher ΔF[%] and a lower lesion area (p<0.05) relative to the non-rotated control image after debonding (0°md-0°bl AD). This applied to the incisors and canines, separate and combined. The canines showed overall a lower ΔF[%] and a lower lesion area relative to the incisors.

Fluorescence loss (ΔF[%])

The influence of rotation during assessment on fluorescence loss is shown in figure 3. In figure 3 striped patterns represent a significantly different fluorescence loss relative to the non-rotated control image with the corresponding color. In additional table S1 and S2 the descriptive data are presented.

Incisors

An increased ΔF[%] was seen for rotations towards lingual compared to the non-rotated control, showing a higher ΔF[%] with a larger angle towards lingual. This was significant for the series with bracket (WB): F(7.40, 391.97)=18.75, p=0.0; with elastic and ligature (WE): F(8.91, 472.36)= 24.50, p=0.0; and without bracket (AD): F(6.17, 326.97)=56.31, p=0.0.

No significant differences in fluorescence loss were seen for rotations towards buccal. This significance was seen for all the lingual rotations compared to 0° mesiodistal as well as for the lingual rotations compared to the corresponding 10° or 20° mesial rotation.

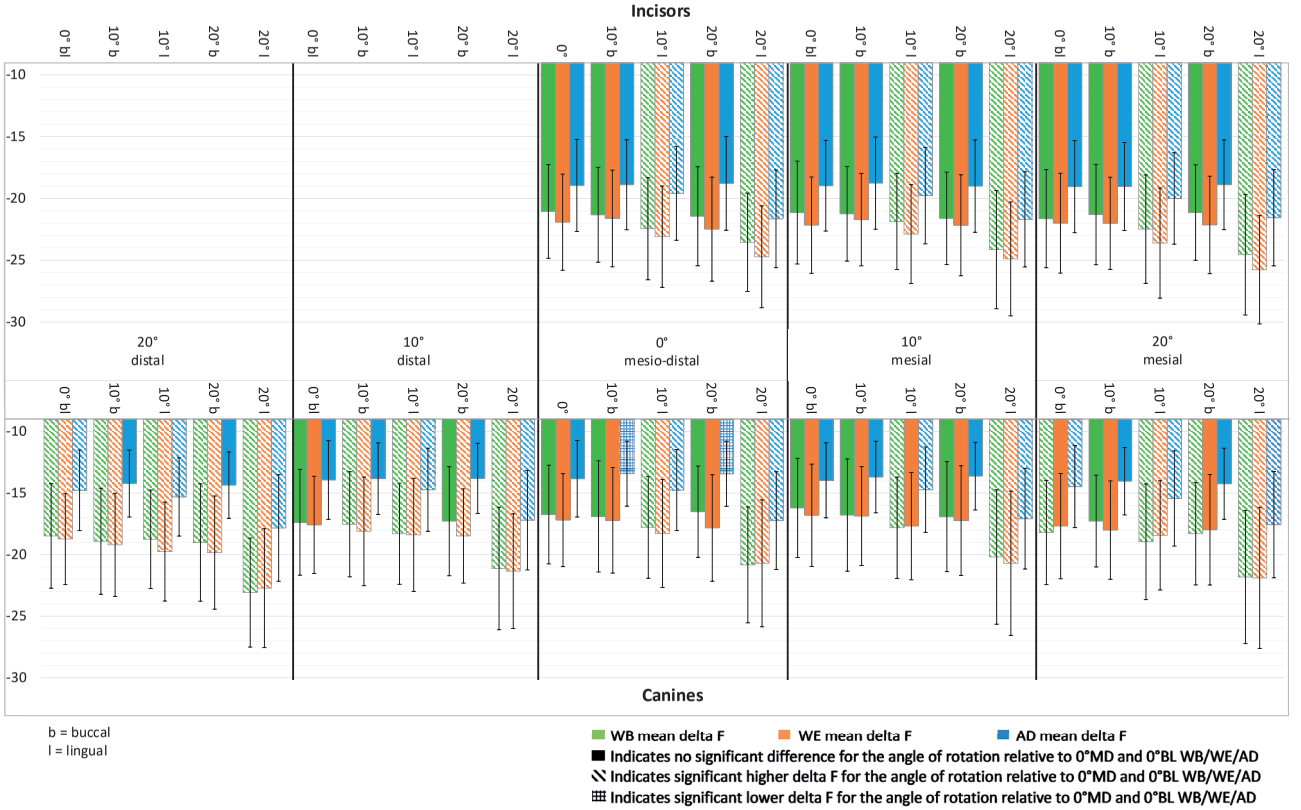

Figure 3. Average ΔF[%] (y-axis) at the different rotation angles [^{0}] (x-axis).
Average ΔF[%] on the y-axis and on the x-axis the different rotation angles for the teeth photographed. The incisors are represented in the upper part and the canines in the lower part. The data are presented in green for WSL images with bracket (WB), in orange for WSL images with elastic ligature and wire (WE) and in blue WSL images after debonding (AD).

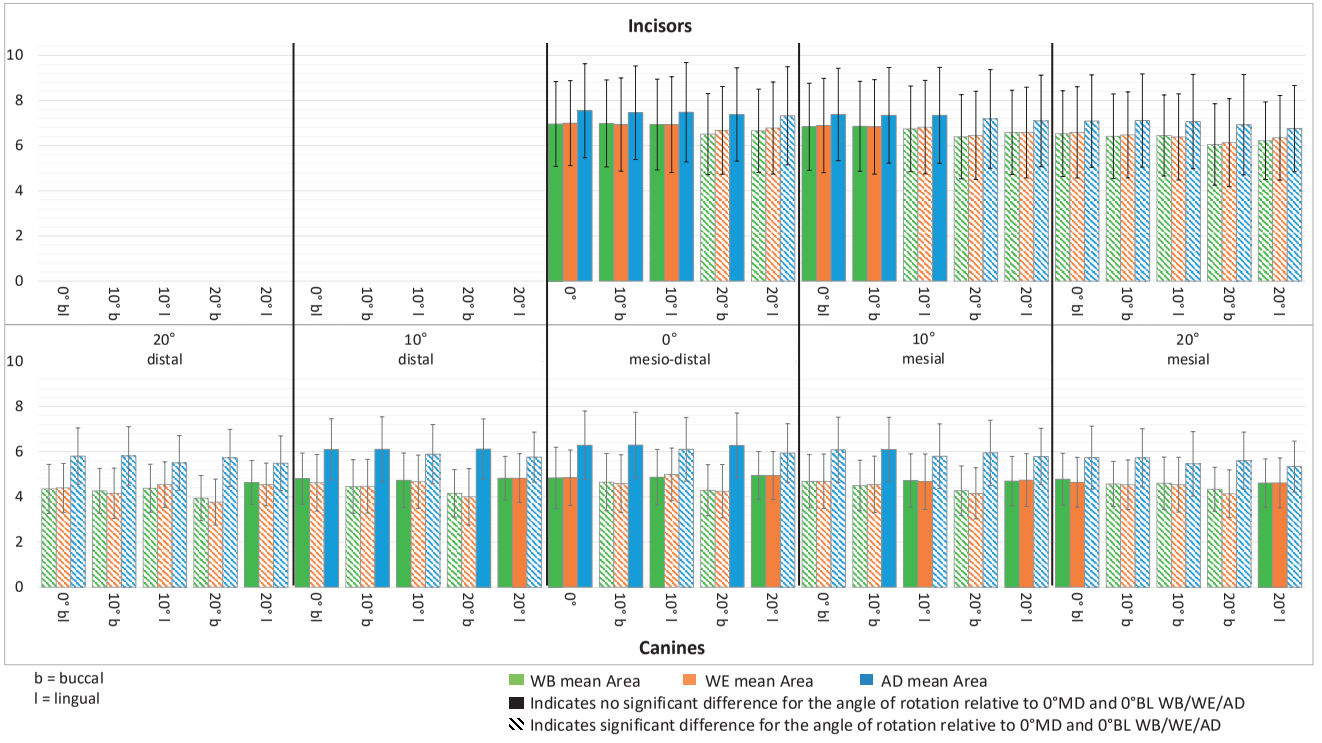

Figure 4. Average lesion area [mm^2] (y-axis) at the different rotation angles [^{0}] (x-axis).

Average lesion area [mm^2] on the y-axis and on the x-axis the different rotation angles for the teeth photographed. The incisors are represented in the upper part and the canines in the lower part. The data are presented in green for WSL images with bracket (WB), in orange for WSL images with elastic ligature and wire (WE) and in blue WSL images after debonding (AD).

Canines

For the canines a greater fluorescence loss was seen for the increasing angles towards lingual. Towards buccal only a larger angle towards mesial or distal showed a significance.

Incisors and canines combined

Almost all angles of rotation showed a significant higher ΔF[%] between images WB and images WE when comparing the same angles of rotation.

Area

The influence of rotations on the lesion area is shown in figure 4. In figure 4 striped patterns represent a significantly different area relative to the non-rotated control image with the corresponding color. In additional table S3 and S4 the descriptive data are presented.

Incisors

A significantly smaller lesion area relative to that of the non-rotated control images was seen for a mesial rotation of 20° for all groups regardless of the buccolingual rotation. This trend was less pronounced at a mesial rotation of 10° and at 0° mesiodistal. After debonding all rotations of 20° lingual, irrespectively of the mesial rotation resulted in a significantly smaller lesion area relative to the control.

Canines

A significantly smaller lesion area relative to the non-rotated control images was seen for 50 out of 72 angles of rotation towards mesiodistal or buccolingual. When brackets were present, lesion area was influenced more for rotations towards buccal than towards lingual. After debonding the opposite was seen, all rotations towards lingual showed a significantly smaller lesion area. In case of brackets, distal rotations had more influence than mesial rotations. With brackets irrespective of the mesial rotation, rotations towards buccal resulted in smaller lesions size, while such an influence was not seen after debonding.

Incisors and canines combined

No significant differences in lesion areas were found between images WB and images WE when comparing the same angles of rotation.

DISCUSSION

The presence of orthodontic brackets with or without an elastic ligature and wire affected WSL assessment with QLF-D when compared with the situation after debonding. Even when QLF-D images of teeth with brackets were obtained under standardized conditions

the ΔF[%] was overestimated, while the lesion area was underestimated. This result was not consistent with previous reports on light images, which showed that on 20 maxillary central incisors with brackets the area of WSL was reproducibly assessed for lingual rotations up to 20° in comparison to teeth without fixed appliances (Livas *et al.*, 2008). This discrepancy may be caused by the use of QLF images instead of light images and the bigger sample size in this study. To calculate ΔF[%] and area a comparison was made between sound and demineralized enamel (de Josselin de Jong *et al.*, 1995). Due to the presence of a bracket there was no sound enamel available for the assessment on the coronal site. Therefore this site was excluded on the contour around the WSL for the reconstructed sound patch, leading to a higher contrast between sound and demineralized enamel and, as shown in this study, resulting in a higher fluorescence loss and a smaller lesion area (Ando *et al.*, 2004). Besides that, the enamel in the coronal area is thicker than that of the cervical area (Ando *et al.*, 2004).

No significant differences in lesion area, incisors and canines together, were found, for the same rotation angle of group WB relative to group WE. In contrast ΔF[%] was overestimated for almost all angles of rotation when comparing the same angle in group WE to group WB. This indicates that elastic ligatures around and wires through the bracket did not influence the measured area of the WSL, but did have an influence on ΔF[%]. An elastic ligature placed around the bracket with a wire resulted in a shadow on the WSL and hence a darker appearance of the WSL and therefore a further difference in contrast of ΔF[%] (Benson *et al.*, 2000). An elastic ligature around the bracket with a wire thus interfered in the analysis of WSL, due to a distorted light intensity. It is feasible that other materials used during an orthodontic treatment with fixed appliances may also create interference, such as a Kobayashi hook, a chain elastic or an overlay arch.

In this study for all groups of incisors rotations towards lingual always resulted in a significant higher ΔF[%] relative to the corresponding non-rotated control image, but rotations towards buccal did not give significant differences for ΔF[%]. This showed that ΔF[%] is overestimated for all incisors with or without brackets only towards the lingual direction. In the lingual direction thinner cervical enamel is used as a reference in the assessment of the WSL, compared to the thicker and brighter coronal enamel. When the enamel is thinner, the fluorescence is higher due to more reflection of dentin (Ando *et al.*, 2004).

Further, this study showed that for incisors after debonding at 0°md only a lingual rotation of 20° gave a significant smaller lesion area relative to the non-rotated control image after debonding. For canines after debonding at 0°md lingual rotations relative to the non-rotated control image resulted in a significant smaller lesion area, while the angles of rotation towards buccal did not result in a significant smaller lesion area. This result is consistent with previous research which showed that the lesion area of WSL at teeth after debonding can be reproducibly measured at buccal rotations up to 20° (Benson *et al.*, 2000, Ando *et al.*, 2004).

The presence of orthodontic brackets at the incisors or canines, separately or together, with or without an elastic ligature and wire affected WSL assessment. In this study all rotations resulted in a significant higher ΔF[%] and a smaller lesion area in comparison to the non-rotated control image after debonding. In the assessment of WSL the bracket with or without a soldered hook caused a higher ΔF[%] and a smaller area. This shows that a bracket with or without an attached hook makes no difference on the WSL assessment in comparison to teeth after debonding. Whilst, brackets on canines with a, at the mesial site, attached hook show more significance towards distal, regardless of the buccal or lingual angle. This means that the presence of a hook attached to the cervical part of the bracket has the largest effect on rotations towards the side opposite of the location of the hook, due to interference of the hook being projected over the WSL.

To summarize, when a bracket is present on a tooth there is less healthy tooth tissue available to use as a reference in the assessment of a WSL. Such a healthy tooth tissue reference is required to get a reliable calculation of ΔF[%] and area (Aljehani *et al.*, 2004). This resulted for all bracketed teeth in a significantly higher ΔF[%] and a smaller area for all angle combinations when compared to teeth after debonding. For teeth after debonding, the lesion area seemed to be reproducibly measured with QLF-D for rotations up to 0°md and 20°b. In such a situation there is healthy tooth tissue available on the coronal site to use as a reference in the assessment of a WSL. Furthermore, this study showed that the effect of a bracket on a tooth on the mean values for area is bigger than for ΔF[%] and that a hook attached to the bracket has the largest effect on rotations towards the side opposite of the location of the hook.

CONCLUSION

A QLF-D camera can detect WSL adjacent to orthodontic brackets irrespective the presence of an elastic ligature and wire. However, ΔF[%] is overestimated and the lesion area is underestimated, when compared with teeth after debonding, at various mesiodistal and buccolingual rotations (0° and up) under which the WSL is photographed. This is due to the presence of the bracket, where a healthy tissue reference at the coronal part of the tooth is not available to determine the WSL. Furthermore, elastic ligatures and wires around or through brackets in orthodontic treatment resulted in a significant overestimation of ΔF[%], but not for lesion area, both parameters compared to teeth with brackets without elastic ligature and wire. The presence of a hook attached to the cervical part of the bracket had the largest effect on these parameters under rotations towards the side opposite of the location of the hook. This implies that precaution must be taken when assessing WSL over time in patients undergoing treatment with fixed appliances using QLF-D. The images of the tooth should always be made under the same angle of rotation, with the same light

intensity and for example always without a wire. The full extent of WSL developed adjacent to orthodontic brackets will only become apparent after debonding with rotations until 20° towards buccal and 0° mesiodistal. Thus the use of QLF for longitudinal follow-up of WSL is limited clinically, but QLF is very useful for demonstrating purposes, showing patients the presence of WSL, which are earlier detectable by QLF than by visual inspection (Heinrich-Weltzien *et al.*, 2005) and showing the presence of matured plaque as red fluorescence in the images (Tranaeus *et al.*, 2001).

RECOMMENDATION

QLF can be effectively used in identifying and showing demineralizations of teeth with or without orthodontic brackets. A suitable application is for example, the use as a preventive tool and for demonstrating purposes in the dental and orthodontic practice. In research settings for patients with fixed appliances over time QLF-D is less easily used, since too much alterations take place over time thus interfering with the measurements. Also after debonding a standardized method of monitoring lesions, such as a bite block, is recommended in research settings. Further research is needed to investigate whether the effect of rotations of teeth in patients with fixed appliances can be corrected for, for example by a percentage of over- or underestimation of ΔF[%] and area.

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SUPPLEMENTARY MATERIAL

Table S1. Descriptive data and statistical outcome for the effect of rotation on fluorescence loss (ΔF[%]) for the incisors (n=54).

	WB Delta F		WE Delta F		AD Delta F	
Rotation Angle	Mean (sd)	p-value comparison with 0°	Mean (sd)	p-value comparison with 0°	Mean (sd)	p-value comparison with 0°
0°md-20°l	-23.55 (4.0)	0.000*	-24.72 (4.1)	0.000*	-21.65 (4.0)	0.000*
0°md-10°l	-22.45 (4.1)	0.000*	-23.09 (4.1)	0.000*	-19.58 (3.8)	0.000*
0°	-21.05 (3.8)	-	-21.92 (3.9)	-	-18.95 (3.7)	-
0°md-10°b	-21.32 (3.8)	0.252	-21.62 (3.9)	0.389	-18.89 (3.6)	0.692
0°md-20°b	-21.44 (4.0)	0.208	-22.48 (4.2)	0.104	-18.78 (3.8)	0.288
10°m-20°l	-24.14 (4.8)	0.000*	-24.89 (4.6)	0.000*	-21.69 (3.9)	0.000*
10°m-10°l	-21.86 (3.9)	0.017*	-22.88 (4.0)	0.014*	-19.77 (3.9)	0.000*
10°m-0°bl	-21.14 (4.2)	0.776	-22.16 (3.9)	0.545	-18.97 (3.7)	0.861
10°m-10°b	-21.24 (3.8)	0.529	-21.71 (3.7)	0.553	-18.76 (3.7)	0.181
10°m-20°b	-21.61 (3.7)	0.104	-22.17 (4.1)	0.507	-19.00 (3.7)	0.747
20°m-20°l	-24.55 (4.9)	0.000*	-25.77 (4.4)	0.000*	-21.56 (3.9)	0.000*
20°m-10°l	-22.48 (4.4)	0.000*	-23.61 (4.5)	0.000*	-20.00 (3.7)	0.000*
20°m-0°bl	-21.63 (4.0)	0.129	-22.00 (4.0)	0.852	-19.04 (3.7)	0.643
20°m-10°b	-21.30 (4.1)	0.538	-22.01 (3.7)	0.788	-19.03 (3.6)	0.614
20°m-20°b	-21.14 (3.9)	0.798	-22.14 (3.9)	0.528	-18.89 (3.6)	0.787
ANOVA for Repeated measures	$F(7.40, 391.97)=18.75$, $p=0.0$		$F(8.91, 472.36)= 24.50$, $p=0.0$		$F(6.17, 326.97)=56.31$, $p=0.0$	

Table S2. Descriptive data and statistical outcome for the effect of rotation on fluorescence loss (ΔF[%]) for the canines (WB: n=31, WE: n=30, AD: n=31).

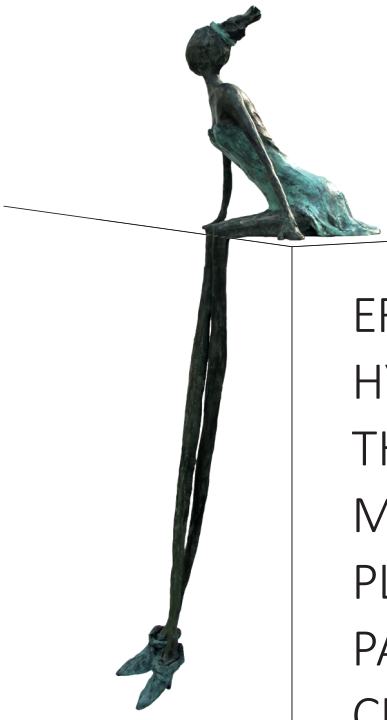
	WB Delta F		WE Delta F		AD Delta F	
Rotation Angle	Mean (sd)	p-value comparison with 0°	Mean (sd)	p-value comparison with 0°	Mean (sd)	p-value comparison with 0°
20°d-20°l	-23.08 (4.4)	0.000*	-22.72 (4.8)	0.000*	-17.83 (4.3)	0.000*
20°d-10°l	-18.75 (4.0)	0.000*	-19.75 (4.0)	0.000*	-15.31 (3.2)	0.000*
20°d-0°bl	-18.48 (4.3)	0.000*	-18.72 (3.7)	0.002*	-14.77 (3.3)	0.000*
20°d-10°b	-18.91 (4.3)	0.000*	-19.20 (4.2)	0.000*	-14.22 (2.7)	0.098
20°d-20°b	-19.01 (4.8)	0.000*	-19.83 (4.6)	0.000*	-14.35 (2.7)	0.058
10°d-20°l	-21.12 (5.0)	0.000*	-21.34 (4.7)	0.000*	-17.20 (4.0)	0.000*
10°d-10°l	-18.30 (4.1)	0.001*	-18.39 (4.6)	0.011*	-14.72 (3.4)	0.000*
10°d-0°bl	-17.37 (4.3)	0.098	-17.58 (4.0)	0.351	-13.93 (3.2)	0.580
10°d-10°b	-17.53 (4.3)	0.013*	-18.10 (4.4)	0.020*	-13.82 (2.9)	0.962
10°d-20°b	-17.28 (4.4)	0.116	-18.48 (3.8)	0.002*	-13.80 (2.9)	0.873
0°md-20°l	-20.83 (4.7)	0.000*	-20.70 (5.2)	0.000*	-17.23 (4.0)	0.000*
0°md-10°l	-17.78 (4.1)	0.003*	-18.28 (4.4)	0.002*	-14.75 (3.3)	0.000*
0°	-16.74 (4.0)	-	-17.19 (3.8)	-	-13.83 (3.1)	-
0°md-10°b	-16.89 (4.5)	0.642	-17.21 (4.3)	0.282	-13.42 (2.6)	0.016*
0°md-20°b	-16.51 (3.7)	0.514	-17.83 (4.3)	0.122	-13.43 (2.6)	0.027*
10°m-20°l	-20.19 (5.5)	0.000*	-20.70 (5.9)	0.000*	-17.08 (4.1)	0.000*
10°m-10°l	-17.81 (4.1)	0.005*	-17.68 (4.4)	0.230	-14.72 (3.5)	0.000*
10°m-0°bl	-16.20 (4.0)	0.108	-16.80 (4.2)	0.296	-13.96 (3.1)	0.354
10°m-10°b	-16.78 (4.6)	0.911	-16.86 (4.0)	0.469	-13.69 (2.9)	0.405
10°m-20°b	-16.91 (4.5)	0.676	-17.23 (4.5)	0.938	-13.62 (2.8)	0.294
20°m-20°l	-21.82 (5.4)	0.000*	-21.89 (5.7)	0.000*	-17.56 (4.3)	0.000*
20°m-10°l	-18.95 (4.7)	0.000*	-18.43 (4.4)	0.020*	-15.43 (3.9)	0.000
20°m-0°bl	-18.20 (4.2)	0.006*	-17.68 (4.3)	0.281	-14.47 (3.3)	0.014*
20°m-10°b	-17.27 (3.7)	0.285	-18.00 (4.0)	0.100	-14.03 (2.7)	0.462
20°m-20°b	-18.30 (4.2)	0.009*	-17.98 (4.5)	0.151	-14.24 (2.9)	0.213
ANOVA for Repeated measures	$F(8.86, 265.89)=22.85$, $p=0.0$		$F(8.65, 250.93)=17.51$, $p=0.0$		$F(4.48, 134.31)=45.30$, $p=0.0$	

Table S3. Descriptive data and statistical outcome for the effect of rotation on lesion area [mm] for the incisors (n=54).

	WB area		WE area		AD area	
Rotation Angle	Mean (SD)	p-value comparison with 0°	Mean (SD)	p-value comparison with 0°	Mean (SD)	p-value comparison with 0°
0°md-20°l	6.66 (1.8)	0.001*	6.78 (2.0)	0.039*	7.32 (2.2)	0.020*
0°md-10°l	6.94 (2.0)	0.818	6.93 (2.1)	0.445	7.48 (2.2)	0.564
0°	6.96 (1.9)	-	7.00 (1.9)	-	7.55 (2.1)	-
0°md-10°b	6.98 (1.9)	0.731	6.93 (2.1)	0.330	7.46 (2.1)	0.238
0°md-20°b	6.51 (1.8)	0.000*	6.68 (1.9)	0.000*	7.38 (2.1)	0.179
10°m-20°l	6.59 (1.9)	0.000*	6.58 (2.0)	0.000*	7.09 (2.0)	0.001*
10°m-10°l	6.74 (1.9)	0.008*	6.82 (2.1)	0.037*	7.34 (2.1)	0.055
10°m-0°bl	6.83 (1.9)	0.148	6.89 (2.1)	0.130	7.38 (2.0)	0.123
10°m-10°b	6.86 (2.0)	0.238	6.83 (2.1)	0.061	7.34 (2.1)	0.088
10°m-20°b	6.39 (1.9)	0.000*	6.46 (2.0)	0.000*	7.18 (2.2)	0.001*
20°m-20°l	6.22 (1.7)	0.000*	6.35 (1.9)	0.000*	6.76 (1.9)	0.000*
20°m-10°l	6.45 (1.9)	0.000*	6.38 (1.9)	0.000*	7.07 (2.1)	0.000*
20°m-0°bl	6.53 (1.9)	0.000*	6.59 (2.0)	0.000*	7.09 (2.0)	0.000*
20°m-10°b	6.41 (1.9)	0.000*	6.48 (1.9)	0.000*	7.11 (2.1)	0.000*
20°m-20°b	6.05 (1.8)	0.000*	6.13 (1.9)	0.000*	6.93 (2.2)	0.000*
ANOVA for Repeated measures	$F(6.57, 348.43)=20.66$, $p=0.0$		$F(6.14, 325.51)=15.89$, $p=0.0$		$F(6.25, 331.38)=9.54$, $p=0.0$	

Table S4. Descriptive data and statistical outcome for the effect of rotation on lesion area [mm] for the canines (WB: n=31, WE: n=30, AD: n=31).

	WB area		WE area		AD area	
Rotation Angle	Mean (sd)	p-value comparison with 0°	Mean (sd)	p-value comparison with 0°	Mean (sd)	p-value comparison with 0°
20°d-20°l	4.64 (1.0)	0.161	4.55 (0.9)	0.005*	5.49 (1.2)	0.000*
20°d-10°l	4.38 (1.1)	0.000*	4.54 (1.0)	0.001*	5.51 (1.2)	0.000*
20°d-0°bl	4.35 (1.1)	0.000*	4.40 (1.1)	0.000*	5.81 (1.2)	0.003*
20°d-10°b	4.27 (1.0)	0.000*	4.16 (1.1)	0.000*	5.82 (1.3)	0.003*
20°d-20°b	3.95 (1.0)	0.000*	3.77 (1.0)	0.000*	5.73 (1.3)	0.001*
10°d-20°l	4.83 (1.0)	0.887	4.83 (1.1)	0.806	5.76 (1.1)	0.003
10°d-10°l	4.74 (1.2)	0.240	4.67 (1.2)	0.039*	5.89 (1.3)	0.008*
10°d-0°bl	4.81 (1.1)	0.753	4.62 (1.3)	0.007*	6.10 (1.4)	0.141
10°d-10°b	4.46 (1.2)	0.001*	4.47 (1.2)	0.000*	6.11 (1.4)	0.080
10°d-20°b	4.16 (1.0)	0.000*	4.00 (1.2)	0.000*	6.12 (1.3)	0.200
0°md-20°l	4.95 (1.1)	0.353	4.94 (1.1)	0.267	5.94 (1.3)	0.001*
0°md-10°l	4.87 (1.2)	0.735	4.99 (1.2)	0.020*	6.11 (1.4)	0.014*
0°	4.84 (1.4)	-	4.85 (1.2)	-	6.29 (1.5)	-
0°md-10°b	4.65 (1.3)	0.032*	4.59 (1.3)	0.000*	6.29 (1.5)	0.934
0°md-20°b	4.29 (1.1)	0.000*	4.25 (1.2)	0.000*	6.28 (1.4)	0.952
10°m-20°l	4.70 (1.1)	0.162	4.74 (1.2)	0.318	5.79 (1.3)	0.000*
10°m-10°l	4.73 (1.2)	0.126	4.67 (1.2)	0.074	5.80 (1.4)	0.000*
10°m-0°bl	4.69 (1.2)	0.047*	4.69 (1.2)	0.017*	6.09 (1.4)	0.038*
10°m-10°b	4.50 (1.1)	0.001*	4.55 (1.3)	0.001*	6.10 (1.4)	0.061
10°m-20°b	4.28 (1.1)	0.000*	4.16 (1.1)	0.000	5.95 (1.4)	0.016*
20°m-20°l	4.61 (1.1)	0.080	4.62 (1.1)	0.090	5.35 (1.1)	0.000*
20°m-10°l	4.61 (1.2)	0.002*	4.54 (1.2)	0.004*	5.46 (1.4)	0.000*
20°m-0°bl	4.78 (1.1)	0.368	4.65 (1.1)	0.072	5.74 (1.4)	0.000*
20°m-10°b	4.57 (1.0)	0.019*	4.54 (1.1)	0.016*	5.73 (1.3)	0.000*
20°m-20°b	4.34 (1.0)	0.000*	4.14 (1.1)	0.000*	5.60 (1.3)	0.000*
ANOVA for Repeated measures	$F(6.77, 202.95)=12.01$, $p=0.0$		$F(5.77, 167.17)=16.23$, $p=0.0$		$F(5.97, 178.98)=11.07$, $p=0.0$	



CHAPTER 5

EFFECT OF REPEATED ORAL HYGIENE INSTRUCTIONS WITH THREE DIFFERENT FEEDBACK METHODS ON THE LEVEL OF PLAQUE IN ORTHODONTIC PATIENTS: A RANDOMIZED CLINICAL TRIAL

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Submitted for publication.

ABSTRACT

Introduction: In preventing caries formation during fixed appliance treatment, maintaining good oral hygiene is important.

Methods: A randomized clinical trial was performed starting six months after placement of fixed appliances. During four study visits the participants received feedback and, on the first three visits, also oral hygiene instructions. Before and after feedback and brushing fluorescence images were made of the anterior teeth. The feedback methods used involved: 1) fluorescence images, 2) erythrosine and 3) a mirror and probe (control).

Results: A total of 87 participants participated. Between the three groups no differences were found in the level of plaque before or after feedback and brushing as assessed on the fluorescence images. A significant decrease in plaque between the four visits was found overall, although this was not clinically relevant. Dichotomizing the group showed that the group with a low level of plaque at start showed no significant differences over time. The group with a moderate to high level of plaque at start showed a significant and clinically relevant decrease in plaque over time. There is also a direct improvement seen in the efficacy of brushing directly after feedback.

Conclusions: Repeated oral hygiene feedback and instructions, started six months into treatment with fixed appliances, showed a significant and clinically relevant decrease in plaque when a moderate to high level of plaque at start was present. This was regardless of the feedback method used.

INTRODUCTION

One of the main disadvantages of fixed appliance treatment is the formation of white spot lesions (WSL). WSL are the early stage of dental caries that develop when plaque remains on the surface for a prolonged period of time. As a result of the placement of fixed appliances there are more plaque retention sites around the brackets at the buccal surfaces. These WSL are therefore mostly found on the incisors and canines, in the aesthetic zone. Normally these surfaces show a low prevalence of caries. The prevalence of WSL after treatment ranges from 50%-97% (Gorelick *et al.*, 1982, Boersma *et al.*, 2005, Julien *et al.*, 2013, van der Kaaij *et al.*, 2015), depending on the method of assessment. Risk factors are long duration of treatment, young age at start of treatment, pre-existing caries or restorations, use of carbonated food and drinks, and inadequate oral hygiene before or during treatment (Chapman *et al.*, 2010, Al Maaitah *et al.*, 2011, Khalaf, 2014).

The oral hygiene of patients having fixed appliances should therefore be reinforced with instructions and with dietary advice. Assessing a patient's oral hygiene at each visit is part of the routine oral examination performed by the orthodontist. The plaque can be shown to the patients using a mirror and a probe. In order to improve the oral hygiene during orthodontic treatment several techniques can be used. Repeated oral hygiene instructions were shown to reduce the plaque accumulation (Acharya *et al.*, 2011). Also visual aids, such as showing an image of the severe consequences of biofilm accumulation were shown to improve the oral hygiene in an orthodontic population (Peng *et al.*, 2014). Methods used during chairside instructions and feedback include plaque disclosing solutions or Quantitative Light-induced Fluorescence (QLF).

Since plaque is generally colorless, it can be stained for a better assessment and visibility. Commonly used disclosing agents are erythrosine, a pink-dye (E127), sometimes combined with a blue-dye (E133). Erythrosine adheres to the plaque and remains visible for the patient after water rinsing. In a previously mentioned study by Peng et al (Peng *et al.*, 2014) the use of disclosing tablets was ineffective, with no significant differences compared to a control group that received only the routine oral hygiene instructions. Other studies have shown that oral hygiene instructions together with plaque self-visualization through disclosing agents and a mirror resulted in an improvement of oral hygiene and reduced gingivitis in non-orthodontically treated children (Bellini *et al.*, 1974, Telford and Murray, 1974).

The QLF technique is based on the property of tooth-tissue to autofluoresce when illuminated by visible light. Changes in mineral content of tooth-tissue can be made visible because of a reduced green fluorescence (de Josselin de Jong *et al.*, 2009). A different form of fluorescence, red, may be formed as a result of porphyrins in bacterial plaque. In vivo this property was demonstrated to be a reliable tool for assessing plaque accumulation (Pretty *et al.*, 2005). Showing QLF image as oral hygiene evaluation tool was proven to be as effective as a white light image for patients having fixed appliances (Miller *et al.*, 2016).

Specific objectives or hypothesis

In the current randomized controlled clinical trial (RCT) three oral hygiene evaluation methods were evaluated using a repeated instruction and evaluation approach. The study starts from six months into treatment, when the novelty of having fixed appliances has gone and the extensive instructions, usually given at start, might have been forgotten. In four consecutive appointments the participants received oral hygiene instruction using one of the following evaluation methods: 1) showing a QLF image of their own teeth, 2) using a disclosing agent to stain their own teeth, 3) using a mirror and probe to indicate the presence of plaque. In the Netherlands these methods are included in the orthodontic guidelines for oral hygiene instructions. Our null hypothesis was that there would be no significant difference in amount of plaque between participants in one of the three groups.

MATERIAL & METHODS

Trial design and any changes after trial commencement

A RCT-study, with three parallel groups, was performed in a private orthodontic practice, aimed to measure oral hygiene levels after repeated instructions with different methods of feedback during treatment with fixed orthodontic appliances. Since the methods used in this study are imbedded in the normal orthodontic care in the Netherlands there was no need to act under the Medical Research Involving Human Subjects Act. The study was approved by the ethical committee of the Academic Centre of Dentistry Amsterdam (number 2017015).

Participants, eligibility criteria, and setting

Patients who were treated with full fixed appliances for a period of 5-7 months were eligible to participate after providing written informed consent and were free to withdraw consent at any stage. The patients were treated at a private orthodontic practice by three qualified orthodontists. To participate the patient needed to fulfill the following inclusion criteria: 1) 12-18 years of age at start of study, 2) good general health. The participants were randomly assigned to one of three study groups with different evaluation methods for oral hygiene. A consent was obtained.

Interventions

During each of four visits first a QLF photograph (TX.1) was taken of the six upper and lower anterior teeth in an end-to-end frontal bite (fig. 1a). Then the feedback was given: Group 1 received feedback via a QLF photograph of their own teeth (group ‘QLF’), showing old plaque as red fluorescence. In group 2 a mirror and the discoloring agent erythrosine were used (group ‘erythrosine’). In group 3 plaque was shown to the patient using a mirror and a probe (group ‘control’).

The participants received oral hygiene instructions together with the chosen feedback during three visits (T0, T1, T2) and only received the feedback during the last visit (T3).

After the feedback and, on T0, T1 and T2, instructions, the participants brushed their teeth and another QLF photograph was taken (TX.2) (fig. 1b). They could brush their teeth with a manual or electric toothbrush (Oral B or Philips Sonicare were provided) and use interdental brushes. Only to the QLF group the QLF image was shown during the study visit. The four study visits were performed immediately prior to the regular appliance check-up, planned approximately every 6 weeks.

Trained dental students or oral hygiene students made the photographs and gave the oral hygiene instructions.

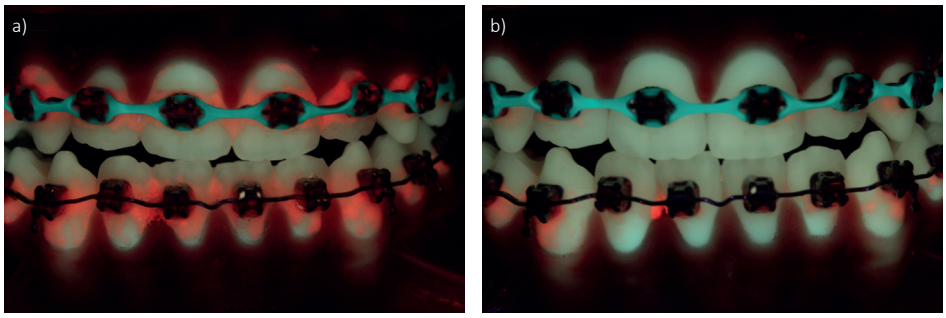

Figure 1. QLF-image of the anterior teeth: (a) before feedback and instructions; (b) after feedback, instructions and subsequently brushing.

Outcomes

The primary outcome was the amount of red fluorescent plaque, assessed on the QLF images, before and after the instructions and/or feedback at each of the four visits.

The amount of plaque was assessed using a modified plaque index: Elements of the modified Navy-index (Rustogi *et al.*, 1992) and of the modified Quigley-Hein index (Turesky *et al.*, 1970) were combined for this new index. The buccal site of the upper and lower anterior teeth (from canine to canine) were classified in eight regions (fig. 2), excluding the site where the bracket was placed. Each site was given a score from 0-3 for the amount of plaque present: 0-no plaque, 1-separate flecks of plaque, 2-a continuous band of plaque, 3-plaque covering more than 50% of the site. Per patient and image the percentage of plaque was calculated.

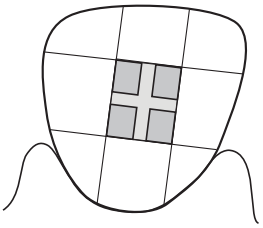

Figure 2. Schematic representation of the regions for the plaque assessment per tooth.

Sample size calculation

A power analysis was performed to determine the necessary number of participants in the study. The effect of 0.35 was chosen (with a power of 0.9 and a significance level of 0.05), resulting in a total minimally required inclusion of 84 participants (three groups of 28 participants; G*power 3.1). This effect size was based on previous studies by Acharya et al (Acharya *et al.*, 2011) and Peng et al (Peng *et al.*, 2014).

Randomization

Assignment of participants to the three study groups was done using a predefined randomization list (made in Microsoft Office Excel). Eligible patients were placed in the order of their appointment on the allocation list by the examiner. They were phoned before their appointment and the consent was send by email. At appointment the written consent was obtained when they decided to participate. Participants were informed that they received oral hygiene instructions with one of the three feedback methods.

Blinding

The trial was single-blinded, it was not possible to mask the subjects giving the feedback and instructions or the participants to the type of feedback method used. All images were blinded before scoring and were assessed by two calibrated examiners (N.K. and M.V.). Each examiner received the blinded images of half of the participants (eight images per patient) to ensure that the same examiner scored all images of a single patient.

Examiners were trained and calibrated for plaque scoring using a set of ten randomly chosen QLF images from the study data set. The intra-examiner intraclass correlation coefficient (ICC) was 0.74 for the plaque scores per site.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 25. Because plaque percentage on baseline (T0.1) was non-normally distributed, non-parametric tests were used. Kruskal-Wallis H rank test was used for baseline comparisons and to compare differences between groups. To test for effects over time the Friedman test was used, with Wilcoxon post-hoc tests.

RESULTS

Participant flow and baseline data

A total of 222 participants were enrolled in this study between October 2017 and October 2018. Since this was a practice-based study, some participants were lost to follow-up due to their appointments being moved. At the end of the study, additional participants were excluded who had attended four research visits but did not have these on four consecutive

regular check-up appointments. A flowchart regarding follow-up is shown in figure 3. The overall loss to follow-up was 34% (QLF 24; erythrosine 17; control 20).

On baseline there were no significant differences between the groups in plaque percentage (P[%]). Table I depicts the baseline data. The mean red-fluorescence P[%] was 22%. There were no differences in P[%] at start between the three groups. A total of 87 participants were included in the analyses.

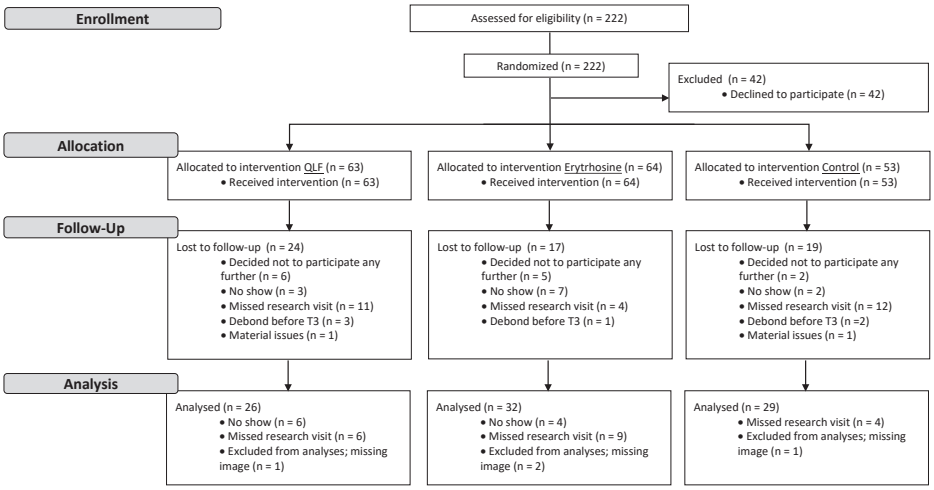

Figure 3. CONSORT flowchart, representing participant follow-up through the trial.

Table 1. Baseline characteristics for the different groups and overall.

	QLF (n = 26)	Erythrosine (n = 32)	Control (n = 29)	All (n = 87)
Age, y (range)	13.3 (12.0-16.2)	13.7 (12.0-16.0)	13.5 (12.1-17.2)	13.5 (12.0-17.2)
Male gender, n (%)	8 (30.8)	19 (59.4)	13 (44.8)	40 (46)
Toothbrush, n (%)				
Hand	14 (53.8)	19 (59.4)	17 (58.6)	50 (57.5)
Electric	6 (23.1)	12 (37.5)	11 (37.9)	29 (33.3)
Combination	4 (15.4)	1 (3.1)	1 (3.4)	6 (6.9)
Missing	2 (7.7)	0 (0)	0 (0)	2 (2.3)
Treatment duration at baseline	6.2	5.9	5.9	6.0
Plaque baseline (T0.1), % (sd)	26 (19)	18 (15)	21 (17)	22 (17)

Outcome analysis

Group results

There were no differences between the three groups on each visit before feedback. After feedback only on T3 there was a significant difference between the groups, χ^2 (2) = 6.186, p=0.046, with a mean rank of P[%] of 43.67 for QLF, 51.78 for erythrosine and 35.71 for the control.

The QLF and control group showed an improvement of P[%] before and after feedback on all four visits. For erythrosine on visit T2 and T3 no such improvement was seen.

Overall results

Before feedback there is a significant difference between the four visits regarding P[%], χ^2 (3) = 11.092, *p*=0.011 (fig. 4a). Significant differences were found between visit T0 and T2 (*Z*=-3.009, *p*=0.003) and between T0 and T3 (*Z*=-2.290, *p*=0.022). This was a non-clinically relevant decrease in plaque. After feedback no differences were found between the four visits (fig. 4b).

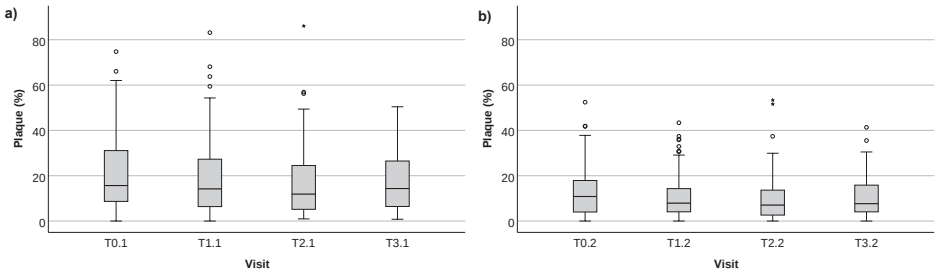

Figure 4. Box plots representing the overall difference in P[%] over time: (a) before feedback and instructions; (b) after feedback, instructions (at T0, T1, T2) and subsequently brushing.

The group was dichotomized into participants with a low start P[%], a level of plaque below 20%, with a total of 50 participants, and a moderate to high start P[%], with a level of plaque from 20%, with a total of 37 participants. The participants with a P[%] below 20% at start (T0.1) showed no decrease in plaque over time (fig. 5a). While the group with a P[%] from 20% showed a decrease in plaque over time, χ^2 (3) = 15.097, *p*=0.02 (fig. 5b). Significant differences were found between visit T0 and T1 (*Z*=-2.240, *p*=0.025) and between T0 and T2 (*Z*=-3.477, *p*=0.001) and between T0 and T3 (*Z*=-3.145, *p*=0.002). This decrease in plaque was clinically relevant.

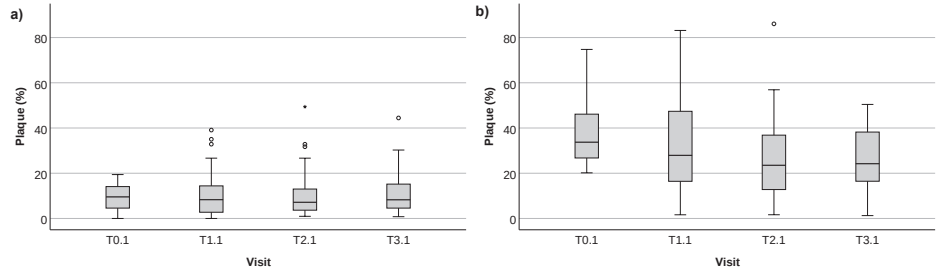

Figure 5. Box plots representing the difference in P[%] over time before feedback and instructions: (a) group (n=50) with a P[%] below 20%; (b) group (n=37) with a P[%] above 20%.

DISCUSSION

Main findings

This study showed that reinforcement of oral hygiene instructions starting from six months into fixed appliance treatment did not give a clinically relevant difference in plaque level for the overall group. However, when the oral hygiene level at start was poor a significant decrease of plaque was seen over time. This was the first study that focuses on reinforcement of oral hygiene starting several months after start of treatment. Most previously conducted studies started at the time of placement of the appliances and had a duration of six months. The study of Peng et al (Peng *et al.*, 2014) reported that the control group and the disclosing agent group both had a significant plaque increase in the first three months of treatment. This level was sustained in the next three months, regardless of the instructions given every month. This finding is comparable with the results of our study. In our study, starting six months into treatment, the P[%] showed no significant differences between groups and remained at the same level even though feedback and instructions were given. Similarly, the study by Acharya (Acharya *et al.*, 2011) showed no difference in plaque between baseline (before placement of the appliances) and 6 months into treatment.

In the 4th session only the feedback was given, without further instructions. Directly after brushing the P[%] decreased in the QLF and control group, thus indicating that the participants knew how to interpret the feedback without instructions.

Limitations and generalizability

At the start of the study the mean P[%] was 22%, this was lower than originally anticipated and lower than in other studies assessing plaque during orthodontic treatment (Acharya *et al.*, 2011, Peng *et al.*, 2014). This low level at start might contribute to the non-clinically relevant difference in plaque, since a decrease of an already low level of plaque is not likely.

Dichotomizing the group, the subjects with a P[%] from 20% did show a clinically relevant decrease in plaque. A clinician should therefore give extra feedback and instructions to those patients that have a moderate to poor oral hygiene during treatment with fixed appliances. Since the extra attention did not decrease the level of plaque in patients already showing a low level of plaque, time can be saved during an appointment by only giving instructions to the subjects that benefit from the feedback.

In the erythrosine group after two of the four sessions we found no difference between the plaque score before and after feedback and brushing. We speculate that this might be due to the remnants of erythrosine still present after brushing. On the QLF images the differences between red autofluorescence and remnants of erythrosine are difficult to be discerned. It has been reported that on QLF images of disclosed plaque a higher level of plaque is assessed than on the image before applying the disclosing fluid (Pretty *et al.*, 2005). A recommendation for future research would be to make the QLF image before

feedback but after disclosing. This was not chosen in our study since a comparison with the QLF and control group would be difficult.

We did not test the role of feedback at home, in between the four sessions. From previous research it is known that at home use of a disclosing agent did not play a substantial role in the improvement of oral hygiene. However, repeated instructions, tested in a non-orthodontic study group, were of importance (Tan and Wade, 1980). At home use of QLF, for example by using the Qscan device (Inspektor Research Systems, Amsterdam, the Netherlands), has not been tested so far. Although just as with erythrosine and the use of a rinse, compliance might be a problem (Geiger *et al.*, 1988).

CONCLUSIONS

Based on these results we conclude that repeated oral hygiene feedback and instructions, started during treatment with fixed appliances, show a statistical and clinically relevant decrease of plaque when a moderate to high level of plaque was present at start. This was seen regardless of the feedback method used. There is a direct improvement seen in the efficacy of brushing directly after feedback using a QLF image or a mirror and probe. However the improvement is not maintained over time for the whole group.

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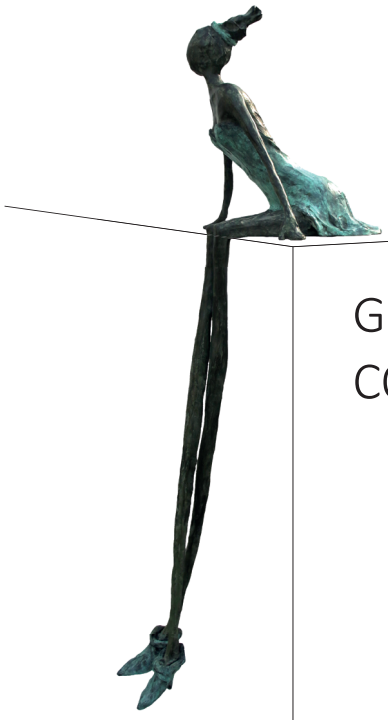

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

GENERAL DISCUSSION AND CONCLUSIONS

A large proportion of the youth receives orthodontic treatment. In the Netherlands, between 60% and 80% of the group of 17 and 23 year olds has received orthodontic treatment (Schuller *et al.*, 2018). During treatment with fixed appliances an impaired dental hygiene and altered microbiome are seen. This, in combination with a diet with a high-frequency intake of carbohydrates, typical for the high-school population, can lead to dental caries.

In the Netherlands the caries prevalence of five year olds has declined in the last decades, while the caries prevalence of eleven year olds has increased (Schuller *et al.*, 2018). For the 17 and 23 year olds the caries prevalence was stable. In the previous epidemiological study of 2011 the caries prevalence had declined in both groups (Schuller *et al.*, 2013); the current data show that this decline has come to a halt. These numbers indicate that during orthodontic treatment there is a need for measures to maintain good oral health which can reduce the development of white spot lesions (WSL).

Altered microbiome and gingivitis during treatment

In this thesis it is shown that placement of fixed appliances is associated with a change in the oral microbiome: More periopathogens, such as the genus *Prevotella* and *Selenomonas,* were observed in the first three months after placement. The differences that were found in bacterial composition between the subjects who received the fluoride rinse and those in the placebo rinse group had little effect. This is similar to the conclusion that the main effect of fluoride is on the demineralization and remineralization processes in the oral cavity (van Loveren, 2001, ten Cate, 2009, Rosin-Grget *et al.*, 2013, ten Cate, 2013).

Gingivitis during orthodontic treatment is attributed to plaque accumulation caused by the increased number of retention sites and consequently impaired oral hygiene (Naranjo *et al.*, 2006, Ren *et al.*, 2014). Not only the orthodontic treatment is related to the onset of gingivitis in these patients, puberty is often also associated with increased gingivitis (Morishita *et al.*, 1988, Mombelli *et al.*, 1990, Nakagawa *et al.*, 1994). Generally, orthodontic treatment takes place during puberty. During this period, the human body experiences hormonal changes (Vetter-O'Hagen and Spear, 2012). In this thesis, it is shown that in subjects with gingivitis more *Porphyromonas* were detected in the first three months of fixed appliance treatment in comparison to persons with a healthy gingiva. This difference disappeared towards the end of treatment and after debonding, although the percentage of subjects with gingivitis increased. A possible explanation for the decrease of *Porphyromonas* towards the end of treatment might be due a reduction in retention sites because of alignment of the teeth. Also removal of the appliances or hormonal changes could explain the difference. This thesis shows that the bacteria that were associated with periodontal pathogenesis decreased at the end of treatment and after removal of the fixed appliances, while the prevalence of health related bacteria increased, suggesting that

orthodontic treatment during puberty does not have a lasting negative effect on the oral microbiome. We note that our study did not include a control group of adolescents that did not receive orthodontic treatment. Therefore it is difficult to discern which microbial changes are related to orthodontic treatment, and which to the onset of puberty.

Preventive measures during treatment: additional fluoride

Efforts to prevent the development of WSL should be implemented by orthodontists, dentists, the patients and their parents/caretakers. One of these measures is the administration of extra fluoride during treatment with fixed appliances. It has been reported that the use of a high-fluoride toothpaste instead of regular fluoride toothpaste resulted in fewer WSL (Sonesson *et al.*, 2014). The latter is a home-care procedure and therefore relies on compliance, an action to be taken by the patient and their parents/caretakers. It is cost-effective compared to professionally applied fluoride varnish, which has also been shown to reduce the incidence of WSL (Stecksen-Blicks *et al.*, 2007). An advantage of the latter is that it relies less on the compliance of the patient as it is applied periodically by a professional; either the orthodontist, dentist or oral hygienist. In the Netherlands, questionnaires taken in 2004 and 2008 showed that orthodontists seldom prescribed a fluoride gel or varnish, in spite of evidence that it is effective (Derks *et al.*, 2007, Kerbusch *et al.*, 2010). This might be due to regulations in the Netherlands, where orthodontists cannot invoice these methods of prevention. These questionnaires also showed that a fluoride rinse was prescribed frequently. Fluoride rinses are home-care products, which are easily available in contrast to the earlier mentioned high-fluoride toothpaste, which in the Netherlands can only be obtained with prescription. Next to only being available on prescription it is registered only for the group above 16 years of age and not fully covered by the insurance companies. Our study concluded that using a fluoride rinse daily, in the evening after brushing, reduces the incidence of WSL. A disadvantage of using a rinse is the above mentioned issue of compliance. It is reported that there is a significant association between compliance and WSL formation (Geiger *et al.*, 1988). A poor compliance was reported in over 50% of the subjects in that study. The investigators gave verbal instructions or instructions on paper and occasionally during treatment. Especially when oral hygiene was poor, they urged patients to improve their oral hygiene and use the rinse. Compliance was not assessed in our study and it is expected to be similar in the fluoride group and in the placebo group. Nevertheless, a positive effect of the fluoride rinse on WSL formation was found. The compliance in our study is expected to be slightly better than in the previously mentioned study (Geiger *et al.*, 1988). The instructions given in our study were given in the same frequency as in the study of Geiger. Compliance is presumed to be better in our study since the patients were seen on research visits every six months and the rinse was supplied and handed out by the researchers. Besides the lower WSL formation the bleeding scores were unaltered during treatment in the group using fluoride rinse, while in the placebo group bleeding was increased from

one year into treatment. Therefore, it is concluded that using an additional fluoride rinse results in fewer WSL and helps to maintain better oral health. Prescribing a fluoride rinse is a good option to prevent WSL during orthodontic treatments in the Netherlands, since it is easily available and cost-effective for the Dutch orthodontist.

Preventive measures during treatment: oral hygiene instructions

A healthy mouth and good oral hygiene help to prevent the formation of WSL (Artun and Brobakken, 1986, Chapman *et al.*, 2010, Khalaf, 2014). It is customary to give oral hygiene and dietary instructions to the patient at the start of treatment with fixed appliances. Repeated oral hygiene instructions, beginning from the start of treatment showed a decreased plaque index (Marini *et al.*, 2014, Peng *et al.*, 2014). The instructions were repeated every four weeks in the study by Marini et al (Marini *et al.*, 2014), in which also the plaque level without instructions decreased over time. A possible explanation for this was not given, but a Hawthorne effect could be responsible, since plaque was measured every 4 weeks in both groups. In the study of Peng *et al.* (Peng *et al.*, 2014), which also started at placement of fixed appliances, the oral hygiene improved by showing images with severe consequences of biofilm accumulation. An elevation in plaque was seen for the group receiving instructions together with the use of a discoloring agent, which remained at the same level independent of the repeated instructions. The current study showed repeated instructions, starting at 6 months into treatment, did not have a clinically relevant effect on the level of plaque. This was found irrespective the method of feedback used. For the group starting with a high level of plaque, a decrease was found over time after repeated oral hygiene instructions. Therefore, it seems important to assess risk factors before treatment start and give attention at the beginning of treatment, as well as further on, to the patients with inadequate oral hygiene. It is well documented that inadequate oral hygiene at start and during treatment are risk factors for the development of WSL (Al Maaitah *et al.*, 2011).

The use of QLF for the purpose of research and in daily practice

In research settings, longitudinal assessment of WSL is often required. QLF can be used for longitudinal observation in a non-bracketed population (Tranaeus *et al.*, 2002). Images of teeth with a lesion could be taken in a reproducible way by different persons, just as the subsequent analyses of the images were found to be reproducible. A longitudinal assessment of demineralizations during fixed appliance treatment is hampered by practical limitations: during orthodontic treatment, brackets and other accessories are present, which can all obscure part of the lesion. This interference is enhanced by the fact that healthy surrounding enamel should be present for QLF measurements. This is not the case when brackets are present. Moreover, positions of teeth will change during the treatment period, which hampers the comparison of pictures taken at various time points. Orthodontic studies showed that QLF images captured under similar circumstances, that is using the

same camera angle, can be reproducibly quantified in vitro (Benson *et al.*, 2003, Pretty *et al.*, 2003, Aljehani *et al.*, 2004). This thesis presents in vitro research, in which changes that may occur during orthodontic treatment are mimicked. For that purpose, the images were made under various conditions, that is, with brackets with or without wire, without brackets and under different angles of rotation, mimicking the change in tooth position. The results showed that fluorescence loss and lesion size are influenced by the angle of rotation under which the WSL is photographed. The full extent of demineralizations is only apparent after removal of the fixed appliances, when photographed at rotations of 0° mesiodistal and up to 20° buccal.

QLF is also used and promoted to visualize the presence of plaque. Porphyrins in matured bacterial plaque form a red fluorescence, which can be shown on a QLF image. The patient is able to remove plaque after seeing such a QLF image with instructions how to interpret the image. In an adolescent orthodontic population, the repeated use of in office feedback with QLF did not decrease the amount of plaque over time (Miller *et al.*, 2016). For research purposes assessing plaque on QLF images in combination with a discoloring agent was found to be difficult. Discriminating between the remnants of the discoloring agent and red fluorescence was found to be problematic. After staining, the plaque level assessed on QLF images was higher than without staining (Pretty *et al.*, 2005). Our study concluded that, when a high level of plaque was present, the plaque decreased in time after repeated oral hygiene instructions, independently of the method of feedback used. From the above, we conclude that when no precautions are taken with respect to standardization, QLF can be used in normal practice settings for the purpose of instructing the patient regarding oral hygiene and to make the WSL visible. However, for research purposes during orthodontic treatment QLF was found insufficiently reliable for monitoring WSL over time.

Clinical advices

During treatment with fixed appliances the orthodontist, dentist, oral hygienist, patient and parents/caretakers have joint responsibility. First of all the orthodontist should prescribe the use of a fluoride rinse during treatment with fixed appliances. This should become a routine in all orthodontic practices, and this procedure should be emphasized by the whole staff, and other dental professionals, throughout the treatment in order to motivate the patient to be compliant. Regarding maintaining good oral health during orthodontic treatment this thesis showed that starting extra oral hygiene instructions already into treatment did not have an effect on the level of plaque for the patients who already have satisfactory oral hygiene. However, when oral hygiene was inadequate repeated instructions were found to decrease the level of plaque over time.

Both these advices can be easily incorporated in daily practice throughout the Netherlands and can help to lower the WSL formation in orthodontic patients.

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CHAPTER 7

SUMMARY

SUMMARY

Orthodontic treatment aims towards a stable occlusion and masticatory function, but also on achieving an aesthetical end result in forms of smile and profile. Fixed orthodontic appliances may lead to formation of dental caries. During treatment there is an increased number of plaque retention sites and oral environment changes, resulting in different plaque composition. Because of these alterations, decalcifications can form around the orthodontic brackets, at locations normally showing a low prevalence of caries. These so-called white spot lesions (WSL) are obviously an (aesthetically) unwanted side effect of orthodontic treatment. To prevent the formation of WSL during fixed orthodontic appliance treatment several methods have been studied. Patients can use a daily rinse with, for example, fluoride, or can use high-fluoride toothpaste. Moreover, practitioners can apply varnishes containing fluoride during every check-up visit.

In this thesis two randomized clinical trials (RCTs) are described with the overall topic the prevention of WSL formation during fixed appliance treatment. The first chapters describe a RCT about the effects of the use of a fluoride rinse on the formation of white spot lesions and the microbiome during treatment. The second RCT aims at the oral hygiene instructions given during treatment to maintain good oral health.

In **chapter 2** the use of a fluoride rinse (Elmex caries protection with a combination of 150 ppm sodium-fluoride and 100 ppm amine-fluoride) was compared with a placebo rinse. This study was performed as a randomized clinical trial (RCT). The rinse was used every evening after tooth brushing starting at placement of fixed appliances and lasting till the end of treatment. A total of 81 participants (mean age 13.3 years) completed the study. Before treatment and around six weeks after debonding WSL and Decayed, Missing and Filled Surfaces (DMFS) were assessed. Bleeding scores were measured before start, during and post treatment. The mean treatment period with fixed appliances was 24.5 months. For the measurements of the demineralizations the method of Quantitative Light-Induced Fluorescence (QLF) was used. In the fluoride group 31% of the participants developed at least one demineralization, compared to 47% in the placebo group. A maximum of five lesions per participant was seen in the fluoride group, compared to a maximum of 15 lesions in the placebo group. Gingival bleeding increased significantly in the placebo group from one year after start of treatment compared to the bleeding scores before placement of the fixed appliances. In the fluoride group the bleeding scores during treatment were not different from those before start of treatment. We conclude that using a fluoride rinse, containing sodium- and amine-fluoride, daily at home, during treatment with fixed appliances has a positive preventive effect. Fewer WSL are formed and gingival health, measured as bleeding, remains stable.

Because of the increased number of plaque retention sites, in combination with a more difficult oral hygiene, more plaque is formed. The effect of fixed appliances on the oral microbiome is investigated in **chapter 3**, with data from the RCT described in chapter 2. The changes in the oral microbiome were investigated before, during and after orthodontic treatment combined with the use of either a fluoride rinse or a placebo. At six time-points during the study plaque samples were taken; once before placement of the appliances, twice in the first phase of treatment, just prior to debonding and twice after removal of the appliances. From 91 participants (mean age 13.3 years) one or more supragingival plaque samples were obtained. The microbial changes were assessed using next-generation sequencing of the bacterial 16S rRNA gene. The fluoride rinse had little effect on the oral microbiome composition during treatment with fixed appliances compared to the placebo. The microbial changes observed in relation to gingival health, measured as bleeding, and orthodontic treatment and time were more pronounced. In the first three months of orthodontic treatment periodontal pathogens (e.g. *Selenomonas* and *Porphyromonas*) were highest in abundance. Most genera, such as *Steptococcus*, *Rothia* and *Haemophilus*, which increased in time are associated with a healthy oral cavity. This increase in healthy associated genera was the only, though minor, change that remained after end of treatment. We conclude that the orthodontic treatment during puberty does not have a lasting negative effect on the oral microbiome. A drawback of our study is the fact that we lack an age-related control group not receiving orthodontic treatment. This means that we cannot discriminate between changes in microbiome over time caused by age or by the fixed appliances.

In the above-mentioned RCT the use of QLF to asses WSL longitudinally during treatment with fixed appliances appeared to be difficult. The presence of a bracket, wire and other auxiliaries in combination with the movement of the teeth (i.e. rotations) obscured parts of the lesion. This is further investigated in an in vitro study described in **chapter 4**. The reproducibility of WSL assessments was investigated when using a QLF-Digital (QLF-D) camera to detect and monitor the area and fluorescence loss of WSL. Reproducibility was assessed for different angles of rotation of teeth with or without brackets, and with or without an attached hook to the bracket or containing an elastic ligature and wire. The brackets were bonded on maxillary incisors or maxillary canines. Demineralizations were formed in vitro directly cervical of the bracket. Images of the lesions were captured using a QLF-D camera mounted on an optical bench, equipped with a goniometer on a turntable. The teeth were placed in the goniometer simulating buccolingual rotations and the turntable was used for mesiodistal rotations. The images were captured at combinations of different angles of rotation before (with and without a wire) and after debonding, the brackets of the canines contained a hook on the bracket. The image after debonding without rotation served as a control. The presence of a bracket (with or without an elastic ligature and wire) resulted is

a significantly higher fluorescence loss, yet a smaller lesion area ($p < 0.05$) in comparison to the control. A significant higher fluorescence loss was seen for rotations towards lingual, this was less explicit for the rotations towards buccal. We conclude that fluorescence loss and lesion size are influenced by the angle of rotation under which the WSL is photographed during orthodontic treatment. In research settings, during treatment with fixed appliances, QLF-D should therefore to be used with precaution, since too many alterations take place over time, and because of the presence of auxiliaries.

Next to the use of additional fluoride, maintaining good oral health during orthodontic treatment is important to prevent the formation of WSL. In **chapter 5** the effect of repeated oral hygiene instruction with three different feedback methods on the level of plaque is tested in a RCT during orthodontic treatment. This study started around six months after placement of the fixed appliances. During four study visits participants received feedback (on all four visits) and oral hygiene instructions (only during the first three visits). The four study visits took place just prior to the regular check-up visits, planned around every six weeks. After feedback or instructions the participants brushed their teeth. They could either use a manual or electric toothbrush, and interdental brushes. The feedback methods involved 1) showing a QLF image of their own teeth, 2) using a disclosing agent to stain their own teeth, 3) using a mirror and probe to indicate the presence of plaque. Before and after feedback and brushing QLF-images of the anterior teeth were made. A total of 87 participants completed the study. Between the three groups no differences in the level of plaque before and after feedback and brushing were seen as assessed on the QLF images. A non-clinically relevant, yet statistically significant decrease in plaque was found between the four study visits. When dichotomizing the total patient group the group starting with a moderate to high level of plaque present showed a statistically significant and clinical relevant decrease in plaque. A direct improvement was also seen in the efficacy of brushing immediately after feedback using a QLF image or a mirror and a probe. We conclude that repeated instructions during orthodontic treatment can help to lower the level of plaque in patients with a moderate to high level of plaque. This finding was made regardless of the feedback method used.

Based on the above-mentioned RCT's we have two clinical advises, that can be easily incorporated in daily practice in the Netherlands. In the first place the daily use of a fluoride rinse should be promoted during treatment with fixed appliances. Secondly, if oral hygiene is inadequate during treatment, repeated instructions should be given in order to decrease the level of plaque.

CHAPTER 8

SAMENVATTING

SAMENVATTING

Een orthodontische behandeling heeft als doel een stabiele occlusie en kauwfunctie, maar daarnaast het behalen van een esthetisch eindresultaat met betrekking tot lach en profiel. Een behandeling met vaste apparatuur kan leiden tot het ontstaan van cariës. Tijdens de behandeling zijn er meer plaqueretentieplaatsen en is het mondmilieu veranderd, waardoor er een andere plaquesamenstelling is. Vanwege deze veranderingen kunnen er decalcificaties ontstaan rondom de slotjes, plekken waar normaal weinig cariës voorkomt. Deze zogeheten witte vlek laesies ('white spot lesions' – WSL) zijn daarom een esthetisch nadelig effect van beugelbehandelingen. Om het ontstaan van WSL tegen te gaan worden diverse methodes gebruikt gedurende de behandeling met vaste apparatuur. Patiënten kunnen dagelijks spoelen met een spoelmiddel, met bijvoorbeeld fluoride, of kunnen poetsen met een tandpasta met een hoge concentratie fluoride. Daarnaast kunnen behandelaars een lak of gel met fluoride aanbrengen tijdens de controle afspraak.

In deze thesis worden twee gerandomiseerde klinische onderzoeken ('randomized clinical trial' – RCT) beschreven met als algeheel doel het voorkomen van het ontstaan van WSL tijdens behandeling met vaste apparatuur. In de eerste hoofdstukken wordt een RCT beschreven over de effecten van een fluoride spoelmiddel op het ontstaan van WSL en het microbioom tijdens de behandeling. De tweede RCT gaat over de mondhygiëne instructies, gegeven tijdens de behandeling om goede mondhygiëne te behouden.

In **hoofdstuk 2** wordt het gebruik van een fluoride spoelmiddel (Elmex anti-cariës met een combinatie van 150 ppm natriumfluoride en 100 ppm aminfluoride) vergeleken met een placebo spoelmiddel. Het betrof een gerandomiseerd klinisch onderzoek. Het spoelmiddel werd dagelijks gebruikt, 's avonds na het tandenpoetsen vanaf plaatsing van de vaste apparatuur tot aan het einde van de behandeling. In totaal ronden 81 proefpersonen (gemiddelde leeftijd 13,3 jaar) het onderzoek af. Voorafgaand aan de behandeling en ongeveer 6 weken na het verwijderen van de apparatuur werden WSL en 'Decayed Missing Filled Surfaces'-index (DMFS) onderzocht. DMFS staat voor de som van het aantal 'decayed' (aangetaste of onbehandelde), 'filled' (gevulde), 'missing' (ontbrekende of door cariës geëxtraheerde) 'surfaces' (tandvlakken). Bloedingsscores werden gemeten voorafgaand, tijdens en na de behandeling. De gemiddelde behandelduur was 24,5 maanden. Voor de meting van de demineralisaties werd gebruikt gemaakt van kwantitatieve licht-geïnduceerde fluorescentiebeeldvorming ('Quantitative light-Induced Fluorescence' – QLF). In de fluoride groep ontwikkelde 31% van de proefpersonen ten minste één laesie, ten opzichte van 47% in de placebo groep. Een maximum van 5 laesies per proefpersoon werd gezien bij de fluoride groep, terwijl er bij de placebo groep een maximum van 15 laesies werd gezien. Bloedend tandvlees nam significant toe in de placebo groep vanaf één jaar in behandeling ten opzichte van de bloeding voorafgaand aan plaatsing van de vaste apparatuur. In de

fluoride groep was de bloeding niet anders tijdens de behandeling indien vergeleken met voorafgaand aan de behandeling. We concluderen dat het dagelijks thuis gebruiken van een fluoride spoelmiddel, met een combinatie van natrium- en aminfluoride, een preventief effect heeft. Er worden minder WSL gevormd en het tandvlees blijft stabiel, gemeten in hoeveelheid bloeding.

Vanwege het feit dat er meer plaqueretentieplaatsen zijn, in combinatie met een moeizamere mondhygiëne, wordt er meer plaque gevormd. Het effect van vaste apparatuur op het orale microbioom is onderzocht in **hoofdstuk 3**, met data van de RCT beschreven in hoofdstuk 2. De veranderingen in het orale microbioom werden onderzocht voor, tijdens en na de behandeling met vaste apparatuur in combinatie met het gebruik van een fluoride spoelmiddel of een placebo. Op zes meetmomenten werd tandplaque verzameld gedurende de studie; voor het plaatsen van de beugel, tweemaal in de eerste periode na plaatsen van de beugel, vlak voor verwijdering van de beugel en tweemaal na verwijdering. Van 91 proefpersonen werden één of meerdere supragingivale plaquemonsters verzameld. De microbiële samenstelling van de tandplaque werd bepaald door het sequencen van het 16S rRNA gen. Het fluoride spoelmiddel had weinig effect op de microbiële samenstelling gedurende de behandeling met vaste apparatuur ten opzichte van de placebo. De microbiele veranderingen in relatie tot de gezondheid van het tandvlees, gemeten als hoeveelheid bloeding, de orthodontische behandeling en de tijd waren meer zichtbaar. In het begin van de orthodontische behandeling kwamen de paropathogenen (zoals *Selenomonas* en *Porphyromonas*) het meest voor. De genera, als *Steptococcus*, *Rothia* en *Haemophilus*, welke toenamen in tijd worden geassocieerd met een gezonde mond. Deze toename van met gezondheid geassocieerde genera was het enige kleine blijvende effect na behandeling. We concluderen dat de orthodontische behandeling gedurende de pubertijd geen blijvende nadelige effecten heeft op het orale microbioom. Een nadeel van deze studie is dat een leeftijds-gerelateerde controle groep ontbreekt, zonder orthodontische behandeling. Dit betekent dat we geen duidelijk onderscheid kunnen maken tussen de veranderingen in de tijd van het microbioom veroorzaakt door de leeftijd of door de vaste apparatuur.

In hierboven beschreven RCT bleek het lastig om QLF te gebruiken voor de longitudinale metingen van WSL gedurende de behandeling met vaste apparatuur. De aanwezigheid van het slotje, de draad en andere extra benodigdheden in combinatie met de veranderingen in tandstand, zoals rotaties, blokkeerden delen van de laesie. Dit is onderzocht in een in vitro onderzoek beschreven in **hoofdstuk 4**. De reproduceerbaarheid van WSL metingen bij het gebruik van een QLF-Digital (QLF-D) camera is onderzocht bij het detecteren en monitoren van de oppervlakte en fluorescentieverlies van WSL. De reproduceerbaarheid werd gemeten voor diverse rotatiehoeken bij tanden met of zonder slotje, en met of zonder een vastzittend haakje aan het slotje of een draad vastgezet (‘ingeligeerd’) met een elastiekje.

De slotjes waren geplakt op boven voortanden of boven hoektanden. Demineralisaties werden in vitro gevormd direct cervicaal van het slotje. Foto's van de laesies werden gemaakt met behulp van de QLF-D camera gemonteerd op een optische bank, met een goniometer en draaitafel erop. De tanden werden geplaatst op de goniometer, welke buccolinguale rotaties simuleerde en de draaitafel werd gebruikt voor mesiodistale rotaties. De foto's werden gemaakt met combinaties van de rotatiehoeken voor (met en zonder draad) en na verwijderen van het slotje, de slotjes van de hoektanden waren voorzien van een haakje. De foto na verwijdering van het slotje, gemaakt zonder rotatie, diende als controle. De aanwezigheid van een slotje (met of zonder ingeligeerde draad) resulteerde in een significant hoger fluorescentieverlies, maar een kleiner laesie oppervlak ($p < 0,05$) in vergelijking tot de controle. Een significant hoger fluorescentieverlies werd gezien bij de rotatiehoeken richting linguaal, waarbij dit minder duidelijk was bij de rotatiehoeken naar buccaal. We concluderen dat fluorescentieverlies en laesieoppervlakte worden beïnvloed door de rotatiehoek waaronder de WSL wordt gefotografeerd tijdens de orthodontische behandeling. In onderzoek omgeving moet QLF-D, tijdens de behandeling met vaste apparatuur, met voorzorg worden gebruikt, omdat er teveel veranderingen plaatsvinden gedurende de tijd en vanwege de aanwezigheid van extra benodigdheden.

Naast het gebruik van additionele fluoride, is het behouden van een goede mondhygiëne tijdens de orthodontische behandeling van belang om het ontstaan van WSL tegen te gaan. In **hoofdstuk 5** is het effect van herhaalde mondhygiëne instructie met drie verschillende terugkoppelingsmethodes op de hoeveelheid plaque onderzocht in een RCT tijdens de orthodontische behandeling. Deze studie startte ongeveer 6 maanden na plaatsing van de vaste apparatuur. Tijdens vier onderzoek afspraken ontvingen de deelnemers terugkoppeling (bij alle vier de afspraken) en instructie (alleen tijdens de eerste drie afspraken). De vier onderzoek afspraken vonden plaats direct voorafgaand aan de normale controle afspraken, gepland ongeveer om de zes weken. Na de terugkoppeling of instructies poetsten de deelnemers hun tanden. Ze konden daarbij gebruik maken van een hand- of elektrische tandenborstel en interdentale borstels. De terugkoppelingsmethoden hielden in 1) het laten zien van de QLF foto van hun eigen tanden, 2) het gebruik van een plaqueverklikker om de tanden te kleuren, 3) het gebruik van een spiegel en sonde om de plaque te laten zien. Voor en na de terugkoppeling en het poetsen werden QLF foto's van de voortanden gemaakt. In totaal rondden 87 deelnemers het onderzoek af. Voor en na de terugkoppeling en het poetsen werden geen verschillen gezien qua hoeveelheid plaque tussen de drie groepen, zoals beoordeeld op de QLF foto's. Een niet klinisch, maar wel statistisch significant verschil in plaque werd gezien tussen de waarnemingen tijdens de vier afspraken. Bij het in tweeën delen van de groep werd bij de groep die begon met een matige tot hoge plaquescore een statistisch significante en klinisch relevante afname in de hoeveelheid plaque gezien. Daarnaast is er een directe verbetering te zien van het poetsen na terugkoppeling met

een QLF foto of met een spiegel en sonde. We concluderen dat herhaald instructie geven tijdens de behandeling kan helpen de hoeveelheid plaque te verminderen wanneer en een matige tot hoge hoeveelheid plaque aanwezig is. Hierbij maakt de methode van terugkoppeling geven niet uit.

Gebaseerd op de beide bovengenoemde RCTs hebben we twee klinische adviezen, welke makkelijk kunnen worden toegepast in de praktijk in Nederland. In de eerste plaats moet het dagelijks gebruiken van een fluoride spoelmiddel worden gepromoot gedurende de behandeling met vaste apparatuur. In de tweede plaats moet er herhaald instructie worden gegeven in het geval van onvoldoende mondhygiëne.

CHAPTER 9

LIST OF PUBLICATIONS
AUTHOR CONTRIBUTIONS
DANKWOORD
CURRICULUM VITAE

LIST OF PUBLICATIONS

Related to this thesis:

van der Kaaij, N. C. W., van der Veen, M. H., van der Kaaij, M. A. E., and ten Cate, J. M. (2015). A prospective, randomized placebo-controlled clinical trial on the effects of a fluoride rinse on white spot lesion development and bleeding in orthodontic patients. *Eur J Oral Sci* 123, 186-193.

Koopman, J. E., van der Kaaij, N. C.W., Buijs, M. J., Elyassi, Y., van der Veen, M. H., Crielaard, W., ten Cate, J. M., and Zaura, E. (2015). The Effect of Fixed Orthodontic Appliances and Fluoride Mouthwash on the Oral Microbiome of Adolescents - a Randomized Controlled Clinical Trial. *PLoS One* 10, e0137318.

van der Kaaij, N. C. W., Faaij, M. J., ten Cate, J. M., and van der Veen, M. H. (2018). The reproducibility of assessment of white spot lesions adjacent to orthodontic brackets, with a quantitative light induced fluorescence digital camera at different rotations of teeth – an in vitro study. *BMC Oral Health* 18, 209.

van der Kaaij, N. C. W., ten Cate, J. M., and van der Veen, M. H. Effect of repeated oral hygiene instructions with three different feedback methods on the level of plaque in orthodontic patients: a randomized clinical trial.*

Other:

van der Kaaij, N. C. W., Maillou, P., van der Weijden, J. J., Naeije, M., and Lobbezoo, F. (2009). Reproducible effects of subjectively assessed muscle fatigue on an inhibitory jaw reflex in humans. *Arch.Oral Biol.* 54, 879-883.

van der Kaaij, N. C. W., Zuurbier, P. C. M. Onzichtbare orthodontie: vacuümgevormde apparatuur, linguale apparatuur en keramische brackets.
Quality Practice Mondhygiëne, 2016 Juni, Jaargang 8, Nummer 4
Quality Practice Tandheelkunde, 2016 December, Jaargang 12, Nummer 3
Quality Practice Assistenten, 2017 Oktober, Jaargang 5, Nummer 1

van der Kaaij, N. C. W., (2018). Orthodontie en de algemeen practicus. *AccreDidact*, nummer 3

* Currently under review for publication

Author contributions

Of the published or submitted manuscripts in this thesis.

Chapter 2

A prospective, randomized placebo-controlled clinical trial on the effects of a fluoride rinse on white spot lesion development and bleeding in orthodontic patients
Nicoline C.W. van der Kaaij, Monique H. van der Veen, Marleen A.E. van der Kaaij, Jacob M. ten Cate
Published in European Journal of Oral Sciences 2015; 123: 186-193

Conceived and designed the study: NvdK, MvdV
Performed the study: NvdK
Analysed the data: NvdK, MvdV, MvdK
Drafted the manuscript: NvdK, MvdV
Critically revised the manuscript: JtC
Conflicts of interest: This study was supported by Elmex research/Colgate-Palmolive Europe, Therwil, Switzerland. Both the placebo and the fluoride rinse were supplied by Elmex research/Colgate-Palmolive Europe, Therwill, Switzerland. Dr M. H. van der Veen is a co-inventor of several patents relating to quantitative light-induced fluorescence. The authors declare that otherwise there is no conflict of interest pertaining to the data presented in this article.
Acknowledgements: The authors thank all the participants joining the study and the dental students, especially Rianne Faaij and Merlijn Marsman, who participated in collecting the data.

Chapter 3

The effect of fixed orthodontic appliances and fluoride mouthwash on the oral microbiome of adolescents- a randomized controlled clinical trial
Jessica E. Koopman*, Nicoline C.W. van der Kaaij*, Mark J. Buijs, Yassaman Elyassi, Monique H. van der Veen, Wim Crielaard, Jacob M. ten Cate, Egija Zaura
* Both authors contributed equally to this work
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Conceived and designed the study: NvdK, MvdV, EZ
Performed the study: NvdK
Analysed the data: JK, MB, YE, MvdV
Drafted the manuscript: JK, NvdK, EZ
Critically revised the manuscript: MvdV, JtC, WC, EZ

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Chapter 4

The reproducibility of assessment of white spot lesions adjacent to orthodontic brackets, with a quantitative light induced fluorescence digital camera at different rotations of teeth – an *in vitro* study

Nicoline C.W. van der Kaaij*, Maria J. Faaij*, Jacob M. ten Cate, Monique H. van der Veen.

* Both authors contributed equally to this work

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Conceived and designed the study: NvdK, MF, MvdV, JtC

Performed the study: MF, NvdK

Analysed the data: MF, NvdK

Drafted the manuscript: NvdK, MF, MvdV

Critically revised the manuscript: JtC

Conflicts of interest: Dr M. H. van der Veen is a co-inventor of several patents relating to quantitative light-induced fluorescence. The authors declare that otherwise there is no conflict of interest pertaining to the data presented in this article.

Chapter 5

Effect of repeated oral hygiene instructions with three different feedback methods on the level of plaque in orthodontic patients: a randomized clinical trial

Nicoline C.W. van der Kaaij, Jacob M. ten Cate, Monique H. van der Veen.

Submitted for publication.

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CURRICULUM VITAE

Nicoline (N.C.W.) van der Kaaij was born in Heemstede, the Netherlands on april 29th 1983. She received her secondary school diploma in 2001 at the Stedelijk Gymnasium Haarlem. In 2007 she completed her master's degree in dentistry at the Academic Centre for Dentistry Amsterdam. During her study her interest in research was raised, doing research at the Department of Oral Kinesiology and in Brasil for the Department of Pediatric Dentistry. In 2008 she started her specialization in orthodontics at ACTA, from which she graduated in 2012. During her specialization she started a randomized clinical trial, a collaboration between the Department of Orthodontics and Preventive Dentistry, as a part of her PhD. After her graduation she continued working at the Department of Orthodontics, to finish the research but also as a lecturer for the undergraduate dental students and the residents in orthodontics. In 2015 she started as an orthodontist for the cleft- and craniofacial team at the Erasmus MC- Sophia Childrens's Hospital. In 2016 she became a co-owner of Orthodontisten Heemstede, a private orthodontic practice she runs together with Anne Karsten and Petra Zuurbier.

Nicoline currently lives in Haarlem and next to her work as an orthodontist she enjoys playing the viola. She plays in several orchestras and is a member of the Concorde String Quartet.