

# The Diagnosis of White Spot Lesions in Orthodontic Patients



THE UNIVERSITY  
*of* ADELAIDE

A thesis submitted in partial fulfilment of the requirements for the  
degree of Doctor of Clinical Dentistry (Orthodontics)

by

**Dr Balya SRIRAM**

Orthodontic Unit

School of Dentistry

Faculty of Health Sciences

The University of Adelaide

July 2013

# 1. Thesis Abstract

**Objectives:**(i) To investigate the associations between the presence, number and severity of white spot lesions (WSLs) and patient characteristics. (ii)To investigate the associations between the presence, number and severity of WSLs and the saliva properties tested using the Saliva-Check BufferKit (GC Corp., Belgium).(iii) To evaluate the use of the DIAGNOdent pen (KaVo, Biberach, Germany) as an aid in the identification of WSLs in orthodontic patients.

**Method:** With ethics approval, 91 orthodontic patients had de-identified parameters recorded which included date of birth, sex, postcode, age at banding, time in bands, failure to attend (FTA) rate, type of bracket used, reported oral hygiene regimen and number of restored molars. All participants were examined for WSLs on their upper and lower anterior teeth using a visual index outlined by the International Caries Detection and Assessment System II (ICDAS II) and a laser-based caries detection device (DIAGNOdent pen).Of the 91 participants, 50 had saliva properties tested which included hydration, consistency, resting pH, stimulated flow, stimulated pH and buffering capacity.

**Results Paper 1:** Brushing fewer than 14 times a week and the presence of restored molars were significant variables for the development and severity of WSLswhen the severity was  $\geq$  ICDAS II grading of 2 ( $p < 0.05$ ).When WSLs were ICDAS II  $\geq$  3 grading, the FTA rate and

brushing fewer than 14 times per week were significant variables ( $p < 0.05$ ). The number of WSLs increased when participants brushed fewer than 14 times per week or had an increased FTA rate ( $p < 0.05$ ). Comparisons between ICDAS II scores and DIAGNOdent pen scores were statistically significant ( $p < 0.0001$ ).

**Results Paper 2:** When using the Saliva-Check Buffer Kit, the pH of stimulated saliva was a significant diagnostic variable in identifying WSLs ( $p < 0.05$ ). The pH of stimulated saliva and the quantity of saliva produced in 5 minutes were significant variables of WSL severity when the grading was greater than or equal to an ICDAS II score of 2 ( $p < 0.05$ ). When the grading was greater than or equal to an ICDAS II score of 3, the pH of unstimulated saliva was a significant variable ( $p < 0.05$ ). No relationship was found between the number of WSLs in a patient and the saliva properties tested with the Saliva-Check Buffer Kit.

**Conclusions:** A patient's report of brushings per week indicates the presence, severity and number of white spots they may experience. The number of restored molars may indicate the presence and severity of their white spot lesion experience. Patients who fail to attend appointments are likely to have a larger number of WSLs with greater severity. The DIAGNOdent Pen corresponds significantly to the ICDAS II system to grade WSLs in orthodontic patients. The pH of stimulated saliva, the pH of unstimulated saliva and saliva flow rate may indicate orthodontic patients who are susceptible to WSLs and may also indicate the severity

of the lesions. The Saliva-Check Buffer Kit is unable to distinguish between patients who have many or those who have few WSLs.

## TABLE OF CONTENTS

Thesis Abstract.....	2
List of Figures and Tables.....	7
Declaration.....	9
Acknowledgements.....	10
Literature Review.....	11
White Spot Lesions in Orthodontic Treatment.....	11
Clinical Evaluation of White Spot Lesions.....	18
The Effect of Orthodontic Appliances on the Oral Environment	26
Saliva Properties and Dental Caries.....	28
The Saliva-Check Buffer Kit.....	33
The Efficacy of the Saliva-Check Buffer Kit in Caries Prediction .....	35
Other Saliva Tests Used to Determine Caries Risks in Orthodontic Patients.....	37
Management of White Spot Lesions.....	38
Conclusion.....	48
References.....	50
Statement of Purpose .....	71
Aims and Hypothesis .....	73
Article 1.....	75
The DIAGNOdent pen and Patient Factors to Diagnose White Spots in Orthodontic Patients	
Abstract.....	76
Introduction.....	77
Materials and Methods.....	80

Results.....	88
Discussion.....	95
Conclusions.....	101
References.....	102
Article 2.....	108
Saliva Tests to Determine the Risk of White Spot Lesions During Orthodontic Treatment	
Abstract.....	109
Introduction.....	110
Materials and Methods.....	113
Results.....	118
Discussion.....	126
Conclusions.....	128
References.....	130
Concluding Remarks.....	134
Appendix.....	136
Saliva-Check Buffer Kit.....	136
Information Sheet for Participants.....	141
Standard Consent Form.....	142
Consent Form for Minors.....	143
Independent Complaints Information.....	144

# LIST OF FIGURES AND TABLES

## LITERATURE REVIEW

### *Tables*

1. The ICDAS II Caries Scoring System 21

## ARTICLE 1

### *Figures*

1. Summary of Methodology 81
2. Central Incisors Divided into “Mini-Quadrants” 85

### *Tables*

1. The ICDAS II Caries Scoring System 86
2. Type of WSLs 89
3. Severity of WSLs 89
4. The Relationship Between Patient Factors and the Presence of WSLs 90
5. The Relationship Between Patient Factors and the Severity of WSLs  $\geq$  ICDAS II Grade 2 92
6. The Relationship Between Patient Factors and the Severity of WSLs  $\geq$  ICDAS II Grade 3 93
7. The Relationship Between Patient Factors and the Number of WSLs 94
8. Least Squares Means 95

## **ARTICLE 2**

### *Figures*

1. Presentation of White Spot Lesions	111
2. Buffering Capacity Points	115
3. Central Incisors Divided into “Mini-Quadrants”	116

### *Tables*

1. The ICDAS II Caries Scoring System	117
2. Score of WSLs	119
3. Relationship Between the Presence of WSLs and the Saliva-Check Buffer Kit	120
4. Relationship Between the Severity of WSLs $\geq$ ICDAS II Grade 2 and the Saliva-Check Buffer Kit	122
5. Relationship Between the Severity of WSLs $\geq$ ICDAS II Grade 3 and the Saliva-Check Buffer Kit	123
6. Relationship Between the Number of WSLs a Patient Experiences and the Saliva-Check Buffer Kit	125

## **APPENDIX**

### *Figures*

1. Labial Gland Saliva Secretion	137
2. pH Colour Chart	138
3. Buffering Capacity Points	139



## 2. Thesis Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution in my name and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

I give consent for this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue and also through web search engines, unless permission has been granted by the University to restrict access for a period of time

Dr Balya Sriram

22<sup>nd</sup> July 2013

### 3. Acknowledgements

I would like to thank the following people for their academic support over the duration of my candidature

Professor Wayne Sampson, P.R. Begg Chair in Orthodontics, The University of Adelaide.

Associate Professor Craig Dreyer, Orthodontics, The University of Adelaide.

Dr Neville Gully, Senior Lecturer in Microbiology, The University of Adelaide.

Associate Professor John Kaidonis, Craniofacial Biology, The University of Adelaide.

Thomas Sullivan, Statistician, Data Management & Analysis Centre, Discipline of Public Health, The University of Adelaide.

The Australian Orthodontic Society Research Foundation for their financial assistance.

The Australian Dental Research Foundation for their financial assistance.

To David Bachmayer, for being my mentor since I had my braces.

To my parents Dak and Kalpana, and my sister Divya for their ongoing emotional support .

To my husband Ruchit, for providing me with strength and encouragement throughout my journey.

# 4. Literature Review

## WHITE SPOT LESIONS IN ORTHODONTIC TREATMENT

### Definition

White spot lesions (WSLs) are a precursor to decay. Their presence shows that the healthy balance between demineralisation and remineralisation on the enamel surface has shifted towards demineralisation with subsequent loss of tooth structure. Like dental caries, WSLs may be considered a carbohydrate induced, bacterial infectious disease(1). Bacteria, in the presence of sugars, produce acids which lead to the demineralisation of enamel structure.

The dissolution of the enamel's mineral structure creates an alteration of the refractive index when light shines upon it creating the opaque white appearance(2, 3). The generated WSL may be remineralised back to normal, remain stable or progress to a cavitation depending on the oral environment (4). Even if a WSL does not progress to cavitation requiring restoration, it can leave a permanent unaesthetic blemish on the tooth's surface which is seen as a chalky white or brown lesion(5-7). WSLs are a common complication of orthodontic treatment and are of concern because the enamel on the buccal surface of teeth would otherwise have low susceptibility to caries.

## **Incidence of WSLs**

The incidence of WSLs varies greatly ranging from 2% to 97%(3, 8-14). This large variation is due to heterogeneous methods of diagnosis and grading of WSLs, differences in the sample size of teeth examined, geographic location of the study sample, time period of the study, age at the start of treatment, duration of the study and materials used in the study(15). Using visual examination, Gorelick et al. (9) found an incidence rate of 50%. Using quantitative light fluoroscopy, Boersma et al. (8) found the incidence after orthodontic treatment to be 97%. A lesion may become noticeable via examination within one month of banding (16). After treatment, the number of WSLs decrease within the first two years (17) but can continue to be an aesthetic problem even 5 years or 12 years post treatment (3, 4). Quantitative light-induced fluorescence studies examining the severity of WSLs have shown significant progression in approximately 10% of lesions within 6-12 months after appliance removal, 30% regress and the majority remain stable(5, 18).

## **Risk Factors in the Formation of WSLs**

### ***Gender***

There is no clear sexual predisposition to WSLs with both males and females being equally at risk for disease development(6, 11, 19). Several studies have found that males develop more white spot lesions than females (7, 8, 15)while

others have determined that females display a higher incidence than males (9). Additionally, males tend to have a greater severity of demineralisation than females once the disease develops (6, 15). However, it is unlikely that a true gender-based risk exists. The apparent gender influences may be due to compliance with hygiene and preventive measures.

### *Age*

Geiger et al. have reported that the incidence of a WSL does not correlate positively with age; however, there are more advanced cavitated lesions in those less than 13 years of age (11). Boersma et al. have found age is not a significant factor in the incidence of WSLs (8). Alternatively, other studies have found that younger patients develop WSLs more than older patients (6, 15).

### *Location of WSLs*

No significant differences in WSL formation or prevalence have been found between right and left sides of the maxilla and mandible (3, 9, 15). The maxillary arch may be at greater risk to WSLs than the mandibular arch (9, 20, 21). The frequency is reported to be greatest on maxillary lateral incisors (3, 9, 15, 20) although one study has found that the prevalence is similar on all tested teeth (7). The latter study assessed patients wearing fixed orthodontic appliances which may have caused difficulty in the identification of WSLs. In the lower arch, the canine is most likely to be affected (9, 22). Access to the

flow of saliva and a reduced distance from the bracket to the free gingival margin, which impedes tooth brushing, are identified reasons cited for discrepancies in the incidence of WSLs between teeth(9). This may explain why a smaller tooth, such as a maxillary lateral incisor, has much greater incidence of demineralisation compared to a maxillary central incisor (9). Lesions tend to occur on the buccal surfaces of orthodontically treated teeth, adjacent to the gingival margins and close to the brackets(3, 9, 12).

### *Treatment Duration*

Some reports suggest that the duration of orthodontic treatment correlates positively with the severity of the WSL while others have found that treatment duration does not seem to affect the incidence or severity of WSLs (6, 9, 11, 20). This may be explained by the method of assessment of the WSL, the number of subjects or the actual treatment times examined in the study. One study of large sample size that has assessed WSL prevalence via photos has found a significantly greater number of white spots in patients treated for over 36 months compared with a 24-36 month group (20).

### *Oral Hygiene*

Patients with poor oral hygiene before and during treatment are a greater risk for developing WSLs (3, 6, 9, 11, 15, 20, 21). When oral hygiene levels decrease during treatment, the incidence of WSLs significantly increases(20).

A positive correlation has been found between gingival bleeding scores and the presence of WSLs (8).

### *Caries Experience*

Risk factors for caries have also been shown to be effective in predicting the formation of WSLs. Children who are at increased risk of demineralisation without orthodontic appliances have a much greater chance of developing further demineralisation when appliances are placed (20, 21, 23). One study has found that the presence of a restored first molar increases the development and severity (graded by degree of mineralisation) of WSLs during treatment (6). Al Mulla et al. have found patients with a high rate of decayed, missing or filled surfaces prior to treatment have a greater risk of caries during treatment (24). The natural protective effects of saliva with regard to caries development are also significant. Lower lingual surfaces with fixed retainers, rarely experience WSLs due to free flowing saliva which protects against decalcification (9, 12).

### *Fluoride Exposure*

Patients with pre-treatment fluorosis marks on their teeth seem to have a decreased prevalence of WSLs compared with children who do not have any fluorosis (20). This may be because a history of increased fluoride exposure

during tooth development has resulted in enamel which is more resistant to demineralisation.

### *Operator Differences and Socio Economic Status*

Experience or qualification of the operator also does not predict the development of a WSL(6). Other factors that do not show correlation with the disease include appointment attendance and the socioeconomic status of the patient (6, 8).

### *Appliance Type*

Al Maatiah et al. has found no difference in incidence between patients undergoing full fixed appliance therapy, those combined with surgery or functional appliance treatment, those treated with preadjusted edgewise (0.022x 0.028-in slot) or a Tip-Edge Plus appliance (0.022x 0.028-in slot)(6). There is no difference in WSL incidence between self-ligation and conventional pre-adjusted edgewise brackets (25, 26) although there are reports of decreased plaque and microbial counts with self-ligation brackets (27, 28).

A recent study surveying orthodontic patients, their parents, dentists and orthodontists has revealed that all groups accept that WSLs are unattractive,



the patient is most responsible for prevention and treatment should be managed by the general dentist (29). Interestingly, the study also has found that most parents would recommend orthodontic treatment even if WSLs were to be found on their children after treatment and over 50% feel that their children would benefit from more instruction on the prevention of WSLs.

In relation to risk factors for WSLs, most studies have compared risk factors to the presence or absence of the disease. Very few studies have been able to adequately grade the severity of WSLs in regards to degree of demineralisation of each lesion. Although a few studies have been able to do so, others have considered the total number of WSLs and use this to describe the severity of the disease experienced. One study has defined severity as an increase in the surface area of tooth covered (15). This void in the literature may be due to the absence of appropriate clinical grading of early smooth surface carious lesions in the past. The risk factors that indicate the presence, number and severity of WSLs in the South Australian orthodontic population is unknown.

## **CLINICAL EVALUATION OF WHITE SPOT LESIONS**

The ideal method for the detection of WSLs should have a high level of sensitivity (the ability to detect disease when present) and specificity enabling the ability to confirm that disease is absent. Diagnostic methods that are available to diagnose carious lesions include visual or visual/tactile methods, radiographic methods, fibre optic transillumination, electric conductance and laser fluorescence (30). A review in 2002 is inconclusive regarding the efficacy of these methods in detecting caries in anterior teeth or on buccal surfaces (30), both of which are common in orthodontic related WSLs. Since WSLs in orthodontic patients are usually located adjacent to brackets, radiographs are not used as part of the standard clinical diagnosis. The common clinical methods available in the literature to assess WSLs are considered below.

### **Visual Inspection**

The most commonly used method of WSL detection is via visual clinical examination. The use of a magnifying visual aid such as loupes with a minimum of 2.5 times enlargement has been found to improve the detection of early carious lesions (31, 32). A significant correlation has been found between visual caries assessment and the histological extent of a lesion (33).

Visual inspection means that assessors must be calibrated prior to and at regular times during a study(34, 35). Problems arise if the operator is also the

assessor which means that blinding is not possible(35). Furthermore, studies should record the initial appearance of the tooth as well as the appearance at the end of the experiment to give both incidence and severity. This could mean a very long experimental time of 18-30 months over a course of orthodontic treatment(34).

A common method of recording decay in the literature uses the DMFT index (decayed, missing, filled teeth) or DMFS index (decayed, missing, filled surfaces). The problems with these two indices are that missing and filled teeth are not always due to caries experience and hence may cause an overestimation(36). This is especially true in orthodontic patients in whom extraction of permanent teeth is commonly performed and removed teeth may or may not have experienced decay. Missing and filled teeth can also be a sign of past, as opposed to current disease experience. Furthermore, WSLs are normally very early signs of demineralisation and the DMFT and DMFS would not allow sufficient categorisation of the lesions.

A popular index for WSLs in the literature is one described by Gorelick et al. which uses a numerical scale (9):

- 0 = No lesion
- 1 = Slight white spot formation
- 2= Severe white spot formation

- 3 = Excessive white spot formation (cavitation)

This index provides a guideline on severity, presence and absence but does not indicate which area of the tooth is affected by the white spot. An alternative index developed by Banks et al. in 2000 also uses a numerical scale but includes an assessment of the area covered (13):

- 0 = No visible change
- 1 = Slight wet colour change, only visible after air drying
- 2 = Slight colour change with certain marked white areas
- 3 = White consistent colour change
- 4 = Distinct white colour change

## **The ICDAS II**

The International Caries Detection and Assessment System (ICDAS) describes a visual index for caries detection (37). The original system, ICDAS I developed in 2003, has been modified to the ICDAS II which has been used since 2007. The improvement has involved changing the codes so that the index appropriately indicates the increasing severity of lesions (38, 39). The advantage of this index over conventional methods such as DMFT and DMFS is its ability to further categorise early enamel demineralisation(38, 39). This is particularly significant with WSLs. The ICDAS II assessment of caries requires a dental light, triplex syringe to dry the tooth and a blunt probe to examine the

tooth. Because of its clear categorisation of lesions and use of standard clinical equipment, the ICDAS II has gained increasing popularity in caries related epidemiological studies. The ICDAS II is also increasingly popular in studies involving WSLs in orthodontic patients due to these described advantages over the traditional clinical indices. The code and criteria are as follows:

**Table 1: The ICDAS II Caries Scoring System (40)**

CODE	CRITERION
0	Sound tooth surface: No evidence of caries after 5 seconds of air drying
1	First visual change in enamel. Opacity of discolouration is visible after prolonged air drying
2	Distinct visual change in enamel visible when wet. Lesion must be visible when dry
3	Localised enamel breakdown (without clinical visual signs of dentinal involvement). Seen when wet and after prolonged drying
4	Underlying dark shadow from dentine
5	Distinct cavity with visible dentine
6	Extensive (more than half the surface). Distinct cavity with visible dentine

## Laser fluorescence- DIAGNOdent and DIAGNOdent pen

Fluorescence is light emission due to the movement of molecules in response to the absorption of high energy light (32). Natural fluorescence occurs in every tooth due to the proteins in enamel and dentine. Laser fluorescence (LF) is a quantitative method of caries detection based on the emission of light from a diode laser ( $\lambda = 655\text{nm}$ ) and the recording of fluorescence emitted from teeth (41). Specifically, bacterial metabolites in caries, assumed to be porphyrins, emit fluorescence that the LF device measures (42). Detection of white spot lesions using a quantitative LF method is much more sensitive than direct visualisation (8). There is evidence that the use of LF devices may be appropriate in diagnosis and grading of WSLs in orthodontic patients (43, 44). The most commonly used LF devices in the market are the DIAGNOdent (Kavo, Biberach Germany) and the DIAGNOdent pen (Kavo, Biberach Germany).

The DIAGNOdent and the DIAGNOdent pen were developed as a result of research by Hibst and Paulus in the 1990's (32). The return fluorescence emissions produced by these devices are regulated to show the level of mineralisation on a tooth surface using a scale ranging from 1 to 99. The older version, DIAGNOdent was launched and first promoted to detect occlusal and smooth surface caries. The newer device is the DIAGNOdent pen. The main clinical advantage of the latter is that the handpiece used over the tooth is not

physically connected to a monitor. The pen version consists of a monitor and handpiece combined.

The older DIAGNOdent has moderate sensitivity (0.71) and good specificity (0.88) when compared with photographic evaluation to detect the presence of WSLs (26). When compared with direct visual evaluation using the method described by Gorelick in 1982 (9), it is less reliable for detecting WSLs during orthodontic treatment (45). A recent study found the DIAGNOdent pen to be a useful aid which correlates well to the ICDAS II system in grading WSLs in orthodontic patients (46). Authors in this study have recommend its use for grading WSLs in orthodontic patients.

The use of fluorescent dyes has been proposed to improve the performance of both old and the new LF devices for *in vitro* studies in which bacteria are not present (41, 47). The use of a fluorescent dye in combination with the DIAGNOdent pen increases sensitivity without decreasing specificity when compared with the old device with dye (41). However, this was a study which examined artificial teeth and the utility of fluorescence dyes in combination with LF *in vivo* is still largely unknown.

## Photographic Images to Detect White Spot Lesions

Photographs are a popular method of detecting WSLs as they are simple to use, inexpensive and are often standard practice in orthodontic treatment.

They have the advantage that diagnosis can be made by several different people and at different times. They may be viewed in random order, the assessors can be blinded and error analysis can be carried out (35).

Furthermore, a permanent record of the appearance of the tooth is taken (34, 35). Also, the problem of examiner drift, where an assessor might make subtle changes of their assessment over time, is reduced (34).

The problems with photographic analysis are associated with technical factors including lighting, developing and producing reflections which are similar to WSLs while taking the photographs (35). Careful management of potential complications is required. To reduce the effects of glare and reflection, photos should be taken at slight angulations and parallel to each other for comparisons. A published standardised technique describes using a two-armed jig constructed with a greyscale to establish a fixed parallel distance from the buccal surface of the tooth to the camera lens and allow for colour calibration of digital images(48). An angulation between 20° and 40°decreases the area of demineralisation seen on the tooth. An angle of exactly 20° to the perpendicular of the buccal surface of the tooth is ideal to reduce reflection and maintain perspective of the tooth(49).One unique study uses photographs to determine the percentage of tooth surface area covered by WSLs on



individual teeth(15). Although this study provides information on severity based on surface area, it does not give information on severity based on extent of mineralisation. However, it is only information that photographic analysis would provide.

### **Optical Methods**

Optical methods of WSL detection are costly but can provide an accurate measurement of the extent of demineralisation. One method is quantitative light fluorescence which involves using a charge-coupled device in an intraoral camera to emit light in blue to green wavelengths onto the tooth.

Demineralisation is seen as a dark coloured spot. The main disadvantage of this system is the time taken for an image to form which makes it impractical for clinical use (32). Another technique involves using LED technology in intraoral cameras to illuminate a tooth and record its fluorescence (32). Long-term clinical studies on the effectiveness of these technologies compared to clinical diagnosis are not available, especially in relation to orthodontic patients and WSLs.

An accepted standard for detecting WSLs is directly quantifying the tooth in question. When direct quantitative methods, such as microradiography or hardness testing are used to measure mineral loss or the depth of caries, the tooth must be extracted. Hence this method is not ideal for many clinical

studies and not often used for orthodontic patients. In regards to simple but affective clinical grading, the ICADS II seems to have the most advantages over other clinical indices. The major disadvantage with LF devices includes their cost, time and that their use in orthodontic patients is still under validation. In this regard, the DIAGNOdent pen shows some promise over other devices. Advantages this device offers over the ICDAS II is the large scale (between 1 and 99) it offers in determining levels of demineralisation and the lack of subjectivity with its use. The use of the DIAGNOdent pen in the orthodontic population for the detection of WSLs requires further study.

## **THE EFFECT OF ORTHODONTIC APPLIANCES ON THE ORAL**

### **ENVIRONMENT**

Orthodontic appliances increase the accumulation of plaque and food on the smooth surfaces of teeth which would normally experience a very low rate of decay (16). The presence of brackets, wires and attachments increases the accumulation of plaque, makes brushing more difficult and hinders the self-cleansing mechanisms of saliva, muscles and tongue movement (21). The plaque that forms after the placement of fixed appliances has a lower pH than plaque in non-orthodontic patients (50, 51).

In addition, orthodontic treatment alters the oral bacterial flora in plaque by promoting a lower pH thus increasing the concentration of acidogenic bacteria such as *S. Mutans* and *Lactobacillus*(52, 53). Other types of microbiota associated with WSLs are *S wiggisiae*, *G.elegans*, *Veillonellaceae* and *Bifidobacteriaceae*(54). These bacteria produce acid by-products, in the presence of fermentable carbohydrates, further lowering the pH. Once the pH drops below a critical threshold, demineralisation of the enamel occurs (55). This results in more rapid caries progression in orthodontic patients compared with non-orthodontic patients (7).

The increased caries risk that orthodontic patients undergo is counterbalanced, though not to the full extent, by saliva changes after full fixed appliances are introduced. Generally, the saliva flow rate increases, the pH increases and there is improved buffering capacity (56-62). The differences tend to be more pronounced in males than females (62).The increase in flow rate has been found to be similar despite which bracket is used (59). These saliva changes may explain why some patients have hardly any demineralisation despite accumulating a large amount of plaque (1).

## **SALIVA PROPERTIES AND DENTAL CARIES**

Saliva plays an integral role in the maintenance of the oral cavity. Its functions include lubrication during mastication and speech, cleansing, antimicrobial activities, digestion, taste, maintaining health of the oral mucosa, and providing a reservoir for calcium, fluoride and phosphate ions required for buffering and remineralisation(63).

In addition, saliva influences the balance between demineralisation and remineralisation at the tooth's surface. Although bacteria, carbohydrates, a susceptible tooth and time are required for demineralisation, saliva properties such as buffering capacity, pH and flow rate influence the extent of demineralisation and repair via remineralisation(1). Furthermore, a lack of saliva can lead to unusual locations of dental decay (64).

The two main aspects of saliva may be broadly categorised as the quantity and quality. The quantity of saliva is described as its flow rate. The quality may be described by its pH, proteins, viscosity and buffering capacity (65). The relationship between saliva properties and caries is described below.

## Flow Rate of Saliva

An increase in the saliva flow rate increases buffering capacity, accelerates clearance and increases anti-bacterial activity (1). The increase in buffering capacity is due to an increased concentration of bicarbonate produced by more saliva (66). A low saliva secretion rate causes a greater than normal decrease in pH after exposure to fermentable carbohydrates and also hinders pH recovery (67). The term 'flow rate' within the literature incorporates the time it takes for saliva to be produced without stimulation (unstimulated flow rate) as well as the amount of saliva produced after stimulation (stimulated flow rate).

The literature is inconclusive regarding the effect of unstimulated flow rate of saliva and caries rates as several studies have found a positive relationship between a low unstimulated flow rate and caries (68, 69) and others have found no relationship between unstimulated flow rates and caries levels (70, 71).

Similarly, an increased stimulated flow rate is sometimes associated with a decreased caries rate (66, 69, 72, 73) and at other times no association is found (70).

Many papers have been published showing conflicting results with regards to flow rates of saliva. A meta-analysis has found that saliva flow has the strongest association with caries risk compared to other salivary parameters but with poor sensitivity and good specificity (74). Twenty one examined articles suggest saliva flow is a predictive tool for caries but thirty four articles do not. The difference in the literature may be due to the fact that caries is not directly proportional to salivary flow at all rates. Patients with a reduced salivary flow, due to pathology or medications often show a greater increase in caries risk than those with physiologically reduced rates (74). Generally, a high risk individual may have an unstimulated flow rate less than 0.3mL/min (75, 76) and/or a stimulated flow rate less than 0.7-0.8mL/min (64, 74).

### **Salivary Proteins and Viscosity**

Differences have been found in the proteins within saliva in high and low caries risk groups(77-79). Other studies have found differences between mucin levels in high and low risk caries groups with a possible link between reduced level of specific mucins and high caries rates(80).

Some studies have found an increased saliva viscosity, describing it as “frothy and bubbly”, in higher caries risk groups (69, 73). The authors attribute this increase in viscosity to a decrease in water content.

### **Resting and Stimulated pH of Saliva**

Some studies have found a significant correlation between low resting salivary pH and increased incidence of caries (69, 73, 81, 82). Other studies have found no difference between high and low caries groups(70, 83).

Similarly, with regard to stimulated pH, some studies have shown a positive correlation between caries and lower pH levels(84). Others have not indicated any differences between stimulated pH and caries levels(85).

### **Buffering Capacity of Saliva**

The buffering capacity of saliva is its ability to resist pH change (64). It is this feature of saliva which neutralises acids and maintains the pH in the mouth above the critical pH at which demineralisation will occur (86). However, the relationship between the buffering capacity and caries is still controversial.

Some studies have found that an increased buffering capacity is associated with lower caries rates(68-70, 73, 81). Others suggest that there is no significant difference in buffering capacity between higher and lower caries groups(82, 85, 87).

The buffering capacity of saliva depends mainly upon available carbonate ions but also relies on phosphate and protein buffers. The ideal pH value for

carbonate buffers to work is 6.3 and for phosphate buffers 7.2. Buffering in oral environments below a pH of 5 is based on proteins (88).

The gold standard for measuring buffering capacity is known as the Ericsson method. This method involves a laboratory, is expensive and is too complicated to accomplish chair side. Hence, "strip tests" have been developed to simplify the procedure(89). These have a thin layer of acid embedded during manufacture. The acid is neutralised by saliva during the test. The degree of neutralisation by the saliva is represented by colour change and a chart to categorise the patient(65). Examples of the strip type tests include the Saliva-Check Buffer Test (GC Corp.), the Dentobuff Strip (Orion Diagnostica) and CRT® Buffer Test (Ivoclar Vivadent). When saliva buffering capacity is high, there is agreement between the tests. When the buffering capacity is low or medium there are disagreements between the strip tests compared with the Ericsson test(65). In addition, there are differences in categorisations of buffering capacity between the different manufacturers of strip tests(65, 89). Viscosity of the saliva sample may alter the colour strip result by influencing the volume of the saliva drop, its ability to wet the strip and the ability to remove excess saliva from the strip with more viscous saliva (89). Colours may also be difficult to read and assign a category, are subject to visual disturbances such as colour blindness, can be influenced by lighting and are subjective(65, 89).



## Why Are There Conflicting Results?

From the above, it is evident that the role of saliva and its effect on caries has been extensively studied. Although a direct link is difficult to establish, saliva is likely to play an important part in caries because of its role in the remineralisation and demineralisation balance. The difference in results between the various studies may be due to the different age groups being examined, the type of study design, differing methods of identifying and classifying decay, other risk factors of caries significantly outweighing the role of saliva in the sample group, or the methods used to identify salivary properties. It might be that the role of saliva is of greater importance when considering very early carious lesions, like those seen in orthodontic patients with WSLs.

## THE SALIVA-CHECK BUFFER KIT

The Saliva-Check Buffer Kit (GC Corp., Belgium) provides 6 results regarding saliva function that are proposed to indicate a patient's expected caries risk(90, 91). Please see attached appendix (pg. 136) for full explanation of this test.

**Test 1:** *Visual inspection of the level of hydration:* This involves assessing the lower lip gland secretion. The time taken for the visible production of saliva is recorded.

**Test 2: Saliva Consistency:** The assessment of resting saliva consistency and its categorisation as either (a) sticky and frothy or (b) frothy and bubbly or (c) watery and clear.

**Test 3: Resting pH Measurement:** A pH strip is placed into resting saliva expectorated into a cup and the pH recorded.

**Test 4: Testing of Stimulated Saliva Quantity:** The patient chews a piece of wax for 5 mins and regularly expectorates into a cup. The quantity of saliva is measured.

**Test 5: pH of Stimulated Saliva.** Determined by a pH strip placed into the stimulated saliva.

**Test 6: Buffering Capacity:** A pipette is used to draw up saliva from the previous test and dispense 1 drop onto each of the test pads on a buffer test strip. At 2 minutes each of the 3 colour pads is graded according to colour. A score between 0 and 12 is given for buffering capacity using a chart provided by the manufacturer.

## **VALIDITY OF THE SALIVA-CHECK BUFFER KIT IN CARIES**

### **PREDICTION**

A study published in 2008 using the Saliva-Check Buffer Kit on 58 non-orthodontic adult patients has found a negative correlation between resting pH and pre-cavitated (ICDAS II grade 1 and 2) carious lesions (70). The same study also shows a negative correlation between saliva buffering and moderate lesions (ICDAS II grade 3 and 4). This study implies that the resting pH may be useful in diagnosing WSLs which are normally graded an ICDAS II score of 1 or 2.

Cheng et al. examined saliva profiles using the Saliva-Check Buffer Kit on cleft lip and palate children with and without orthodontic treatment and compared their results to non-cleft lip and palate children (60). The study found strong correlations between buffering capacity, the pH of stimulated and non-stimulated saliva and the salivary flow rate. Furthermore, the saliva flow rate correlates with the pH of resting saliva and secretion time. Although the study has collected the DMFT of participants, it does not compare the DMFT with saliva variables.

DMFS were recorded on 34 subjects before and one month after the placement of full fixed appliances and saliva properties examined using the

Saliva-Check Buffer Kit.(62) Properties that protected against an increased DMFS were reported to be stimulated saliva flow, buffering capacity and stimulated salivary pH (62). No statistical difference in DMFS is shown in the one month period and hence no comparison could be made with changes in salivary properties. The authors of this study have recommended assessing these properties to identify caries risk.

An additional study has examined the ability of the Saliva-Check Buffer Kit to distinguish between 20 non-orthodontic adult patients who had a DMFT > 5 compared to 20 patients with a DMFT = 0. The DMFT = 0 group have been shown to have a higher flow rate, viscosity, pH and buffering capacity compared to the DMFT > 5 group (69).

The diagnostic validity of Saliva-Check Buffer Kit in the detection of WSLs via ICDAS II and the DIAGNOdent pen in orthodontic patients is not available in the published literature.

## **OTHER SALIVA TESTS USED TO DETERMINE CARIES RISKS IN ORTHODONTIC PATIENTS**

Two recent studies which have examined orthodontic patients have found no significant differences in the buffering capacity or the stimulated saliva flow rate in both low and high risk caries groups when caries is assessed using DMFT scores (24, 92). Although both studies have not used the Saliva-Check Buffer Kit, the authors question the validity of using these parameters to identify high caries risk individuals in children undergoing orthodontic treatment. It should be noted that these studies have looked at overall DMFT scores and not scores designed for the identification of WSLs.

The Clinpro Cario L- Pop® (3M ESPE), (CCLP) test works by measuring the quantity of lactic acid production. Cariogenic potential is based on the rationale that the greater the lactic acid production, the higher at risk the patient is.(93). The CCLP assesses dental caries risk using the following grading: low (1-3), moderate (4-6) and high (7-9). The test works by placing a sucrose-impregnated swab with the patient's plaque into the L-Pop blister to initiate a chemical reaction (94, 95). When using DMFT and DMFS to assess caries risk Chaussain et al. have found the CCLP test to be acceptable in predicting the caries risk for orthodontic patients (95).

A saliva test that could predict the development, number and severity of WSLs in orthodontic patients would be a useful clinical adjunct as WSLs continue to

be a complication of orthodontic treatment. The Saliva-Check Buffer Kit has shown some positive results in diagnosing risk factors in both the non-orthodontic and orthodontic populations when caries has been assessed via DMFT and DMFS. Overall, there have been very few studies with limited sample sizes and treatment times. Currently, there is no widely accepted clinical test that can differentiate between those patients who will develop WSLs and those who will not. Consideration should also be given to the total number of lesions and to those who develop a severe degree of demineralisation as opposed to those who develop superficial, easily remineralised lesions. As the DMFS and DMFT are not sufficient to categorise early carious lesions, the ICDAS II would be the better method to grade the lesions.

## **MANAGEMENT OF WHITE SPOT LESIONS**

Management involves preventing demineralisation during orthodontic treatment as well as remineralising lesions once they have occurred (96). Generally, prevention is better than cure with any disease process and this holds true in the management of WSLs. Many preventive strategies are under investigation. From the literature available at present, it is apparent that the exact combination of strategies required to prevent the occurrence of WSLs is still unknown.

A systematic review of preventive measures applied during orthodontic treatment has considered the use of fluoride, chlorhexidine, sealants and bonding materials. It has found that toothpastes and gel with high fluoride concentrations as well as chlorhexidine rinses result in the reduction of demineralisation (97). Some of the common strategies known for the management of WSLs are considered below.

### **Oral Hygiene and Dietary Protocols**

A high standard of oral hygiene is required to prevent WSLs during orthodontic treatment (2, 9, 11, 16). Ideal orthodontic patients should have a low caries risk and excellent hygiene habits before the start of treatment (98). Patients are often asked to brush a minimum of three times a day with a fluoridated dentifrice. Brushing and flossing technique should be demonstrated (98). The use of an electric toothbrush can be recommended as it leads to a lower amount of plaque than manual tooth brushing with fixed appliances in place (99).

Professional dental scaling and oral hygiene instructions at regular intervals have been found effective in reducing the incidence of WSLs (11). In addition, this study has found a negative association between compliance of preventive strategies at home and the incidence of WSLs.

A diet that is high in fermentable carbohydrates presents an increased risk of decay. Patients are advised to reduce their intake of complex carbohydrates, carbonated drinks and acids (98).

## Fluoride

Fluoride is widely accepted to have a key beneficial role in the prevention of decay in children. When fluoride is incorporated into enamel, fluorapatite crystals are formed which have an increased resistance to acid attack compared with normal hydroxyapatite crystals (55). During orthodontic treatment fluoride application may be topical (mouthwashes, varnishes, gels, toothpastes etc.) or incorporated into orthodontic materials (cements, modules, adhesives etc.). Various preparations of fluoride such as stannous or sodium fluoride are also available. An initial Cochrane review on fluorides for the prevention of WSLs, published in 2004, has found that the exact treatment prescription that causes a reduction in WSLs in orthodontic patients is still debatable due to insufficient research available at that time (100). A review in 2009 on fluoride use for caries in general has further emphasised that more research using better methodology is required before an exact modality and strength of fluoride can be recommended for orthodontic use (101). Another published review agrees with the above and further comments that high



potency preparations may have additional benefits but firm conclusions cannot be made(102).

### **Topical Fluoride Application**

Topical fluoride application causes the formation of a calcium fluoride-like material on the enamel surface which acts as a fluoride reservoir and is present some weeks after orthodontic treatment starts (103). Many studies have shown that the incidence and severity of WSLs have been found to be reduced when fluoride rinses are administered during orthodontic treatment (11, 14, 100, 104). One systematic review has recommended daily rinsing with 0.05% sodium fluoride mouthwash during treatment (34). However, this recommendation is based on research carried out on non-orthodontic patients of similar age groups. Fluoride rinses are dependent on patient compliance. An alternative that does not depend on the patient may be a fluoride varnish (105). Fluoride varnish provides a protective coat over the tooth, adheres longer to the tooth's surface and has a higher concentration of fluoride compared with rinses(106, 107). A varnish may also be placed in dental areas at higher risk of demineralisation (108). Disadvantages of a varnish include temporary discolouration, limits in frequency of application and increased costs/chair time (55). A randomised controlled trial has found that the application of a 5% sodium fluoride varnish, for treatment of WSLs post orthodontic treatment, is effective and decreases the DIAGNOdent readings

on the enamel surface to a much greater extent than a control group who had saline applied to the enamel(109).

### **Fluoride Releasing Orthodontic Materials**

Fluoride releasing materials have the advantage of not being dependent on compliance but their disadvantage is that many of them release large amounts of fluoride initially but the levels drop down to sub-therapeutic levels throughout orthodontic treatment (98, 100).

Glass ionomer cement (GIC) and resin modified glass ionomer cement (RMGIC) can prevent demineralisation around orthodontic brackets when compared to resin adhesives during treatment (30, 41, 100). After long term follow up (2 or 12 years), one study has found that patients with brackets bonded with GIC experience less WSLs(4). This study concludes that this was due to GIC releasing fluoride, which decreases the formation of WSLs and the fact the resin adhesives are associated with deeper WSLs which remineralise to a lesser extent after treatment (4). A systematic review concerning the question of whether GIC as an orthodontic adhesive results in less demineralisation compared to traditional resin adhesives concludes that evidence is very weak in favour of GIC adhesives being protective (35). This study also mentions that GIC adhesives have a higher rate of debonding which may contraindicate their routine use in orthodontics.

The use of GIC adhesives instead of resin adhesives is not widespread due to their reduction in shear and tensile bond strengths (110). A Cochrane review on orthodontic adhesives recommends the use of composite resin adhesives over GIC adhesives(111).

Fluoride releasing composite resin has been developed but releases less fluoride than RMGIC and GIC adhesives(112). Their survival time, however, is similar to conventional composites (35)

Similar to the adhesives, fluoride releasing elastomers have not seen popular use in orthodontics. This is likely due to their rapidly declining fluoride release after initial placement and their ineffective force levels compared with regular elastomers (113, 114). Some studies show that elastomers can enlarge when placed in the mouth which increases their surface area to which plaque can attach(115, 116).

### **CPP-ACP and CPP-ACFP**

CPP-ACP and CPP-ACFP products involve using casein phosphopeptides (CPP) to stabilise and localise amorphous calcium phosphate (CPP-ACP) or amorphous calcium fluoride phosphate (CPP-ACFP). Both have been shown to have remineralising effects on the enamel surface(43, 117, 118). The added benefit of fluoride in CPP-ACFP has shown more remineralisation (119). The mechanism involves forming nano-complexes of CPP-ACP (or

CPP-ACFP) to act as a reservoir in a soluble format for calcium and phosphate ions adjacent to the tooth's surface to reduce demineralisation and promote remineralisation(120). CPP-ACP has been added to various products including drinks, GIC, chewing gum, mints and topical gels for its beneficial effects(121).

Marchisio et al. have used CPP-ACP topically on 25 orthodontic patients for 3 weeks and then suspend use for 3 weeks. The authors have measured salivary pH, plaque pH and oral hygiene at baseline, 3 weeks and 6 weeks later. They have found no conclusive results showing improvement in any of these areas with use of CPP-ACP(120). This might be because of the short term use of the product or the fact that the study has not tested patient compliance with product use. These are common clinical problems associated with topical use of CCP-ACP.

### **ACP Containing Orthodontic Products**

An in vivo study on the effect of an ACP containing orthodontic composite has found a reduction of enamel mineral loss around the brackets compared to traditional composite resin. However, this is a study in which the adhesives are tested only for four weeks(122). Hence, the long term protective effects remain unknown. The bond strength of the ACP containing adhesive is lacking compared to traditional orthodontic adhesive on healthy tooth structure(123,

124). However, if bonding to a demineralised surface, the CPP-ACP treatment requires higher debonding forces (125).

A comparison of CPP-ACP (used topically) and fluoride gel applied around brackets has found no differences between them in their ability to prevent demineralisation in vivo and in vitro (126).

### Sealers

Similar to sealers that prevent caries in molars by being placed over fissures, resin sealers can be placed over the buccal surface of teeth with orthodontic brackets. This forms a physical barrier against both acid attacks and certain oral bacteria (127). The main problem with resin sealers is their low wear resistance causing them to be abraded by toothbrushes and requiring re-application(127, 128). The advantages include that their preparation may include fluoride or antimicrobial components and that they are not reliant on patient compliance. One in vitro study has found resin sealants and fluoride varnish to be superior in protecting against WSLs compared to CPP-ACP products applied topically and incorporated within cements. The authors in this study warn that evidence from randomised clinical trials is required before firm conclusions can be made (127). Another published study has found no significant difference using a fluoride releasing sealant compared to traditional resin adhesives (129).

## **Bonding Technique**

Leakage and decalcification may be present if cement holding orthodontic bands fails or a band is not contoured adequately around the tooth (98).

Excess bonding material can also cause a junction for extra plaque deposit (98). One study has found that increased etching times and surplus etching of the labial enamel surface (that which is beyond the bracket base) increases the amount of demineralisation; especially, in the absence of adequate hygiene over a long period(130).

## **Other Preventive Methods**

A recent study on extracted premolars has found that antibacterial orthodontic adhesive may have a protective effect on preventing caries when compared with traditional resin adhesives (131). The study is short term (30 days) and hence the long term effects and bond strengths are still unknown.

## **Debonding and Treatment of WSLs**

Before orthodontic treatment begins, it has been recommended that the extent and severity of existing WSLs be noted and a record of all teeth be taken with photographs (55). After orthodontic treatment, prior to debonding, WSLs should be again documented. Removing brackets and adhesive around WSLs may lead to permanent damage and cavitation to a tooth's surface. An *in vitro* study from the Melbourne Dental School found that use of CPP-ACFP to

remineralise WSLs decreases the amount of damage done to a tooth when adhesive is removed with a tungsten carbide bur and a slow speed handpiece (132). A further study shows that regardless of the method of adhesive removal, CPP-ACFP use during treatment leads to a reduction in damage to the enamel in depth and size (133). This study also shows that removing adhesive with an aluminium oxide disc produces less damage to teeth with WSLs (or teeth that have been remineralised after WSLs) compared with using a high speed, slow speed or ultrasonic scaler.

If WSLs are observed after debonding they should initially be treated for a 2-3 month period with good oral hygiene, allowing some natural remineralisation with saliva. Early treatment with high concentration fluoride can have unaesthetic affects (55). After a few months, fluoride can be applied. If a high concentration of fluoride is applied straight away, the outer enamel surface would remineralise earlier than the subsurface lesion. This would mean that although the lesion is arrested, a permanent white opaque appearance may be left on the tooth's surface (98). Leaving the tooth for some time may allow calcium and fluoride penetration to deeper areas at first and then allow superficial areas to mineralise.

The use of CPP-ACP after debonding has shown superior results to fluoride alone in the remineralisation of WSLs leading to better aesthetic scores (43,

134). The difference between the use of CPP-ACP and topical fluoride does not reflect in the DIAGNOdent scores (43). It has been postulated that early use of CPP-ACP may be beneficial as a supplement to natural healing as the nano-clusters of ACP are smaller and may be able to access the demineralised subsurface area through the remineralised surface zone (135). Others have found no benefits with early use of CPP-ACP over tooth brushing with a fluoridated dentifrice to allow natural remineralisation(136).

If time, fluoride and the use of CPP-ACP does not improve the aesthetic concerns of the patient, alternatives such as tooth whitening , microabrasion or resin infiltrations in conjunction with remineralisation aids can be considered (55, 137-140). A last resort may be the placement of prosthetic veneers (55).

## **CONCLUSION**

WSLs continue to be a topic of interest as they are a very common complication of orthodontic treatment with detrimental aesthetic implications to anterior teeth. The extensive literature available concerning this area can roughly be divided into the diagnosis, prevention and treatment of WSLs. This thesis focuses on the diagnosis of WSLs and attempts to further our



knowledge so that significant clinical benefit in treating orthodontic patients might be gained.

## REFERENCES

1. Chang HS, Walsh LJ, Freer TJ. Enamel demineralization during orthodontic treatment. Aetiology and prevention. Aust Dent J. 1997 Oct;42(5):322-7.
2. Ogaard B, Rolla G, Arends J, ten Cate JM. Orthodontic appliances and enamel demineralization. Part 2. Prevention and treatment of lesions. Am J Orthod Dentofacial Orthop. 1988 Aug;94(2):123-8.
3. Ogaard B. Prevalence of white spot lesions in 19-year-olds: a study on untreated and orthodontically treated persons 5 years after treatment. Am J Orthod Dentofacial Orthop. 1989 Nov;96(5):423-7.
4. Shungin D, Olsson AI, Persson M. Orthodontic treatment-related white spot lesions: a 14-year prospective quantitative follow-up, including bonding material assessment. Am J Orthod Dentofacial Orthop. 2010 Aug;138(2):136 e1-8; discussion -7.
5. van der Veen MH, Mattousch T, Boersma JG. Longitudinal development of caries lesions after orthodontic treatment evaluated by quantitative light-induced fluorescence. Am J Orthod Dentofacial Orthop. 2007 Feb;131(2):223-8.
6. Al Maaitah EF, Adeyemi AA, Higham SM, Pender N, Harrison JE. Factors affecting demineralization during orthodontic treatment: a post-hoc

analysis of RCT recruits. *Am J Orthod Dentofacial Orthop.* 2011 Feb;139(2):181-91.

7. Tufekci E, Dixon JS, Gunsolley JC, Lindauer SJ. Prevalence of white spot lesions during orthodontic treatment with fixed appliances. *Angle Orthod.* 2011 Mar;81(2):206-10.

8. Boersma JG, van der Veen MH, Lagerweij MD, Bokhout B, Prahl-Andersen B. Caries prevalence measured with QLF after treatment with fixed orthodontic appliances: influencing factors. *Caries Res.* 2005 Jan-Feb;39(1):41-7.

9. Gorelick L, Geiger AM, Gwinnett AJ. Incidence of white spot formation after bonding and banding. *Am J Orthod.* 1982 Feb;81(2):93-8.

10. Zachrisson BU, Zachrisson S. Caries incidence and oral hygiene during orthodontic treatment. *Scand J Dent Res.* 1971;79(6):394-401.

11. Geiger AM, Gorelick L, Gwinnett AJ, Griswold PG. The effect of a fluoride program on white spot formation during orthodontic treatment. *Am J Orthod Dentofacial Orthop.* 1988 Jan;93(1):29-37.

12. Mitchell L. Decalcification during orthodontic treatment with fixed appliances--an overview. *Br J Orthod.* 1992 Aug;19(3):199-205.

13. Banks PA, Chadwick SM, Asher-McDade C, Wright JL. Fluoride-releasing elastomerics--a prospective controlled clinical trial. *Eur J Orthod.* 2000 Aug;22(4):401-7.

14. Ogaard B, Larsson E, Henriksson T, Birkhed D, Bishara SE. Effects of combined application of antimicrobial and fluoride varnishes in orthodontic patients. *Am J Orthod Dentofacial Orthop.* 2001 Jul;120(1):28-35.
15. Chapman JA, Roberts WE, Eckert GJ, Kula KS, Gonzalez-Cabezas C. Risk factors for incidence and severity of white spot lesions during treatment with fixed orthodontic appliances. *Am J Orthod Dentofacial Orthop.* 2010 Aug;138(2):188-94.
16. Ogaard B, Rolla G, Arends J. Orthodontic appliances and enamel demineralization. Part 1. Lesion development. *Am J Orthod Dentofacial Orthop.* 1988 Jul;94(1):68-73.
17. Mattousch TJ, van der Veen MH, Zentner A. Caries lesions after orthodontic treatment followed by quantitative light-induced fluorescence: a 2-year follow-up. *Eur J Orthod.* 2007 Jun;29(3):294-8.
18. Al-Khateeb S, Forsberg CM, de Josselin de Jong E, Angmar-Mansson B. A longitudinal laser fluorescence study of white spot lesions in orthodontic patients. *Am J Orthod Dentofacial Orthop.* 1998 Jun;113(6):595-602.
19. Mizrahi E. Enamel demineralization following orthodontic treatment. *Am J Orthod.* 1982 Jul;82(1):62-7.
20. Julien KC, Buschang PH, Campbell PM. Prevalence of white spot lesion formation during orthodontic treatment. *Angle Orthod.* 2013 Jan 4.

21. Lovrov S, Hertrich K, Hirschfelder U. Enamel Demineralization during Fixed Orthodontic Treatment - Incidence and Correlation to Various Oral-hygiene Parameters. *J Orofac Orthop.* 2007 Sep;68(5):353-63.
22. Travess H, Roberts-Harry D, Sandy J. Orthodontics. Part 6: Risks in orthodontic treatment. *Br Dent J.* 2004 Jan 24;196(2):71-7.
23. Zimmer BW, Rottwinkel Y. Assessing patient-specific decalcification risk in fixed orthodontic treatment and its impact on prophylactic procedures. *Am J Orthod Dentofacial Orthop.* 2004 Sep;126(3):318-24.
24. Al Mulla AH, Kharsa SA, Kjellberg H, Birkhed D. Caries risk profiles in orthodontic patients at follow-up using Cariogram. *Angle Orthod.* 2009 Mar;79(2):323-30.
25. Polat O, Gokcelik A, Arman A, Arhun N. A comparison of white spot lesion formation between a self-ligating bracket and a conventional preadjusted straight wire bracket. *World J Orthod.* 2008 Summer;9(2):e46-50.
26. Buck T, Pellegrini P, Sauerwein R, Leo MC, Covell DA, Jr., Maier T, et al. Elastomeric-ligated vs self-ligating appliances: a pilot study examining microbial colonization and white spot lesion formation after 1 year of orthodontic treatment. *Orthodontics : the art and practice of dentofacial enhancement.* 2011 Summer;12(2):108-21.
27. Pellegrini P, Sauerwein R, Finlayson T, McLeod J, Covell DA, Jr., Maier T, et al. Plaque retention by self-ligating vs elastomeric orthodontic brackets:

quantitative comparison of oral bacteria and detection with adenosine triphosphate-driven bioluminescence. *Am J Orthod Dentofacial Orthop.* 2009 Apr;135(4):426 e1-9; discussion -7.

28. Pandis N, Papaioannou W, Kontou E, Nakou M, Makou M, Eliades T. Salivary *Streptococcus mutans* levels in patients with conventional and self-ligating brackets. *Eur J Orthod.* 2010 Feb;32(1):94-9.

29. Maxfield BJ, Hamdan AM, Tufekci E, Shroff B, Best AM, Lindauer SJ. Development of white spot lesions during orthodontic treatment: perceptions of patients, parents, orthodontists, and general dentists. *Am J Orthod Dentofacial Orthop.* 2012 Mar;141(3):337-44.

30. Bader JD, Shugars DA, Bonito AJ. A systematic review of the performance of methods for identifying carious lesions. *J Public Health Dent.* 2002 Fall;62(4):201-13.

31. Erten H, Uctasli MB, Akarслан ZZ, Uzun O, Baspinar E. The assessment of unaided visual examination, intraoral camera and operating microscope for the detection of occlusal caries lesions. *Operative dentistry.* 2005 Mar-Apr;30(2):190-4.

32. Guerrieri A, Gaucher C, Bonte E, Lasfargues JJ. Minimal intervention dentistry: part 4. Detection and diagnosis of initial caries lesions. *Br Dent J.* 2012 Dec;213(11):551-7.

33. Ekstrand KR, Ricketts DN, Kidd EA. Reproducibility and accuracy of three methods for assessment of demineralization depth of the occlusal surface: an in vitro examination. *Caries Res.* 1997;31(3):224-31.
34. Benson PE, Shah AA, Millett DT, Dyer F, Parkin N, Vine RS. Fluorides, orthodontics and demineralization: a systematic review. *J Orthod.* 2005 Jun;32(2):102-14.
35. Rogers S, Chadwick B, Treasure E. Fluoride-containing orthodontic adhesives and decalcification in patients with fixed appliances: a systematic review. *Am J Orthod Dentofacial Orthop.* 2010 Oct;138(4):390 e1-8; discussion -1.
36. Broadbent JM, Thomson WM. For debate: problems with the DMF index pertinent to dental caries data analysis. *Community Dent Oral Epidemiol.* 2005 Dec;33(6):400-9.
37. Ismail AI. Visual and visuo-tactile detection of dental caries. *J Dent Res.* 2004;83 Spec No C:C56-66.
38. Ekstrand KR, Martignon S, Ricketts DJ, Qvist V. Detection and activity assessment of primary coronal caries lesions: a methodologic study. *Operative dentistry.* 2007 May-Jun;32(3):225-35.
39. Ismail AI, Sohn W, Tellez M, Amaya A, Sen A, Hasson H, et al. The International Caries Detection and Assessment System (ICDAS): an

integrated system for measuring dental caries. *Community Dent Oral Epidemiol.* 2007 Jun;35(3):170-8.

40. Shoaib L, Deery C, Ricketts DN, Nugent ZJ. Validity and reproducibility of ICDAS II in primary teeth. *Caries Res.* 2009;43(6):442-8.

41. Alencar CJ, Braga MM, de Oliveira E, Nicolau J, Mendes FM. Dye-enhanced laser fluorescence detection of caries lesions around brackets. *Lasers Med Sci.* 2009 Nov;24(6):865-70.

42. Lussi A, Hibst R, Paulus R. DIAGNOdent: an optical method for caries detection. *J Dent Res.* 2004;83 Spec No C:C80-3.

43. Andersson A, Skold-Larsson K, Hallgren A, Petersson LG, Twetman S. Effect of a dental cream containing amorphous cream phosphate complexes on white spot lesion regression assessed by laser fluorescence. *Oral Health Prev Dent.* 2007;5(3):229-33.

44. Aljehani A, Yousif MA, Angmar-Mansson B, Shi XQ. Longitudinal quantification of incipient carious lesions in postorthodontic patients using a fluorescence method. *Eur J Oral Sci.* 2006 Oct;114(5):430-4.

45. Kronenberg O, Lussi A, Ruf S. Preventive effect of ozone on the development of white spot lesions during multibracket appliance therapy. *Angle Orthod.* 2009 Jan;79(1):64-9.



46. Baeshen HA, Lingstrom P, Birkhed D. Effect of fluoridated chewing sticks (Miswaks) on white spot lesions in postorthodontic patients. *Am J Orthod Dentofacial Orthop.* 2011 Sep;140(3):291-7.
47. Mendes FM, Siqueira WL, Mazzitelli JF, Pinheiro SL, Bengtson AL. Performance of DIAGNOdent for detection and quantification of smooth-surface caries in primary teeth. *J Dent.* 2005 Jan;33(1):79-84.
48. Benson PE, Pender N, Higham SM. Quantifying enamel demineralization from teeth with orthodontic brackets--a comparison of two methods. Part 1: repeatability and agreement. *Eur J Orthod.* 2003 Apr;25(2):149-58.
49. Benson PE, Pender N, Higham SM. Enamel demineralisation assessed by computerised image analysis of clinical photographs. *J Dent.* 2000 Jul;28(5):319-26.
50. Chatterjee R, Kleinberg I. Effect of orthodontic band placement on the chemical composition of human incisor tooth plaque. *Arch Oral Biol.* 1979;24(2):97-100.
51. Gwinnett AJ, Ceen RF. Plaque distribution on bonded brackets: a scanning microscope study. *Am J Orthod.* 1979 Jun;75(6):667-77.
52. Lundstrom F, Krasse B. Caries incidence in orthodontic patients with high levels of *Streptococcus mutans*. *Eur J Orthod.* 1987 May;9(2):117-21.

53. Attin R, Thon C, Schlagenhauf U, Werner C, Wiegand A, Hannig C, et al. Recolonization of mutans streptococci on teeth with orthodontic appliances after antimicrobial therapy. *Eur J Orthod.* 2005 Oct;27(5):489-93.
54. Tanner AC, Sonis AL, Lif Holgerson P, Starr JR, Nunez Y, Kressirer CA, et al. White-spot lesions and gingivitis microbiotas in orthodontic patients. *J Dent Res.* 2012 Sep;91(9):853-8.
55. Bishara SE, Ostby AW. White Spot Lesions: Formation, Prevention, and Treatment. *Semin Orthod.* 2008 (14):174-82.
56. Ulukapi H, Koray F, Efes B. Monitoring the caries risk of orthodontic patients. *Quintessence Int.* 1997 Jan;28(1):27-9.
57. Andersson R, Arvidsson E, Crossner CG, Holm AK, Mansson B. The flow rate, pH and buffer effect of mixed saliva in children. *Journal of the International Association of Dentistry for Children.* 1974 Jul;5(1):5-12.
58. Forsberg CM, Oliveby A, Lagerlof F. Salivary clearance of sugar before and after insertion of fixed orthodontic appliances. *Am J Orthod Dentofacial Orthop.* 1992 Dec;102(6):527-30.
59. Jurela A, Repic D, Pejda S, Juric H, Vidakovic R, Matic I, et al. The effect of two different bracket types on the salivary levels of *S mutans* and *S sobrinus* in the early phase of orthodontic treatment. *Angle Orthod.* 2013 Jan;83(1):140-5.

60. Cheng LL, Moor SL, Kravchuk O, Meyers IA, Ho CT. Bacteria and salivary profile of adolescents with and without cleft lip and/or palate undergoing orthodontic treatment. *Aust Dent J.* 2007 Dec;52(4):315-21.
61. Chang HS, Walsh LJ, Freer TJ. The effect of orthodontic treatment on salivary flow, pH, buffer capacity, and levels of mutans streptococci and lactobacilli. *Aust Orthod J.* 1999 Apr;15(4):229-34.
62. Lara-Carrillo E, Montiel-Bastida NM, Sanchez-Perez L, Alanis-Tavira J. Effect of orthodontic treatment on saliva, plaque and the levels of *Streptococcus mutans* and *Lactobacillus*. *Med Oral Patol Oral Cir Bucal.* 2010 Nov;15(6):e924-9.
63. Ranganath LM, Shet RG, Rajesh AG. Saliva: a powerful diagnostic tool for minimal intervention dentistry. *The journal of contemporary dental practice.* 2012 Mar-Apr;13(2):240-5.
64. Guo L, Shi W. Salivary biomarkers for caries risk assessment. *Journal of the California Dental Association.* 2013 Feb;41(2):107-9, 12-8.
65. Cheaib Z, Ganss C, Lamanda A, Turgut MD, Lussi A. Comparison of three strip-type tests and two laboratory methods for salivary buffering analysis. *Odontology / the Society of the Nippon Dental University.* 2012 Jan;100(1):67-75.
66. Stookey GK. The effect of saliva on dental caries. *J Am Dent Assoc.* 2008 May;139 Suppl:11S-7S.

67. Lingstrom P, Birkhed D. Plaque pH and oral retention after consumption of starchy snack products at normal and low salivary secretion rate. *Acta Odontol Scand.* 1993 Dec;51(6):379-88.
68. Vehkalahti M, Nikula-Sarakorpi E, Paunio I. Evaluation of salivary tests and dental status in the prediction of caries increment in caries-susceptible teenagers. *Caries Res.* 1996;30(1):22-8.
69. Gopinath VK, Arzreanne AR. Saliva as a Diagnostic Tool for Assessment of Dental Caries. *Archives of Orofacial Sciences.* 2006;1:57-9.
70. Varma S, Banerjee A, Bartlett D. An in vivo investigation of associations between saliva properties, caries prevalence and potential lesion activity in an adult UK population. *J Dent.* 2008 Apr;36(4):294-9.
71. Gabris K, Nagy G, Madlena M, Denes Z, Marton S, Keszthelyi G, et al. Associations between microbiological and salivary caries activity tests and caries experience in Hungarian adolescents. *Caries Res.* 1999 May-Jun;33(3):191-5.
72. Machiulskiene V, Nyvad B, Baelum V. Caries preventive effect of sugar-substituted chewing gum. *Community Dent Oral Epidemiol.* 2001 Aug;29(4):278-88.
73. Holbrook WP, de Soet JJ, de Graaff J. Prediction of dental caries in pre-school children. *Caries Res.* 1993;27(5):424-30.

74. Leone CW, Oppenheim FG. Physical and chemical aspects of saliva as indicators of risk for dental caries in humans. *Journal of dental education*. 2001 Oct;65(10):1054-62.
75. Larmas M. Saliva and dental caries: diagnostic tests for normal dental practice. *International dental journal*. 1992 Aug;42(4):199-208.
76. Ansai T, Yamashita Y, Shibata Y, Kato Y, Sakao S, Takamatsu N, et al. Relationship between dental caries experience of a group of Japanese kindergarten children and the results of two caries activity tests conducted on their saliva and dental plaque. *International journal of paediatric dentistry / the British Paedodontic Society [and] the International Association of Dentistry for Children*. 1994 Mar;4(1):13-7.
77. Cowman RA, Baron SS, Fitzgerald RJ, Stuchell RE, Mandel ID. Comparative growth responses of oral streptococci on mixed saliva or the separate submandibular and parotid secretions from caries-active and caries-free individuals. *J Dent Res*. 1983 Sep;62(9):946-51.
78. Cowman RA, Baron SS, Fitzgerald RJ, Danziger JL, Quintana JA. Growth inhibition of oral streptococci in saliva by anionic proteins from two caries-free individuals. *Infection and immunity*. 1982 Aug;37(2):513-8.
79. Rosan B, Appelbaum B, Golub E, Malamud D, Mandel ID. Enhanced saliva-mediated bacterial aggregation and decreased bacterial adhesion in caries-resistant versus caries-susceptible individuals. *Infection and immunity*. 1982 Dec;38(3):1056-9.

80. Baughan LW, Robertello FJ, Sarrett DC, Denny PA, Denny PC. Salivary mucin as related to oral *Streptococcus mutans* in elderly people. *Oral microbiology and immunology*. 2000 Feb;15(1):10-4.
81. Bagherian A, Asadikaram G. Comparison of some salivary characteristics between children with and without early childhood caries. *Indian J Dent Res*. 2012 Sep;23(5):628-32.
82. Farsi N. Dental caries in relation to salivary factors in Saudi population groups. *The journal of contemporary dental practice*. 2008;9(3):16-23.
83. Cogulu D, Sabah E, Kutukculer N, Ozkinay F. Evaluation of the relationship between caries indices and salivary secretory IgA, salivary pH, buffering capacity and flow rate in children with Down's syndrome. *Arch Oral Biol*. 2006 Jan;51(1):23-8.
84. Johansson I, Saellstrom AK, Rajan BP, Parameswaran A. Salivary flow and dental caries in Indian children suffering from chronic malnutrition. *Caries Res*. 1992;26(1):38-43.
85. Crossner CG, Holm AK. Saliva tests in the prognosis of caries in children. *Acta Odontol Scand*. 1977;35(3):135-9.
86. Mandel ID. The functions of saliva. *J Dent Res*. 1987 Feb;66 Spec No:623-7.

87. Tenovuo J. Salivary parameters of relevance for assessing caries activity in individuals and populations. *Community Dent Oral Epidemiol.* 1997 Feb;25(1):82-6.
88. Bardow A, Moe D, Nyvad B, Nauntofte B. The buffer capacity and buffer systems of human whole saliva measured without loss of CO<sub>2</sub>. *Arch Oral Biol.* 2000 Jan;45(1):1-12.
89. Kitasako Y, Burrow MF, Stacey M, Huq L, Reynolds EC, Tagami J. Comparative analysis of three commercial saliva testing kits with a standard saliva buffering test. *Aust Dent J.* 2008 Jun;53(2):140-4.
90. GC. Saliva Check Buffer. In: LTD. SKSBBTC, editor. Hong Kong: GC corporation; 2001.
91. Walsh LJ. Saliva testing: Good Practice, Good Sense. GC Asia; 2002.
92. Sanpei S, Endo T, Shimooka S. Caries risk factors in children under treatment with sectional brackets. *Angle Orthod.* 2010 May;80(3):509-14.
93. Gerardu V, Heijnsbroek M, Buijs M, van der Weijden F, Ten Cate B, van Loveren C. Comparison of Clinpro Cario L-Pop estimates with CIA lactic acid estimates of the oral microflora. *Eur J Oral Sci.* 2006 Apr;114(2):128-32.
94. Schiffner U, Torres-Quintero A. Reproducibility of a new caries risk test under different oral conditions. *Clin Oral Investig.* 2005 Sep;9(3):187-91.

95. Chaussain C, Opsahl Vital S, Viallon V, Vermelin L, Haignere C, Sixou M, et al. Interest in a new test for caries risk in adolescents undergoing orthodontic treatment. *Clin Oral Investig*. 2010 Apr;14(2):177-85.
96. Sudjalim TR, Woods MG, Manton DJ. Prevention of white spot lesions in orthodontic practice: a contemporary review. *Aust Dent J*. 2006 Dec;51(4):284-9; quiz 347.
97. Derks A, Katsaros C, Frencken JE, van't Hof MA, Kuijpers-Jagtman AM. Caries-inhibiting effect of preventive measures during orthodontic treatment with fixed appliances. A systematic review. *Caries Res*. 2004 Sep-Oct;38(5):413-20.
98. Noble J, Cassolato S, Karaikos N, Wiltshire WA. Point of care. Preventive and interceptive measures for improving and maintaining good oral hygiene and cariogenic control in orthodontic patients. *J Can Dent Assoc*. 2009 Jul;75(6):441-3.
99. Costa MR, Silva VC, Miqui MN, Sakima T, Spolidorio DM, Cirelli JA. Efficacy of ultrasonic, electric and manual toothbrushes in patients with fixed orthodontic appliances. *Angle Orthod*. 2007 Mar;77(2):361-6.
100. Benson PE, Parkin N, Millett DT, Dyer FE, Vine S, Shah A. Fluorides for the prevention of white spots on teeth during fixed brace treatment. *Cochrane Database Syst Rev*. 2004 (3):CD003809.



101. Marinho VC. Cochrane reviews of randomized trials of fluoride therapies for preventing dental caries. *Eur Arch Paediatr Dent*. 2009 Sep;10(3):183-91.
102. Chadwick BL, Roy J, Knox J, Treasure ET. The effect of topical fluorides on decalcification in patients with fixed orthodontic appliances: a systematic review. *Am J Orthod Dentofacial Orthop*. 2005 Nov;128(5):601-6; quiz 70.
103. Ogaard B. CaF<sub>2</sub> formation: cariostatic properties and factors of enhancing the effect. *Caries Res*. 2001;35 Suppl 1:40-4.
104. Ogaard B, Alm AA, Larsson E, Adolfsson U. A prospective, randomized clinical study on the effects of an amine fluoride/stannous fluoride toothpaste/mouthrinse on plaque, gingivitis and initial caries lesion development in orthodontic patients. *Eur J Orthod*. 2006 Feb;28(1):8-12.
105. Petersson LG. Fluoride mouthrinses and fluoride varnishes. *Caries Res*. 1993;27 Suppl 1:35-42.
106. Arends J, Lodding A, Petersson LG. Fluoride uptake in enamel. In vitro comparison of topical agents. *Caries Res*. 1980;14(6):403-13.
107. Demito CF, Vivaldi-Rodrigues G, Ramos AL, Bowman SJ. The efficacy of a fluoride varnish in reducing enamel demineralization adjacent to orthodontic brackets: an in vitro study. *Orthod Craniofac Res*. 2004 Nov;7(4):205-10.

108. Gontijo L, Cruz Rde A, Brandao PR. Dental enamel around fixed orthodontic appliances after fluoride varnish application. *Braz Dent J.* 2007;18(1):49-53.
109. Du M, Cheng N, Tai B, Jiang H, Li J, Bian Z. Randomized controlled trial on fluoride varnish application for treatment of white spot lesion after fixed orthodontic treatment. *Clin Oral Investig.* 2012 Apr;16(2):463-8.
110. Graf I, Jacobi BE. Bond strength of various fluoride-releasing orthodontic bonding systems. Experimental study. *J Orofac Orthop.* 2000;61(3):191-8.
111. Mandall NA, Millett DT, Mattick CR, Hickman J, Worthington HV, Macfarlane TV. Orthodontic adhesives: a systematic review. *J Orthod.* 2002 Sep;29(3):205-10; discussion 195.
112. Marcusson A, Norevall LI, Persson M. White spot reduction when using glass ionomer cement for bonding in orthodontics: a longitudinal and comparative study. *Eur J Orthod.* 1997 Jun;19(3):233-42.
113. Wiltshire WA. Determination of fluoride from fluoride-releasing elastomeric ligature ties. *Am J Orthod Dentofacial Orthop.* 1996 Oct;110(4):383-7.
114. Storie DJ, Regennitter F, von Fraunhofer JA. Characteristics of a fluoride-releasing elastomeric chain. *Angle Orthod.* 1994;64(3):199-209; discussion 10.

115. Wiltshire WA. Fluoride release from ligature ties. *Am J Orthod Dentofacial Orthop.* 1997 Aug;112(2):17A.
116. Wiltshire WA. In vitro and in vivo fluoride release from orthodontic elastomeric ligature ties. *Am J Orthod Dentofacial Orthop.* 1999 Mar;115(3):288-92.
117. Bailey DL, Adams GG, Tsao CE, Hyslop A, Escobar K, Manton DJ, et al. Regression of post-orthodontic lesions by a remineralizing cream. *J Dent Res.* 2009 Dec;88(12):1148-53.
118. Reynolds EC, Cai F, Cochrane NJ, Shen P, Walker GD, Morgan MV, et al. Fluoride and casein phosphopeptide-amorphous calcium phosphate. *J Dent Res.* 2008 Apr;87(4):344-8.
119. Jayarajan J, Janardhanam P, Jayakumar P. Efficacy of CPP-ACP and CPP-ACPF on enamel remineralization - an in vitro study using scanning electron microscope and DIAGNOdent. *Indian J Dent Res.* 2011 Jan-Feb;22(1):77-82.
120. Marchisio O, Esposito MR, Genovesi A. Salivary pH level and bacterial plaque evaluation in orthodontic patients treated with Recaldent products. *Int J Dent Hyg.* 2010 Aug;8(3):232-6.
121. Sudjalim TR, Woods MG, Manton DJ, Reynolds EC. Prevention of demineralization around orthodontic brackets in vitro. *Am J Orthod Dentofacial Orthop.* 2007 Jun;131(6):705 e1-9.

122. Uysal T, Amasyali M, Ozcan S, Koyuturk AE, Akyol M, Sagdic D. In vivo effects of amorphous calcium phosphate-containing orthodontic composite on enamel demineralization around orthodontic brackets. *Aust Dent J.* 2010 Sep;55(3):285-91.
123. Foster JA, Berzins DW, Bradley TG. Bond strength of an amorphous calcium phosphate-containing orthodontic adhesive. *Angle Orthod.* 2008 Mar;78(2):339-44.
124. Dunn WJ. Shear bond strength of an amorphous calcium-phosphate-containing orthodontic resin cement. *Am J Orthod Dentofacial Orthop.* 2007 Feb;131(2):243-7.
125. Uysal T, Baysal A, Uysal B, Aydinbelge M, Al-Qunaian T. Do fluoride and casein phosphopeptide-amorphous calcium phosphate affect shear bond strength of orthodontic brackets bonded to a demineralized enamel surface? *Angle Orthod.* 2011 May;81(3):490-5.
126. Uysal T, Amasyali M, Koyuturk AE, Ozcan S. Effects of different topical agents on enamel demineralization around orthodontic brackets: an in vivo and in vitro study. *Aust Dent J.* 2010 Sep;55(3):268-74.
127. Behnan SM, Arruda AO, Gonzalez-Cabezas C, Sohn W, Peters MC. In-vitro evaluation of various treatments to prevent demineralization next to orthodontic brackets. *Am J Orthod Dentofacial Orthop.* 2010 Dec;138(6):712 e1-7; discussion -3.

128. Knosel M, Forslund L, Jung K, Ziebolz D. Efficacy of different strategies in protecting enamel against demineralization during fixed orthodontic treatment. *J Orofac Orthop.* 2012 May;73(3):194-203.
129. Leizer C, Weinstein M, Borislow AJ, Braitman LE. Efficacy of a filled-resin sealant in preventing decalcification during orthodontic treatment. *Am J Orthod Dentofacial Orthop.* 2010 Jun;137(6):796-800.
130. Knosel M, Bojes M, Jung K, Ziebolz D. Increased susceptibility for white spot lesions by surplus orthodontic etching exceeding bracket base area. *Am J Orthod Dentofacial Orthop.* 2012 May;141(5):574-82.
131. Uysal T, Amasyali M, Ozcan S, Koyuturk AE, Sagdic D. Effect of antibacterial monomer-containing adhesive on enamel demineralization around orthodontic brackets: an in-vivo study. *Am J Orthod Dentofacial Orthop.* 2011 May;139(5):650-6.
132. Mayne RJ, Cochrane NJ, Cai F, Woods MG, Reynolds EC. In-vitro study of the effect of casein phosphopeptide amorphous calcium fluoride phosphate on iatrogenic damage to enamel during orthodontic adhesive removal. *Am J Orthod Dentofacial Orthop.* 2011 Jun;139(6):e543-51.
133. Cochrane NJ, Ratneser S, Reynolds EC. Effect of different orthodontic adhesive removal techniques on sound, demineralized and remineralized enamel. *Aust Dent J.* 2012 Sep;57(3):365-72.

134. Reynolds EC, Cai F, Shen P, Walker GD. Retention in plaque and remineralization of enamel lesions by various forms of calcium in a mouthrinse or sugar-free chewing gum. *J Dent Res.* 2003 Mar;82(3):206-11.
135. Iijima Y, Cai F, Shen P, Walker G, Reynolds C, Reynolds EC. Acid resistance of enamel subsurface lesions remineralized by a sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. *Caries Res.* 2004 Nov-Dec;38(6):551-6.
136. Brochner A, Christensen C, Kristensen B, Tranaeus S, Karlsson L, Sonnesen L, et al. Treatment of post-orthodontic white spot lesions with casein phosphopeptide-stabilised amorphous calcium phosphate. *Clin Oral Investig.* 2011 Jun;15(3):369-73.
137. Caglaroglu M, Gelgor IE. Microabrasion technique for treatment of demineralization after debonding. *J Clin Orthod.* 2012 Dec;46(12):734-7.
138. Ardu S, Castioni NV, Benbachir N, Krejci I. Minimally invasive treatment of white spot enamel lesions. *Quintessence Int.* 2007 Sep;38(8):633-6.
139. Al-Khateeb S, Exterkate R, Angmar-Mansson B, ten Cate JM. Effect of acid-etching on remineralization of enamel white spot lesions. *Acta Odontol Scand.* 2000 Feb;58(1):31-6.
140. Paris S, Meyer-Lueckel H. Masking of labial enamel white spot lesions by resin infiltration--a clinical report. *Quintessence Int.* 2009 Oct;40(9):713-8.

## 5. Statement of Purpose

Numerous studies have investigated the risk factors associated with the incidence of WSLs in the orthodontic population. Few studies have investigated risk factors associated with different degrees of severity of WSLs. In such studies, severity has been defined as the number of lesions present in an individual or the extent of surface area of the tooth covered by a WSL. Severity described by the amount of demineralisation of the WSL has gone largely unreported due to difficulties in clinical grading. This can now be overcome with the development of the ICDAS II index.

This study will examine patient related factors and their association with the presence, severity and number of white spot lesions. The grading of WSLs will be done with the accepted standard clinical method of diagnosis, the ICDAS II method. The other component of this section compares the use of a hand held laser device, the DIAGNOdent pen (KaVo, Biberach, Germany), to the ICDAS II method.

The use of salivary properties to predict the development of dental caries has been studied extensively with many conflicting results. A saliva test in orthodontic patients to assess the risk of developing WSLs would be a useful clinical indicator. The second part of this study evaluates the relationship between saliva parameters tested with the Saliva-Check Buffer Kit (GC Corp., Belgium) and WSLs in orthodontic patients. The

saliva parameters that are to be examined include hydration, saliva consistency, resting saliva pH, stimulated saliva quantity, stimulated saliva pH and buffering capacity. These parameters will be compared to the presence, severity and number of WSLs in orthodontic patients.



# 6. Aims and Hypothesis

## AIMS

Paper 1, written in the style of submission to the Angle Orthodontist

addresses the following aims:

- To investigate patient characteristics which are associated with the presence of WSLs.
- To investigate patient characteristics which are associated with the increase in number of WSLs.
- To investigate patient characteristics which are associated with an increased severity of WSLs.
- To evaluate the use of the DIAGNOdent Pen (KaVo, Biberach, Germany) as an aid in the identification of WSLs in orthodontic patients.

Paper 2, written in the style of submission to the Angle Orthodontist

addresses the following aims:

- To investigate the associations between the presence of white spot lesions (WSLs) and the saliva properties tested with the Saliva-Check Buffer Kit (GC Corp., Belgium).
- To investigate the associations between an increase in number of WSLs and the saliva properties tested with the Saliva-Check Buffer Kit.

- To investigate the associations between the severity of WSLs and the saliva properties tested with the Saliva-Check Buffer Kit.

## **NULL HYPOTHESES**

### Paper 1:

- The patient characteristics examined cannot be associated with the presence of WSLs in orthodontic patients
- The patient characteristics examined cannot be associated with the number of WSLs an orthodontic patient has.
- The patient characteristics examined cannot be associated with the severity of WSLs an orthodontic patient has.
- The DIAGNOdent pen is not suitable to examine WSLs in orthodontic patients compared to the ICDAS II system.

### Paper 2:

- The Saliva-Check Buffer Kit cannot differentiate between orthodontic patients with WSLs and those without.
- The Saliva-Check Buffer Kit cannot differentiate between orthodontic patients with a high number of WSLs and those with a few WSLs.
- The Saliva-Check BufferKit cannot differentiate between orthodontic patients who have severe WSLs compared to those with mild WSLs.

**THE DIAGNODENT PEN AND PATIENT  
FACTORS TO DIAGNOSE WHITE SPOTS IN  
ORTHODONTIC PATIENTS**



THE UNIVERSITY  
*of* ADELAIDE

**Article 1**

Written in the format for submission to:

The Angle Orthodontist

**Dr Balya SRIRAM**

Orthodontic Unit

School of Dentistry

Faculty of Health Science

The University of Adelaide

June 2013

# 8. Article 1

## ABSTRACT

**Objectives:** To investigate possible associations between patient-related risk factors and the presence, severity and number of white spot lesions (WSLs) experienced during orthodontic treatment. A secondary aim was to evaluate the use of the DIAGNOdent Pen (KaVo, Biberach, Germany) as an aid in the identification of WSLs in orthodontic patients.

**Materials and Methods:** Following ethics approval, 91 orthodontic patients were recruited to this study. De-identified parameters were recorded and included: date of birth, sex, postcode (to obtain a SEIFA score to indicate socio-economic status), age at banding, failure to attend appointments (FTA) rate, type of bracket used, reported oral hygiene regimen and the number of filled molars. All participants were examined for WSLs on their upper and lower anterior teeth using a visual index outlined by the International Caries Detection and Assessment System (ICDAS II) and the DIAGNOdent pen (KaVo, Biberach, Germany). Patient variable data were assessed by univariate logistic, ordinal logistic and binomial regression models using odds ratios, ratio of means and 95% confidence limits with a significance set at  $p < 0.05$ . Comparison between ICDAS II and DIAGNOdent pen was performed utilising a linear mixed effects model.

**Results:** Patients who brushed fewer than 14 times per week and those who had restored molars during treatment were more likely to present with WSLs ( $p < 0.0001$  and  $p = 0.0283$  respectively). These variables were also significant in diagnosing WSLs with a grading of ICDAS II  $\geq 2$  ( $p < 0.0001$  and  $p = 0.0374$ ). When WSLs were ICDAS II  $\geq 3$  severity, the FTA rate ( $p = 0.0063$ ) and brushing less than 14 times per week ( $p = 0.0129$ ) were significant positive associations. The FTA rate and brushing fewer than 14 times per week also provided an indication of the total number of WSLs experienced ( $p = 0.0088$  and  $p < 0.0001$ ). Comparisons between the ICDAS II scores and the DIAGNOdent scores were statistically significant ( $p < 0.0001$ ).

**Conclusions:** The reported number of brushings per week was seen as a significant indicator of the presence, severity and number of white spot lesions. The number of restored molars may indicate the presence and the severity of white spot lesions during orthodontic treatment. Patients who fail to attend appointments are more likely to have a greater number and severity of WSLs. All comparisons between the DIAGNOdent pen and ICDAS II categories were statistically significant.

## INTRODUCTION

Early dental caries on the enamel surface of teeth are commonly referred to as white spot lesions (WSLs). The term WSL is commonly accepted

because the dissolution of the enamel's crystalline structure creates an alteration in the refractive index which produces an opaque chalky white appearance under light(1, 2). WSLs are a common complication of orthodontic treatment and are of concern because of their appearance and their possible progression to a frank cavitation particularly on the labial surface of anterior teeth.

No clear evidence for sexual predisposition to WSLs exists as most studies indicate that males and females are equally at risk (3-5).

However, there are additional reports which suggest that males develop more WSLs than females (6-8). No significant correlation has been found between age and incidence of WSLs (4, 7) though there are reports that younger patients develop WSLs more than older patients (3, 8).

Patients with poor oral hygiene before and during treatment are known to be at greater risk for developing WSLs (2-4, 8-11). Children who are at increased risk of demineralisation without appliances have a much greater chance of developing further demineralisation when appliances are placed (10-12). The type of appliance placed does not appear to affect the incidence of WSLs (3, 13, 14). Other factors which have an unrelated correlation with the disease include attendance compliance and the socioeconomic status of the patient (3, 7).

The incidence of WSLs varies greatly and ranges from 2% to 97% (2, 4, 7, 9, 15-18). This large variation is likely explained by the varying methods of diagnosing and grading WSLs (8). The most commonly used method of WSL detection is via visual clinical examination. This includes the DMFT index (decayed, missing, filled teeth) and DMFS index (decayed, missing, filled surfaces). The disadvantage of these indices is that missing or filled teeth are not necessarily due to caries, especially in orthodontic patients, and may lead to an overestimation (19). In addition, WSLs are the early signs of caries and the DMFT and DMFS would not allow sufficient categorisation of the lesions. Gorelick et al. have described a numerical WSL grading index from 0-3 (9). This provides an indication on severity and presence but does not grade the severity sufficiently or provide information regarding the location of the WSL on the tooth. The International Caries Detection and Assessment System (ICDAS) describes a widely recognised visual index for caries detection (20). The original criteria, ICDAS I, was developed in 2003 and later modified to the ICDAS II in 2007. The improvement has involved changing the codes so that the index appropriately and reliably indicates the increasing severity of lesions (21, 22). The advantage of this index over conventional methods such as DMFT and DMFS is its ability to further categorise early enamel demineralisation (21, 22).

The DIAGNOdent pen (KaVo, Biberach, Germany) uses laser fluorescence (LF) as a quantitative method of caries detection (23).

Fluorescence is the emission of light due to the movement of molecules in response to the absorption of high-energy light (24). Natural fluorescence occurs in every tooth due to the proteins comprising enamel and dentine. Additionally, bacterial metabolites in caries, assumed to be porphyrins, emit fluorescence measurable by LF (25). The fluorescent emissions from teeth are regulated via the DIAGNOdent pen to indicate the level of demineralisation on a tooth surface ranging from a scale of 1 to 99 (24). The detection of white spot lesions using a quantitative LF method is much more sensitive than direct visualisation (7). There is evidence that the use of LF devices might be a useful clinical adjunct in the diagnosis and grading of WSLs in orthodontic patients (26-28).

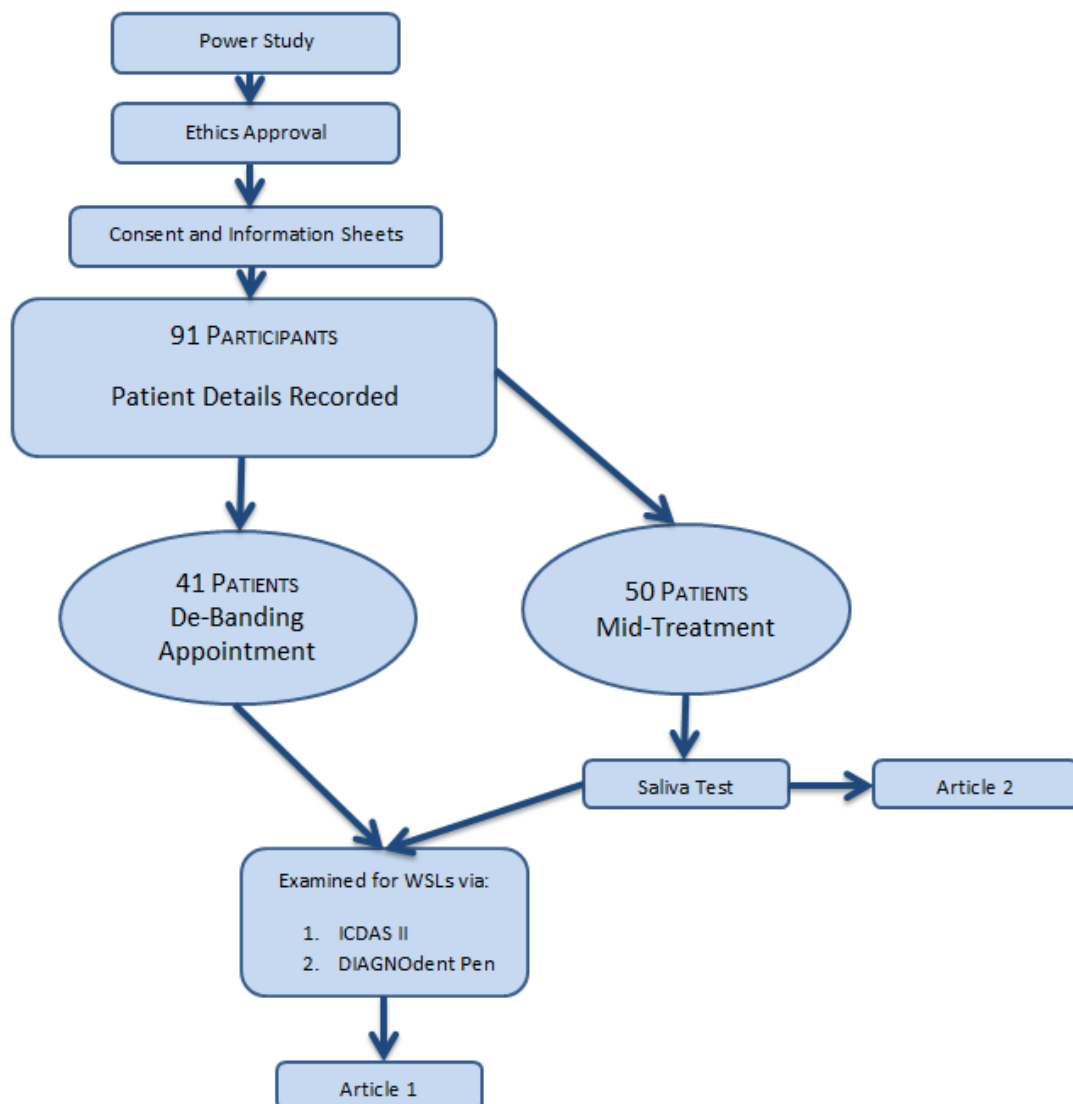
The main aim of the present study was to identify associations between patient related factors and WSLs in orthodontic patients. A determination of the presence, in addition to, the severity and the total number of lesions was to be examined via the ICDAS II. A further aim was to validate the use of the DIAGNOdent pen compared with the ICDAS II in the identification of WSLs in orthodontic patients.

## **MATERIALS AND METHODS**

A power study was conducted to determine an adequate sample size after which ethics approval of the main study was granted through the South Australian Dental Service and the University of Adelaide.



Patients were invited to take part in the study from an orthodontic patient pool treated at the Adelaide Dental Hospital. People were recruited as seen at random. A total of 91 patients fulfilled selection criteria (see below) and agreed to participate. Of the 91 patients, 41 were seen only at their debanding appointment. The remaining 50 patients were also participants in a saliva test study (Article 2) and were mid-way through orthodontic treatment (see Figure 1).



**Figure 1: Summary of Methodology**

Written consent was obtained from all patients or their parent/legal guardian in the case of minors and information sheets were provided. Inclusion criteria required (i) no missing anterior teeth (ii) full fixed appliance treatment for a minimum of six months. Exclusion criteria involved (i) antibiotic use within one month (ii) fluoride treatment, professional dental prophylaxis or CPP-ACP (casein phosphopeptide amorphous calcium phosphate) use within the previous two weeks (iii) those who received antibiotic prophylaxis prior to the appointment (iv) those on medications which affect salivary flow or function (e.g. antidepressants, diuretics, antihistamines, narcotics, and  $\beta$ -adrenoreceptor agonists) (v) smokers (vi) patients who had consumed food or drink within an hour of the test (vii) those who brushed their teeth or used a mouthwash within an hour of the test.

De-identified patient details were recorded by a single examiner and included:

- a) Current age
- b) Age at which orthodontic appliances were placed
- c) The postcode of their residential address was recorded and used to determine their Socio-Economic Indices For Area (SEIFA) score. The SEIFA is an index developed by the Australian Bureau of Statistics to determine the social and economic well-being of an area. The data used

in this study were derived from the 2006 Census of Population and Housing and used the SEIFA Index of Relative Socio-Economic Disadvantage.

d) Gender (female or male)

e) Failure to Attend (FTA) percentage rate. This was calculated by dividing the number of missed appointments by the total number of attended appointments multiplied by one hundred. Appointments that were cancelled were not included.

f) Bracket type. All patients in the study were either bracketed with Tip-Edge Plus™ (TP Orthodontics Inc. 0.022x 0.028-in slot) or Victory Series™ (3M Unitek 0.022x 0.028-in slot).

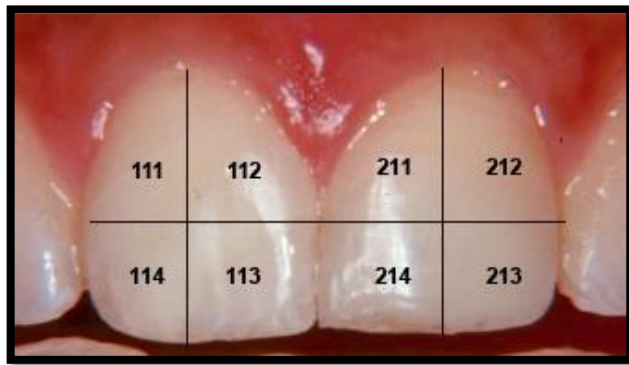
g) Number of filled molars. This was calculated on all erupted molars present at the time of the study. Direct clinical evaluation was used. If there was uncertainty regarding whether a restoration or preventive sealant was present, radiographs and the patient's dental history record was evaluated.

h) Reported oral hygiene regimen. Patients were questioned regarding the number of times a week they brushed, and whether a mouthwash and dental floss were regularly used. The examiner was not the patient's treatment provider and all patients were reassured that their response would remain confidential. If they provided a daily response in terms of brushing such as "twice daily" they were then asked if their routine

changed during the weekend, holidays or on certain days of the week so that they could reconsider and then estimate the total number of brushings during a typical week. None of the participants in the study reported flossing their teeth.

### *Recording WSLs*

Following data collection, the 50 patients who were mid-way through orthodontic treatment underwent a saliva test performed by the examiner (refer to Article 2 -“Saliva Tests to Diagnose White Spot Lesions During Orthodontic Treatment”) after which their WSLs were assessed. The remaining 41 patients who were at their debanding appointment had their appliances removed by their treating operator before returning to the examiner for the assessment of WSLs. For all the participants, a single examiner recorded the WSLs. The teeth were lightly pumiced and anterior teeth (canine to canine) in the upper and lower arches were examined. The 12 anterior teeth were subdivided into 4 “mini quadrants” by the construction of two lines drawn through the centre of the tooth crown following the long axis and the central perpendicular which divided the tooth into four “mini-quadrants” (Figure 2). The first two numbers of the “mini-quadrant” are the FDI notation for that tooth. The third number changed in a clockwise direction from 1 through to 4 starting at the top right quarter of the tooth (beginning from the patient’s right). This system generated 48 sites from the 12 teeth per patient.



***Figure 2: Central Incisors Divided Into “Mini-Quadrants”***

Each of the 48 sites were then assigned an ICDAS II score by the same examiner who used loupes and the aid of a mirror, clinical lighting, a triplex syringe and a blunt probe. The ICDAS II scores were voice recorded so that once a score was assigned, the examiner was not able to refer back to previous scores. The ICDAS II caries scoring system is shown in Table 1.

**Table 1: The ICDAS II Caries Scoring System(29)**

CODE	CRITERION
0	Sound tooth surface: No evidence of caries after 5 seconds of air drying
1	First visual change in enamel. Opacity of discolouration is visible after prolonged air drying. Cannot be seen when wet
2	Distinct visual change in enamel visible when wet. Lesion must be visible when dry
3	Localised enamel breakdown (without clinical visual signs of dentinal involvement). Seen when wet and after prolonged drying
4	Underlying dark shadow from dentine
5	Distinct cavity with visible dentine
6	Extensive (more than half the surface). Distinct cavity with visible dentine

Immediately after the visual examination, the examiner used a DIAGNOdent pen to record mineralisation levels on all 48 sites in each patient. Before each patient, the instrument was calibrated on a ceramic reference disc supplied by the manufacturer and then subsequently calibrated on sound palatal enamel surfaces of central incisors. The peak value for each “mini-quadrant” was recorded. The same DIAGNOdent

pen and the same design of light probe were used on all patients throughout the study. A total of 4368 sites in 91 patients were examined and each generated an ICDAS II score as well as a DIAGNOdent pen reading.

### *Statistical Method*

Associations between patient-related risk factors and the presence and severity of white spots were assessed using logistic regression models, with relationships described using odds ratios and 95% confidence intervals. The most severe WSL was defined as the highest ICDAS II score across all “mini quadrants” in an individual. For analysis purposes patients were dichotomised at scores of 2 and 3 into non-severe and severe groups. Associations between patient factors and the total number of white spots were assessed using negative binomial regression models, with effects described using ratios of means and 95% confidence intervals. The total number of WSLs was defined as the number of “mini-quadrants” with an ICDAS II score of one or higher in each individual. A linear mixed effects model was used to compare mean DIAGNOdent pen scores in regions scored as 1, 2 and 3 with ICDAS II. In the model, patient was included as a random effect to account for the dependence due to repeated DIAGNOdent pen scores within patients. Statistical analysis was performed by an independent statistician using SAS Version 9.3 (SAS institute Inc., Cary, NC, USA). The level of statistical significance was set at  $p < 0.05$  for all statistical tests.

## RESULTS

There were 54 females (59.34%) and 37 males (40.66%) who took part in the study. Of these, 57 participants (62.64%) were banded using Tip-Edge Plus™ (TP Orthodontics Inc. 0.022x 0.028-in slot) appliances and 34 participants (37.36%) were banded with Victory Series™ brackets (3M Unitek 0.022x 0.028-in slot). Sixty-seven participants (73.63%) used a mouthwash regularly while the remaining 24 did not (26.37%). The mean age at appliance placement was 15.66yrs (range 12-20yrs) while the mean age of participants was 17.52yrs (range 12-22yrs). The average FTA rate was 10.3% of appointments (range 0-52%). The number of filled molars was an average of 0.66 (range 0-4). The participants brushed an average of 11.69 times per week (range 1-25 times). A total of 49 patients had observable WSLs (53.85%) while 42 did not (46.15%). A total of 398 “mini quadrants” with WSLs were observed in these 49 patients. The most common type of WSL and most common greatest severity in an individual was an ICDAS II grade 2 lesion (Table 2 and Table 3). Table 2 considers the frequency of WSL by the total number of “mini-quadrants” in the study. Table 3 considers only the most severe WSL in each patient. Tooth 12 “mini-quadrant” 2 identified as the most likely to have a WSL (25.27% of the time). In all 91 patients, tooth 32 “mini-quadrant” 1 did not have a WSL and was the only examined area always free of WSLs. Tables 4 to 8 are presented up to 4 decimal places to accurately reflect the p-value of the variables tested.



**Table 2: Type of WSLs**

TYPE OF WSL	FREQUENCY	PERCENT	CUMULATIVE	CUMULATIVE
			FREQUENCY	PERCENT
1	121	30.40	121	30.40
2	243	61.06	364	91.46
3	32	8.04	396	99.5
4	2	0.5	398	100

**Table 3: Severity of WSLs**

SEVERITY OF WHITE SPOTS	FREQUENCY	PERCENT	CUMULATIVE	CUMULATIVE
			FREQUENCY	PERCENT
0	42	46.15	42	46.15
1	8	8.79	50	54.95
2	27	29.67	77	84.62
3	12	13.19	89	97.80
4	2	2.20	91	100.00

***Patient Factors Compared to the Presence of WSLs***

The presence of WSLs was defined as a patient who had a “mini quadrant” with an ICDAS II score of 1 or higher. This was used as a baseline to assess whether patient factors could determine if WSLs were present regardless of number or severity. Tooth brushing per week was

set at a threshold of 14 times as this was the minimum number advised for orthodontic patients at the Adelaide Dental Hospital. The presence of filled molars ( $p=0.0283$ ) and tooth brushing less than 14 times per week ( $p<0.0001$ ) had significant associations with the presence of WSLs (Table 4). The odds of white spots being present were 83.2 times higher in those patients who brushed fewer than 14 times per week compared with those who brushed 14 or more times per week.

**Table 4: The Relationship between Patient Factors and the Presence of WSLs**

PATIENT FACTOR	ODDS RATIO	LOWER 95% CONFIDENCE LIMIT	UPPER 95% CONFIDENCE LIMIT	P-VALUE
AGE AT TEST	1.0388	0.8512	1.2677	0.7081
AGE AT BANDS ON	1.0086	0.8108	1.2547	0.9385
SEIFA	1.0042	0.9991	1.0094	0.1033
SEX: F vs. M	0.8205	0.3537	1.9033	0.6449
FTA %	1.0219	0.9880	1.0569	0.2080
BRACKET: TIP-EDGE vs. VICTORY	1.0598	0.4523	2.4835	0.8936
NUMBER OF FILLED MOLARS ABOVE 0	1.6629	1.0555	2.6197	*0.0283
BRUSHING PER WEEK: <14 vs. ≥14	83.2498	20.8423	332.5226	***<0.0001
MOUTHWASH: Y vs. N	1.5486	0.6066	3.9531	0.3604

### *Patient Factors Compared to the Severity of WSLs*

Table 5 shows the relationship between the severity of WSLs with an ICDAS II score  $\geq 2$  and the patient factors examined. The presence of filled molars ( $p=0.0374$ ) and brushing fewer than 14 times per week ( $p<0.0001$ ) was effective in predicting patients with an ICDAS II score  $\geq 2$ . Table 6 shows the relationship between the severity of WSLs with an ICDAS II score  $\geq 3$  and the patient factors examined. The FTA percentage ( $p=0.0063$ ) and brushing fewer than 14 times per week ( $p=0.0129$ ) was effective in predicting patients with an ICDAS II score  $\geq 3$ .

**Table 5: The Relationship Between Patient Factors and the Severity of WSLs  $\geq$  ICDAS II Grade 2**

ICDAS II SCORE	PATIENT FACTOR	ODDS RATIO	LOWER 95% CONFIDENCE LIMIT	UPPER 95% CONFIDENCE LIMIT	P-VALUE
SEVERITY $\geq$ 2	Age at test	0.9982	0.8178	1.2183	0.9857
SEVERITY $\geq$ 2	Age at bands on	0.9509	0.7637	1.1841	0.6530
SEVERITY $\geq$ 2	SEIFA	1.0032	0.9981	1.0083	0.2160
SEVERITY $\geq$ 2	Sex: F vs. M	0.7832	0.3379	1.8151	0.5687
SEVERITY $\geq$ 2	FTA %	1.0237	0.9906	1.0579	0.1618
SEVERITY $\geq$ 2	Bracket: Tip-Edge vs. Victory	1.2857	0.5450	3.0334	0.5661
SEVERITY $\geq$ 2	Number of filled molars above 0	1.5540	1.0261	2.3536	*0.0374
SEVERITY $\geq$ 2	Brushing per week: <14 vs. $\geq$ 14	40.1111	10.4748	153.5972	***<0.0001
SEVERITY $\geq$ 2	Mouthwash: Y vs. N	2.5022	0.9186	6.8157	0.0728

**Table 6: The Relationship Between Patient Factors and the Severity of WSLs  $\geq$  ICDAS II Grade 3**

ICDAS II SCORE	PATIENT FACTOR	ODDS RATIO	LOWER 95% CONFIDENCE LIMIT	UPPER 95% CONFIDENCE LIMIT	P-VALUE
SEVERITY $\geq$ 3	Age at test	1.0356	0.7852	1.3660	0.8042
SEVERITY $\geq$ 3	Age at bands on	0.7779	0.5659	1.0691	0.1216
SEVERITY $\geq$ 3	SEIFA	0.9973	0.9907	1.0039	0.4173
SEVERITY $\geq$ 3	Sex: F vs. M	0.6383	0.2034	2.0026	0.4416
SEVERITY $\geq$ 3	FTA %	1.0566	1.0157	1.0991	**0.0063
SEVERITY $\geq$ 3	Bracket: Tip-Edge vs. Victory	1.0875	0.3320	3.5620	0.8898
SEVERITY $\geq$ 3	Number of filled molars above 0	1.3559	0.8470	2.1707	0.2047
SEVERITY $\geq$ 3	Brushing per week: <14 vs. $\geq$ 14	14.0541	1.7514	112.7771	*0.0129
SEVERITY $\geq$ 3	Mouthwash: Y vs. N	0.8772	0.2472	3.1126	0.8393

***Patient Factors Compared to the Total Number of WSLs Experienced***

The FTA percentage ( $p=0.0088$ ) and brushing fewer than 14 times per week ( $p<0.0001$ ) were significant factors in predicting patients with a large number of WSLs (Table 7). The mean number of white spots was

31.82 times higher in those who brushed fewer than 14 times per week compared with those who brushed 14 or more times per week.

**Table 7: The Relationship Between Patient Factors and the Number of WSLs**

PATIENT FACTOR	RATIO OF MEANS	LOWER 95% CONFIDENCE LIMIT	UPPER 95% CONFIDENCE LIMIT	P-VALUE
AGE AT TEST	1.0623	0.8832	1.2775	0.5213
AGE AT BANDS ON	0.9475	0.7923	1.1332	0.5549
SEIFA	1.0038	0.9985	1.0091	0.1614
SEX: F vs. M	1.0629	0.4765	2.3710	0.8815
FAIL TO ATTEND %	1.0351	1.0087	1.0621	**0.0088
BRACKET: TIP-EDGE vs. VICTORY	1.6222	0.7210	3.6499	0.2423
NUMBER OF FILLED MOLARS ABOVE 0	1.3043	0.9047	1.8806	0.1546
BRUSHING PER WEEK: <14 vs. ≥14	31.8160	14.6289	69.1958	***<.0001
Mouthwash: Y vs. N	0.9379	0.3839	2.2910	0.8880

### *ICDAS II Compared to the DIAGNOdent Pen*

There was a statistically significant difference ( $p < 0.0001$ ) in the mean DIAGNOdent pen score between regions scored as 1, 2 and 3 with ICDAS II. As Table 8 shows, the mean DIAGNOdent pen score increased with increasing levels of ICDAS II scores. All pairwise comparisons between ICDAS II scores (1 vs. 2, 1 vs. 3 and 2 vs. 3) were statistically significant ( $p < 0.0001$ ).

**Table 8: Least Squares Means**

ICDAS II SCORE	MEAN DIAGNODENT PEN SCORE	LOWER 95% CONFIDENCE LIMIT	UPPER 95% CONFIDENCE LIMIT
1	11.7498	10.5238	12.9758
2	22.1439	21.0453	23.2426
3	35.8455	33.7880	37.9030

## **DISCUSSION**

The prevalence of WSLs in the present study was 53.85%. Using visual examination, Gorelick et al. found an incidence rate of 50% (9). Tooth 12 “mini quadrant” 2 was the site most likely to have a WSL (25.27% of the time). Other studies have also noted that the frequency of WSLs is greatest on the maxillary lateral incisors (2, 8-10). Tooth 12 “mini-

quadrant”2 is the quarter section of the tooth located on the right hand side lateral incisor, between the bracket and the gingival margin on the mesial side. Others studies have also found that lesions usually occur adjacent to the gingival margins and close to the brackets (2, 9, 16). Access to the flow of saliva and a reduced distance from the bracket to the free gingival margin, which impedes tooth brushing, are identified reasons cited for discrepancies in the incidence of WSLs between teeth (9). This may explain why a smaller tooth such as a maxillary lateral incisor, has a markedly higher incidence of demineralisation compared with a maxillary central incisor (9). This may also provide a reason why tooth 32 “mini-quadrant” 1 did not have any WSLs in the entire sample. This tooth, the lower left lateral incisor and “mini-quadrant” 1, is the quarter section located between the bracket and the incisal edge on the mesial side. At this site there is unobstructed access to tooth brushing and free flowing saliva.

The most common type of WSL and most common greatest severity found was an ICDAS II grade 2 lesion. No previous studies have provided data on the most common type or grading of WSLs on orthodontic patients using the ICDAS II.

The average age of participants in the study was 17.52yrs (range from 12-22yrs). The average age at which full fixed orthodontic appliances was placed was 15.66yrs (range from 12-20yrs). No relationship was found between age and the incidence, severity or the presence of WSLs.



This finding agreed with Boersma et al. who reported that age was not a significant factor in the incidence of WSLs (7). Alternatively, other studies have found that younger patients develop WSLs more than older patients (3, 8). Geiger et al. reported that the incidence of a WSL did not correlate positively with age; however, there was a greater number of advanced cavitated lesions in those under 13 years of age (4). The differences in results between the present study and those which found a greater number and severity in younger patients is likely to be due to the age range of the sample. The age range in the present study was similar to Boersma et al. (7). However, other studies have included a higher number of pre-adolescent patients who were younger than 12 years of age (3, 4, 8).

It was determined that the socio-economic status (SES) of an individual, determined by their SEIFA score, did not affect the incidence, severity or number of WSLs experienced. This supports previous finding of other studies which have used postcodes or other methods to determine SEC levels in orthodontic populations(3, 7). The SEIFA index is a standardised method of determining socio-economic status at a population level in Australia (30). Previous Australian studies have shown correlation between a SEIFA score and caries rates measured via DMFT in non-orthodontic, indigenous child and young adult populations (30, 31). No other studies have compared the SEIFA score with WSL occurrence in orthodontic patients.

There were 59.34% females and 40.66% males who took part in the present study. No statistically significant differences were found between the presence, severity and number of WSLs between the genders. The incidence was similar to previous findings(3-5). In contradiction to this, earlier studies have found that males develop WSLs more than females (6-8) while Gorelick et al. found that females displayed a higher incidence than males (9). Others have found that males tend to have a greater severity of demineralisation than females once the disease develops (3, 8).It is unlikely that a true gender-based risk exists for the development of WSLs. The apparent gender influences might be due to compliance with hygiene and preventive measures.

Patients' FTA rate had no significance to the presence of WSLs which supports the findings of Al Maatiah et al.(3). When related to WSLs of greater severity and the total number of WSLs experienced, the FTA rate was a significant factor. To our knowledge, no other studies have examined a patient's FTA rate compared to the total number and severity of WSLs graded by the ICDAS II.

There was no difference between the presence, severity and number of WSLs between patients banded in Tip-Edge Plus™ (TP Orthodontics Inc. 0.022x 0.028-in slot) or Victory Series™ (3M 0.022x 0.028-in slot). Al Maatiah et al. also found no difference in WSL incidence between these two brackets and, therefore, offers support for the present study(3). Al Maatiah et al. also found no difference in incidence between patients

undergoing full fixed appliance therapy, those combined with surgery or functional appliance treatment. Additional reports have found no difference in WSL incidence between self-ligation and conventional pre-adjusted edgewise brackets (13, 14).

Restored molars in a patient indicated both the presence of WSLs and that the severity of the WSLs was  $\geq$  ICDAS II grade 2. This agreed with a previous finding which found the presence of one restored first molar increased the likelihood of development and severity (graded by degree of mineralisation) of WSLs during treatment (3). Other studies have also found that children who have demineralisation unrelated to orthodontic treatment, have a much greater chance of developing further demineralisation when appliances are placed (10-12). Al Mulla et al. found patients with a high DMFS prior to treatment had a greater risk of caries during treatment (32). There are uncertainties when considering the number of filled molars to assess WSL risk in orthodontic patients. Restorations may have been placed due to hypoplastic or hypomineralised molars. Also, previously restored molars may have been extracted as part of orthodontic treatment

The most significant factor found was the reported number of tooth brushings per week described by the patient. It had a significant association with the presence, severity  $\geq$  ICDAS II grade 2 and ICDAS II grade 3 as well as the total number of white spot lesions in an individual. This supports earlier studies which found that patients with poor oral

hygiene before and during treatment are at greater risk of developing WSLs (2-4, 8-11). Although various operator methods of assessing oral hygiene levels have been used in the past, the present study found a significant correlation by simply asking patients to calculate the number of times they tooth brushed in a seven day period.

All comparisons between the DIAGNOdent pen and ICDAS II categories were statistically significant. This was supported by a previous study which also found that the DIAGNOdent pen was useful, reproducible and correlated well with the ICDAS II system of grading WSLs in orthodontic patients(28). The authors recommended that both could be used to study the progression and regression of WSLs. The DIAGNOdent pen has been increasing in popularity as a means to study WSLs in orthodontic patients(26, 27). It's advantages include ease of use, accuracy, reproducibility and that it can be used as a patient education tool (27, 28, 33). In an orthodontic setting, it may be an expensive adjunct to assess mineralisation levels which can be quantified visually. In addition, it was noted that the teeth should be pumiced to avoid false readings, which can be time consuming. Excess composite around the bracket base and gingival bleeding was also a concern and produced false high readings. The ICDAS II system is widely recognised, time efficient, easy to use and inexpensive but its main disadvantage is its subjectivity (28, 34). Both have a role in research involving WSLs in orthodontic treatment.

## CONCLUSIONS

1. The reported number of tooth brushings per week is a valuable indicator of the presence, severity  $\geq$  ICDAS II grade 2 and ICDAS II grade 3 as well as the total number of white spot lesions in an individual.
2. The number of filled molars may indicate the presence and severity  $\geq$  ICDAS II grade 2 of white spot lesions in an individual.
3. Patients who fail to attend appointments are more likely to have severity  $\geq$  ICDAS II grade 3 and an increased number of WSLs.
4. All comparisons between the DIAGNOdent pen and ICDAS II categories were statistically significant.

This study has been the first to consider risk factors and their associations to the presence, extent of demineralisation and number of WSLs graded via the ICDAS II in orthodontic patients. It is also the first to find that the most common type of WSL found in orthodontic patients is an ICDAS II grade 2 lesion.

## REFERENCES

1. Ogaard B, Rolla G, Arends J, ten Cate JM. Orthodontic appliances and enamel demineralization. Part 2. Prevention and treatment of lesions. *Am J Orthod Dentofacial Orthop.* 1988 Aug;94(2):123-128.
2. Ogaard B. Prevalence of white spot lesions in 19-year-olds: a study on untreated and orthodontically treated persons 5 years after treatment. *Am J Orthod Dentofacial Orthop.* 1989 Nov;96(5):423-427.
3. Al Maaitah EF, Adeyemi AA, Higham SM, Pender N, Harrison JE. Factors affecting demineralization during orthodontic treatment: a post-hoc analysis of RCT recruits. *Am J Orthod Dentofacial Orthop.* 2011 Feb;139(2):181-191.
4. Geiger AM, Gorelick L, Gwinnett AJ, Griswold PG. The effect of a fluoride program on white spot formation during orthodontic treatment. *Am J Orthod Dentofacial Orthop.* 1988 Jan;93(1):29-37.
5. Mizrahi E. Enamel demineralization following orthodontic treatment. *Am J Orthod.* 1982 Jul;82(1):62-67.
6. Tufekci E, Dixon JS, Gunsolley JC, Lindauer SJ. Prevalence of white spot lesions during orthodontic treatment with fixed appliances. *Angle Orthod.* 2011 Mar;81(2):206-210.
7. Boersma JG, van der Veen MH, Lagerweij MD, Bokhout B, Prah Andersen B. Caries prevalence measured with QLF after treatment with

fixed orthodontic appliances: influencing factors. *Caries Res.* 2005 Jan-Feb;39(1):41-47.

8. Chapman JA, Roberts WE, Eckert GJ, Kula KS, Gonzalez-Cabezas C. Risk factors for incidence and severity of white spot lesions during treatment with fixed orthodontic appliances. *Am J Orthod Dentofacial Orthop.* 2010 Aug;138(2):188-194.

9. Gorelick L, Geiger AM, Gwinnett AJ. Incidence of white spot formation after bonding and banding. *Am J Orthod.* 1982 Feb;81(2):93-98.

10. Julien KC, Buschang PH, Campbell PM. Prevalence of white spot lesion formation during orthodontic treatment. *Angle Orthod.* 2013 Jan 4.

11. Lovrov S, Hertrich K, Hirschfelder U. Enamel Demineralization during Fixed Orthodontic Treatment - Incidence and Correlation to Various Oral-hygiene Parameters. *J Orofac Orthop.* 2007 Sep;68(5):353-363.

12. Zimmer BW, Rottwinkel Y. Assessing patient-specific decalcification risk in fixed orthodontic treatment and its impact on prophylactic procedures. *Am J Orthod Dentofacial Orthop.* 2004 Sep;126(3):318-324.

13. Polat O, Gokcelik A, Arman A, Arhun N. A comparison of white spot lesion formation between a self-ligating bracket and a conventional

preadjusted straight wire bracket. *World J Orthod.* 2008

Summer;9(2):e46-50.

14. Buck T, Pellegrini P, Sauerwein R, Leo MC, Covell DA, Jr., Maier T, et al. Elastomeric-ligated vs self-ligating appliances: a pilot study examining microbial colonization and white spot lesion formation after 1 year of orthodontic treatment. *Orthodontics : the art and practice of dentofacial enhancement.* 2011 Summer;12(2):108-121.

15. Zachrisson BU, Zachrisson S. Caries incidence and oral hygiene during orthodontic treatment. *Scand J Dent Res.* 1971;79(6):394-401.

16. Mitchell L. Decalcification during orthodontic treatment with fixed appliances--an overview. *Br J Orthod.* 1992 Aug;19(3):199-205.

17. Banks PA, Chadwick SM, Asher-McDade C, Wright JL. Fluoride-releasing elastomerics--a prospective controlled clinical trial. *Eur J Orthod.* 2000 Aug;22(4):401-407.

18. Ogaard B, Larsson E, Henriksson T, Birkhed D, Bishara SE. Effects of combined application of antimicrobial and fluoride varnishes in orthodontic patients. *Am J Orthod Dentofacial Orthop.* 2001 Jul;120(1):28-35.

19. Broadbent JM, Thomson WM. For debate: problems with the DMF index pertinent to dental caries data analysis. *Community Dent Oral Epidemiol.* 2005 Dec;33(6):400-409.



20. Ismail AI. Visual and visuo-tactile detection of dental caries. *J Dent Res.* 2004;83 Spec No C:C56-66.
21. Ekstrand KR, Martignon S, Ricketts DJ, Qvist V. Detection and activity assessment of primary coronal caries lesions: a methodologic study. *Operative dentistry.* 2007 May-Jun;32(3):225-235.
22. Ismail AI, Sohn W, Tellez M, Amaya A, Sen A, Hasson H, et al. The International Caries Detection and Assessment System (ICDAS): an integrated system for measuring dental caries. *Community Dent Oral Epidemiol.* 2007 Jun;35(3):170-178.
23. Alencar CJ, Braga MM, de Oliveira E, Nicolau J, Mendes FM. Dye-enhanced laser fluorescence detection of caries lesions around brackets. *Lasers Med Sci.* 2009 Nov;24(6):865-870.
24. Guerrieri A, Gaucher C, Bonte E, Lasfargues JJ. Minimal intervention dentistry: part 4. Detection and diagnosis of initial caries lesions. *Br Dent J.* 2012 Dec;213(11):551-557.
25. Lussi A, Hibst R, Paulus R. DIAGNOdent: an optical method for caries detection. *J Dent Res.* 2004;83 Spec No C:C80-83.
26. Andersson A, Skold-Larsson K, Hallgren A, Petersson LG, Twetman S. Effect of a dental cream containing amorphous cream phosphate complexes on white spot lesion regression assessed by laser fluorescence. *Oral Health Prev Dent.* 2007;5(3):229-233.

27. Aljehani A, Yousif MA, Angmar-Mansson B, Shi XQ. Longitudinal quantification of incipient carious lesions in postorthodontic patients using a fluorescence method. *Eur J Oral Sci.* 2006 Oct;114(5):430-434.
28. Baeshen HA, Lingstrom P, Birkhed D. Effect of fluoridated chewing sticks (Miswaks) on white spot lesions in postorthodontic patients. *Am J Orthod Dentofacial Orthop.* 2011 Sep;140(3):291-297.
29. Shoaib L, Deery C, Ricketts DN, Nugent ZJ. Validity and reproducibility of ICDAS II in primary teeth. *Caries Res.* 2009;43(6):442-448.
30. Jamieson LM, Armfield JM, Roberts-Thomson KF. Oral health inequalities among indigenous and nonindigenous children in the Northern Territory of Australia. *Community Dent Oral Epidemiol.* 2006 Aug;34(4):267-276.
31. Jamieson LM, Do LG, Bailie RS, Sayers SM, Turrell G. Associations between area-level disadvantage and DMFT among a birth cohort of Indigenous Australians. *Aust Dent J.* 2013 Mar;58(1):75-81.
32. Al Mulla AH, Kharsa SA, Kjellberg H, Birkhed D. Caries risk profiles in orthodontic patients at follow-up using Cariogram. *Angle Orthod.* 2009 Mar;79(2):323-330.

33. Bamzahim M, Aljehani A, Shi XQ. Clinical performance of DIAGnodent in the detection of secondary carious lesions. *Acta Odontol Scand.* 2005 Feb;63(1):26-30.
  
34. Rodrigues JA, Hug I, Diniz MB, Lussi A. Performance of fluorescence methods, radiographic examination and ICDAS II on occlusal surfaces in vitro. *Caries Res.* 2008;42(4):297-304.

# **SALIVA TESTS TO DETERMINE THE RISK OF WHITE SPOT LESIONS DURING ORTHODONTIC TREATMENT**



## **Article 2**

Written in the format for submission to:

The Angle Orthodontist

**Dr Balya SRIRAM**

Orthodontic Unit

School of Dentistry

Faculty of Health Science

The University of Adelaide

June 2013

## 9. Article 2

### ABSTRACT

**Objectives:** To investigate the relationship between the presence, number and severity of white spot lesions (WSLs) and the properties of saliva tested with the Saliva-Check Buffer Kit (GC Corp., Belgium).

**Materials and Methods:** Fifty, full-fixed appliance orthodontic patients were examined after a routine adjustment. Saliva samples were taken and addressed according to hydration, consistency, resting pH, stimulated flow rate, stimulated pH and buffering capacity. All participants were examined for the presence of WSLs on their upper and lower anterior teeth using a visual index outlined by the International Caries Detection and Assessment System (ICDAS II) and the DIAGNOdent pen (KaVo, Biberach, Germany). Data was assessed by univariate logistic, ordinal logistic and binomial regression models using odds ratios, ratio of means and 95% confidence limits with significance set as  $p < 0.05$ .

**Results:** The pH of stimulated saliva was a significant diagnostic variable in identifying WSLs ( $p = 0.0245$ ). The stimulated flow of saliva and its pH were significant variables relating to the presence WSLs with a grading of ICDAS II  $\geq 2$  ( $p = 0.0195$  and  $p = 0.0095$  respectively). When the grading was ICDAS II  $\geq 3$ , the pH of unstimulated saliva was a significant variable

( $p=0.0218$ ). No significant relationships were found between saliva properties and the number of WSLs identified.

**Conclusions:** The pH of stimulated saliva, the pH of unstimulated saliva and quantity of saliva produced in five minutes are factors which may identify orthodontic patients who are susceptible to the development of WSLs and may also indicate the severity of the lesions. However, the saliva properties tested were not able to differentiate between patients who developed a small or large number of WSLs.

## INTRODUCTION

White spot lesions (WSLs) are known to be precursor of dental decay. Their presence suggests that the healthy balance between demineralisation and remineralisation on the enamel surface has shifted towards demineralisation and likely loss of tooth structure. WSLs are a common complication of orthodontic treatment and are a significant aesthetic concern (Figure 1). A lesion might be clinically detectable within one month of the commencement of orthodontic treatment (1). Once formed, a WSL might re-mineralise back to normal, remain stable or progress to a cavitation requiring restoration depending on the characteristics of the oral environment (2). The incidence rate of WSLs during orthodontic treatment is between 2% to 97% (3-10). This large variation is due to heterogeneous methods of diagnosis and grading of

WSLs. Generally, visual methods of diagnosis are less sensitive compared with light-induced fluorescence methods (3).



**Figure 1: Presentation of White Spot Lesions**

A common visual method of recording decay as reported in the literature is the DMFT index (decayed, missing, filled teeth) or DMFS index (decayed, missing, filled surfaces). Missing and filled teeth are not always due to caries experience, and therefore, these indices may produce an overestimation of the problem(11). This is especially true in orthodontic patients in whom the extraction of permanent teeth is performed as part of treatment and extracted teeth may not have experienced decay.

Furthermore, WSLs are normally early signs of demineralisation and the DMFT and DMFS would not allow sufficient categorisation of the lesions. The International Caries Detection and Assessment System II(ICDAS II) has been widely used since its introduction in 2007. Its key strength over conventional detection methods is its ability to further categorise incipient carious lesions according to their severity(12, 13). The DIAGNOdent pen (KaVo, Biberach, Germany) utilises laser fluorescence as a quantitative

method of caries detection. Fluorescence is light emitted by the movement of molecules in response to the absorption of high energy light (14). These variable fluorescence emissions from teeth are evaluated via the DIAGNOdent pen to indicate the level of demineralisation on a tooth surface according to a scale which ranges from 1 to 99 (14).

As the predecessor of dental caries, WSLs are regarded as a carbohydrate induced, bacterial, infectious disease (15). Saliva, apart from having an integral role in the maintenance of the oral cavity, also plays an important role in the balance between demineralisation and remineralisation at the tooth surface. Although bacteria, carbohydrates, a susceptible tooth and time are required for demineralisation, the properties of saliva related to its buffering capacity, pH and flow rate might influence the extent of demineralisation and repair via remineralisation (15).

The Saliva-Check Buffer Kit (GC Corp., Belgium) provides 6 results related to saliva function which are reported to indicate a patient's expected caries risk (16, 17). The test analyses the level of labial hydration, saliva consistency, resting pH, stimulated saliva quantity in a given time (five minutes), stimulated saliva pH and buffering capacity. Studies using the Saliva-Check Buffer Kit have found correlations between saliva properties and caries rates in non-orthodontic populations (18, 19). The efficacy of the Saliva-Check Buffer Kit in the detection of WSLs in orthodontic patients is unknown.



The aim of this study was to investigate the relationship between the properties of saliva tested with the Saliva-CheckBuffer Kit and the presence, severity and number of WSLs diagnosed using the ICADS II system and the DIAGNOdent pen in orthodontic patients.

## **MATERIALS AND METHODS**

Ethics approval for the study was granted through the South Australian Dental Service and the University of Adelaide. A power study was undertaken and completed to determine a suitable sample size. A sample of 50 was estimated as adequate to produce statistically significant results at  $p \leq 0.05$ . The proposed incidence rate of white spot lesions used to determine sample size was 50%.

Patients were randomly selected at the Orthodontic Clinic of Adelaide Dental Hospital following a routine orthodontic appliance adjustment visit and asked whether they would participate in the study. 50 patients agreed and information sheets were provided and written consent was obtained. Inclusion criteria included:

1. No missing anterior teeth.
2. Full fixed appliance treatment involving upper and lower dentitions for a minimum of six months.

Exclusion criteria included:

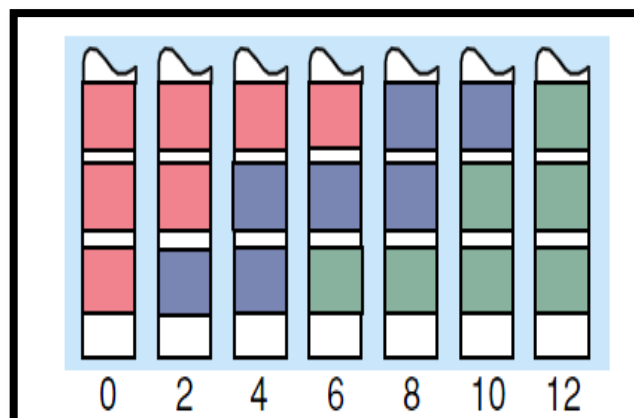
1. Antibiotic use during the preceding month.

2. Fluoride treatment, a professional dental clean or CPP-ACP (casein phosphopeptide amorphous calcium fluoride phosphate) use within the previous two weeks.
3. Patients who received antibiotic prophylaxis prior to the appointment.
4. Patients on medications that affect salivary flow or function (e.g. antidepressants, diuretics, antihistamines, narcotics, and  $\beta$ -adrenoreceptor agonists).
5. Smokers.
6. Patients who had consumed food or drink within an hour of the test.
7. Patients who had brushed their teeth or used a mouthwash within an hour of the test.

Using the GC Saliva-Check Buffer Kit, the following tests were completed for each patient by a single examiner according to the manufacturer's instructions:

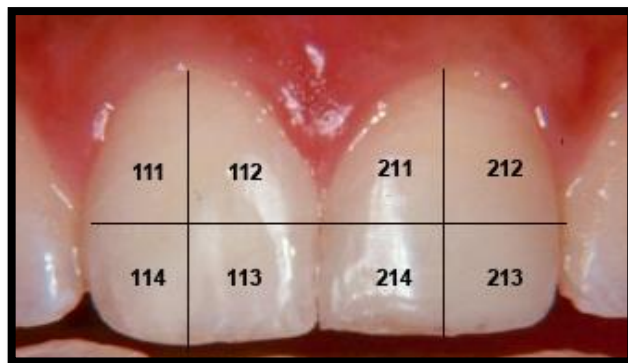
1. *Hydration*: The lower lip was everted, gently wiped with gauze to remove existing saliva and labial gland secretion (the time taken for a saliva drop to form) was timed in seconds.
2. *Saliva Consistency (Viscosity)*: The saliva pooled in the floor of the mouth was assessed as either:
  - a. sticky and frothy (SF) or;
  - b. frothy and bubbly (FB) or;
  - c. watery and clear (WC)

3. *Resting pH Measurement:* The patient was asked to expectorate saliva into a cup provided in the kit. The pH strip and accompanying colour chart was used to determine and record the resting pH.
4. *Testing of Stimulated Saliva Quantity (Flow Rate):* The patient was requested to chew on a piece of wax (1g) for 5 mins and regularly expectorate into a cup. The quantity of saliva was measured.
5. *pH of Stimulated Saliva.* A pH strip was placed into the stimulated saliva and pH recorded.
6. *Buffering Capacity:* A provided pipette was used to draw up saliva from the previous test and dispense 1 drop onto each of the test pads on a buffer test strip. At exactly 2 minutes each of the 3 colour pads was graded according to a colour chart. A score between 0 and 12 was assigned for the buffering capacity using a the manufacturer's scale. (Figure 2)



**Figure 2: Buffering Capacity Points(16)**

A visual examination for the presence of WSLs was performed directly after the saliva tests. All teeth were lightly pumiced and anterior teeth (canine to canine) in the upper and lower arch were examined. These 12 teeth were further subdivided into 4 “mini-quadrants”(Figure 3). Two lines were placed through the centre of the crowns parallel to the long axis of the tooth and perpendicularly halfway between the gingival margin and the incisal edge. This divided the tooth crown into four parts. The first two numbers of the mini-quadrant represent the FDI notation for that tooth. The third number identified quadrants 1 through to 4 in a clockwise direction from the top right quarter of the tooth (starting from the patient’s right). This system generated 48 sites from 12 teeth per patient.



**Figure 3: Central Incisors Divided Into “Mini-Quadrants”**

Each of the 48 sites was assigned an ICDAS II score by the same examiner using loupes and the aid of a mirror, clinical lighting, a triplex syringe and a blunt probe. The ICDAS II scores were voice recorded so that an assigned score was unable to be subsequently referenced to

scores made on previous saliva tests. The ICDAS II caries scoring system is shown in Table 1.

**Table 1: The ICDAS II Caries Scoring System (20)**

<b>CODE</b>	<b>CRITERION</b>
<b>0</b>	Sound tooth surface: No evidence of caries after 5 seconds of air drying
<b>1</b>	First visual change in enamel. Opacity of discolouration is visible after prolonged air drying. Cannot be seen when wet
<b>2</b>	Distinct visual change in enamel visible when wet. Lesion must be visible when dry
<b>3</b>	Localised enamel breakdown (without clinical visual signs of dentinal involvement). Seen when wet and after prolonged drying
<b>4</b>	Underlying dark shadow from dentine
<b>5</b>	Distinct cavity with visible dentine
<b>6</b>	Extensive (more than half the surface). Distinct cavity with visible dentine

Directly following the visual examination, a DIAGNOdent pen was utilised to record mineralisation levels on all 48 sites in each patient. Prior to each use, the instrument was pre-calibrated on a ceramic reference disc supplied by the manufacturer and then calibrated on a sound palatal surface of a central incisor. The peak value for each “mini-quadrant” was recorded. The same DIAGNOdent pen and the same design of light probe was used on all patients throughout the study.

A statistical analysis was performed by an independent statistician using SAS Version 9.3 (SAS institute Inc., Cary, NC, USA). The level of statistical significance was set at  $p < 0.05$  for all statistical tests.

## **RESULTS**

A total of 29 females (58%) and 21 males (42%) took part in the study. The average age at commencement of treatment was 15.62 years (range from 12 to 20 years). The average age at the time of testing was 17.12 years (range from 12-21 years). Hydration score mean was 49.98 seconds (ranging from 15-137 seconds). Mean pH of unstimulated saliva was 6.86 (ranging from 6.2-7.6). The mean pH of stimulated saliva was 7.58 (ranging from 6.8-7.8). The average quantity of saliva produced in five minutes was 6.28mL (ranging from 1 to 16ml). The average buffering capacity score was 9.76 (ranging from 5 to 12). Thirteen patients had an FB saliva type (26%), 7 had a SF saliva type (14%) and 30 had a WC saliva type (60%). A total of 27 out of 50 patients had WSLs (54%) which

were distributed over a total of 177 “mini quadrants”. The type of WSLs can be seen in Table 2. The most prevalent was an ICDAS II score of 2. Tables 3 to 6 are presented up to 4 decimal places to accurately reflect the p-value of the variables tested.

**Table 2: Score of WSLs**

<b>ICDAS II SCORE</b>	<b>FREQUENCY</b>	<b>PERCENT</b>	<b>CUMULATIVE FREQUENCY</b>	<b>CUMULATIVE PERCENT</b>
<b>1</b>	72	40.7	72	40.7
<b>2</b>	95	53.6	167	94.3
<b>3</b>	9	5.1	176	99.4
<b>4</b>	1	0.6	177	100

***The Presence of WSLs and the Saliva-Check Buffer Kit***

The presence of WSLs was defined as a patient who had a “mini-quadrant” with an ICDAS II score of 1 or higher. This was performed to determine whether the Saliva-Check Buffer Kit could detect a patient who had WSLs of a particular severity. Univariate logistic regression models were fitted to the data. The association between each saliva property and the presence of WSLs was described using odds ratios at a confidence limit of 95% (Table 3). The pH of stimulated saliva was a significant variable ( $p=0.0245$ ) of the presence of WSLs. For every 0.1 unit increase

in the pH of stimulated saliva, the odds of detecting white spot lesions decreased by 33%.

**Table 3: Relationship Between the Presence of WSLs and the Saliva-Check Buffer Kit**

SALIVA PROPERTY	ODDS RATIO	LOWER 95% CONFIDENCE LIMIT	UPPER 95% CONFIDENCE LIMIT	P-VALUE
HYDRATION SCORE	1.0105	0.9918	1.0296	0.2725
TYPE OF SALIVA: SF VS. FB	7.0000	0.6470	75.7349	0.1092
TYPE OF SALIVA: WC VS. FB	1.1667	0.3166	4.2993	0.8168
TYPE OF SALIVA: WC VS. SF	0.1667	0.0178	1.5573	0.1161
PH OF UNSTIMULATED SALIVA <sup>∞</sup>	0.9343	0.8166	1.0689	0.3223
QUANTITY OF SALIVA IN 5 MINUTES	0.8812	0.7564	1.0265	0.1045
PH STIMULATED SALIVA <sup>∞</sup>	0.6702	0.4729	0.9497	*0.0245
BUFFERING CAPACITY POINTS	0.9044	0.6929	1.1805	0.4598

<sup>∞</sup> coefficient is for a 0.1 unit increase

***The Severity of WSLs and the Saliva-Check Buffer Kit (GC Corp., Belgium)***

The most severe WSL was defined as the highest ICDAS II score across all mini-quadrants in an individual. As the assumptions of linear regression and proportional odds regression were not satisfied, saliva properties were identified using separate logistic regression models for



each cut-point of severity (severity  $\geq 1$ , severity  $\geq 2$  and severity  $\geq 3$ ). Since the modelling of severity  $\geq 1$  is equivalent to modelling the presence of white spots, the results are not repeated here. Table 4 shows the relationship between the severity of WSLs with an ICDAS II score  $\geq 2$  and the saliva properties examined. The quantity of saliva a patient produces in five minutes and the pH of stimulated saliva was effective in predicting patients with an ICDAS II score  $\geq 2$  ( $p=0.0195$  and  $p=0.0095$  respectively). Table 5 shows the relationship between the severity of WSLs with an ICDAS II score  $\geq 3$  and the saliva properties examined. The pH of unstimulated saliva was effective in predicting patients with an ICDAS II score  $\geq 3$  ( $p=0.0218$ ).

**Table 4: The Relationship Between the Severity of WSLs  $\geq$  ICDAS II Grade2 and the Saliva-Check Buffer Kit**

ICDAS II SCORE	SALIVA PROPERTY	ODDS RATIO	LOWER 95% CONFIDENCE LIMIT	UPPER 95% CONFIDENCE LIMIT	P-VALUE
SEVERITY $\geq 2$	Hydration score	1.0026	0.9852	1.0202	0.7723
SEVERITY $\geq 2$	Type of saliva: SF vs. FB	4.0000	0.5499	29.0962	0.1709
	Type of saliva: WC vs. FB	1.0667	0.2807	4.0530	0.9245
	Type of saliva: WC vs. SF	0.2667	0.0443	1.6054	0.1490
SEVERITY $\geq 2$	pH of unstimulated saliva <sup>∞</sup>	0.9698	0.8484	1.1085	0.6528
SEVERITY $\geq 2$	Quantity of saliva in 5 minutes	0.8053	0.6716	0.9657	*0.0195
SEVERITY $\geq 2$	pH stimulated saliva <sup>∞</sup>	0.6134	0.4241	0.8873	**0.0095
Severity $\geq 2$	Buffering capacity points	0.7729	0.5851	1.0211	0.0698

<sup>∞</sup> coefficient is for a 0.1 unit increase

**Table 5: The Relationship Between the Severity of WSLs  $\geq$  ICDAS II Grade3 and the Saliva-Check Buffer Kit**

ICDAS II SCORE	VARIABLE	ODDS RATIO	LOWER 95% CONFIDENCE LIMIT	UPPER 95% CONFIDENCE LIMIT	P-VALUE
SEVERITY $\geq 3$	Hydration score	1.0031	0.9759	1.0312	0.8237
SEVERITY $\geq 3$	Type of saliva: SF vs. FB	2.0000	0.1057	37.8296	0.6440
	Type of saliva: WC vs. FB	1.3333	0.1255	14.1654	0.8114
	Type of saliva: WC vs. SF	0.6667	0.0587	7.5719	0.7436
SEVERITY $\geq 3$	pH of unstimulated saliva <sup>∞</sup>	1.5628	1.0671	2.2888	0.0218*
SEVERITY $\geq 3$	Quantity of saliva in 5 minutes	0.8849	0.6611	1.1844	0.4110
SEVERITY $\geq 3$	pH stimulated saliva <sup>∞</sup>	1.6280	0.7685	3.4489	0.2032
SEVERITY $\geq 3$	Buffering capacity points	1.1207	0.7092	1.7709	0.6255

<sup>∞</sup> coefficient is for a 0.1 unit increase

### *The Number of WSLs and the Saliva-Check Buffer Kit (GC Corp., Belgium)*

The total number of WSLs was defined as the number of “mini-quadrants” with an ICDAS II score of one or higher in each individual.

This analysis was applied to see if the saliva properties in question could differentiate between those who had a small number versus those who experienced a large number of WSLs. Negative binomial regression models were fitted to the data. The association between each variable and the total number of white spots was described using the ratio of means and the 95% confidence limits level (Table 6). There were no statistically significant relationships between salivary properties and the number of WSLs a patient experienced.

**Table 6: Relationship Between the Number of WSLs a Patient Experiences and the Saliva-Check Buffer Kit**

SALIVA PROPERTY	RATIO OF MEANS	LOWER 95% CONFIDENCE LIMIT	UPPER 95% CONFIDENCE LIMIT	P-VALUE
HYDRATION SCORE	1.0030	0.9897	1.0165	0.6586
TYPE OF SALIVA: SF vs. FB	1.6412	0.3454	7.7993	0.5333
TYPE OF SALIVA: WC vs. FB	0.9271	0.3025	2.8413	0.8947
TYPE OF SALIVA: WC vs. SF	0.5649	0.1404	2.2726	0.4213
PH OF UNSTIMULATED SALIVA <sup>∞</sup>	1.0199	0.9076	1.1461	0.7402
QUANTITY OF SALIVA IN 5 MINUTES	0.9265	0.8365	1.0263	0.1435
PH STIMULATED SALIVA <sup>∞</sup>	0.9245	0.7626	1.1208	0.4244
BUFFERING CAPACITY POINTS	1.0019	0.8019	1.2518	0.9868

<sup>∞</sup> coefficient is for a 0.1 unit increase

As shown in article 1 “Use of the DIAGNOdent pen and Patient Factors to Diagnose White Spots in Orthodontic Patients”, the relationship between the DIAGNOdent pen and ICDAS II is highly significant ( $P < 0.0001$ ).

Hence the result tables comparing the presence, severity and number of WSLs examined via the DIAGNOdent pen and the Saliva-Check BufferKit is not repeated here.

## DISCUSSION

The study was a cross-sectional investigation in which the properties of saliva, tested with the Saliva-Check Buffer Kit, were examined to determine if it was possible to recognise the presence, severity or number of WSLs in mechanically-active orthodontic patients. The efficacy of the Saliva-Check Buffer Kit to detect WSLs using the ICDAS II and the DIAGNOdent pen has not been previously examined.

In the present study, the pH of stimulated saliva was able to recognise patients who displayed WSLs. When the severity of WSLs reached an ICDAS II score  $\geq 2$ , the quantity of saliva produced in 5 minutes and the pH of stimulated saliva were positive variable in those patients. When the severity of WSLs reached an ICDAS II score  $\geq 3$ , the pH of unstimulated saliva was able to distinguish those patients.

Lara-Carrillo et al (21) recorded saliva properties on 34 subjects before and one month after the placement of full fixed appliances. The DMFS index and saliva properties were examined using the Saliva-Check Buffer Kit. It was found that orthodontic treatment increased flow rate, buffering capacity and stimulated salivary pH. No significant changes in the DMFS were reported within one month of treatment which prevented the comparison of saliva properties.

The ideal clinical study of WSL would be longitudinal investigation over an entire course of fixed orthodontic treatment rather than the cross-

sectional design of the present study. The evaluation of WSLs would need to be with a clinical index that is designed to specifically categorise enamel demineralisation; thus the ICDAS II may be more suitable than the DMFT or DMFS. There is positive evidence regarding the use of laser fluorescent devices in the evaluation of WSLs (22-24). Saliva tests would also need to be completed at regular intervals in order to determine general trends rather than short term fluctuations. In practice, however, the tests are used as “one off” markers to identify patients who might be at a higher risk of developing decay. A saliva test at every orthodontic adjustment visit in an 18-24 month period would be time consuming, tax patient compliance and be costly. Although limited, the information obtained in a cross-sectional study is, therefore, valuable.

A study on 40 non-orthodontic patients using the Saliva-Check Buffer Kit has compared saliva properties of patients with a DMFT of zero with patients with a DMFT >5 (19) and found that the DMFT = 0 group have a higher salivary flow rate, viscosity, unstimulated pH and buffering capacity compared to the DMFT > 5 group. Flow rate (or quantity of saliva produced in 5 minutes) and unstimulated salivary pH were also significant diagnostic determinants of WSLs in the present study. A viscosity rating of SF, FB or WC is highly subjective and may account for the variation in results. Furthermore, besides the shortcomings of using the DMFT index for WSLs, the index may indicate past as opposed to present caries risk. The use of the DMFT and DMFS may explain why

salivary parameters have not indicated caries experience in orthodontic patients in the past (25, 26).

A negative correlation between resting pH and ICDAS II 1 and 2 carious lesions has been found in 58 non-orthodontic adult patients(18). In addition, a negative correlation between saliva buffering and moderate lesions at an ICDAS II score of 3 and 4has been found. The present study was unable to identify a relationship between salivary buffering capacity and WSLs; however, very few ICDAS II 3 and 4 lesions were found in the present sample. Resting pH was also only indicative of ICDAS II  $\geq 3$  patients rather than ICDAS II 1 and 2. This may possibly be explained by the age of the subjects or the fact that orthodontic appliances were in place.

None of the saliva properties tested in the present study were able to identify patients who possessed a high number and those who had few WSLs.

## **CONCLUSIONS**

1. The pH of stimulated saliva was able to recognise those patients who exhibited WSLs during orthodontic treatment.
2. The quantity of saliva produced in 5 minutes and the pH of the stimulated saliva was able to distinguish patients who had severe WSLs and an ICDAS II score  $\geq 2$ .



3. When the severity of WSLs increased above an ICDAS II score  $\geq$  3, the pH of unstimulated saliva provided a mechanism to identify those patients.
4. The properties of saliva tested with the GC Saliva-Check Buffer Kit were not able to distinguish between patients who had a high number and those who had a low number of WSLs.

This study was the first to evaluate the use of the Saliva-Check Buffer Kit to detect WSLs in orthodontic patients using the ICDAS II and the DIAGNOdent pen.

## REFERENCES

1. Ogaard B, Rolla G, Arends J. Orthodontic appliances and enamel demineralization. Part 1. Lesion development. *Am J Orthod Dentofacial Orthop.* 1988 Jul;94(1):68-73.
2. Shungin D, Olsson AI, Persson M. Orthodontic treatment-related white spot lesions: a 14-year prospective quantitative follow-up, including bonding material assessment. *Am J Orthod Dentofacial Orthop.* 2010 Aug;138(2):136 e1-8; discussion -7.
3. Boersma JG, van der Veen MH, Lagerweij MD, Bokhout B, Prahl-Andersen B. Caries prevalence measured with QLF after treatment with fixed orthodontic appliances: influencing factors. *Caries Res.* 2005 Jan-Feb;39(1):41-47.
4. Gorelick L, Geiger AM, Gwinnett AJ. Incidence of white spot formation after bonding and banding. *Am J Orthod.* 1982 Feb;81(2):93-98.
5. Zachrisson BU, Zachrisson S. Caries incidence and oral hygiene during orthodontic treatment. *Scand J Dent Res.* 1971;79(6):394-401.
6. Geiger AM, Gorelick L, Gwinnett AJ, Griswold PG. The effect of a fluoride program on white spot formation during orthodontic treatment. *Am J Orthod Dentofacial Orthop.* 1988 Jan;93(1):29-37.

7. Ogaard B. Prevalence of white spot lesions in 19-year-olds: a study on untreated and orthodontically treated persons 5 years after treatment. *Am J Orthod Dentofacial Orthop.* 1989 Nov;96(5):423-427.
8. Mitchell L. Decalcification during orthodontic treatment with fixed appliances--an overview. *Br J Orthod.* 1992 Aug;19(3):199-205.
9. Banks PA, Chadwick SM, Asher-McDade C, Wright JL. Fluoride-releasing elastomerics--a prospective controlled clinical trial. *Eur J Orthod.* 2000 Aug;22(4):401-407.
10. Ogaard B, Larsson E, Henriksson T, Birkhed D, Bishara SE. Effects of combined application of antimicrobial and fluoride varnishes in orthodontic patients. *Am J Orthod Dentofacial Orthop.* 2001 Jul;120(1):28-35.
11. Broadbent JM, Thomson WM. For debate: problems with the DMF index pertinent to dental caries data analysis. *Community Dent Oral Epidemiol.* 2005 Dec;33(6):400-409.
12. Ekstrand KR, Martignon S, Ricketts DJ, Qvist V. Detection and activity assessment of primary coronal caries lesions: a methodologic study. *Operative dentistry.* 2007 May-Jun;32(3):225-235.
13. Ismail AI, Sohn W, Tellez M, Amaya A, Sen A, Hasson H, et al. The International Caries Detection and Assessment System (ICDAS): an

integrated system for measuring dental caries. *Community Dent Oral Epidemiol.* 2007 Jun;35(3):170-178.

14. Guerrieri A, Gaucher C, Bonte E, Lasfargues JJ. Minimal intervention dentistry: part 4. Detection and diagnosis of initial caries lesions. *Br Dent J.* 2012 Dec;213(11):551-557.

15. Chang HS, Walsh LJ, Freer TJ. Enamel demineralization during orthodontic treatment. Aetiology and prevention. *Aust Dent J.* 1997 Oct;42(5):322-327.

16. GC. Saliva Check Buffer. In: LTD. SKSBBTC, editor. Hong Kong: GC corporation; 2001.

17. Walsh LJ. Saliva testing: Good Practice, Good Sense. GC Asia; 2002.

18. Varma S, Banerjee A, Bartlett D. An in vivo investigation of associations between saliva properties, caries prevalence and potential lesion activity in an adult UK population. *J Dent.* 2008 Apr;36(4):294-299.

19. Gopinath VK, Arzreanne AR. Saliva as a Diagnostic Tool for Assessment of Dental Caries. *Archives of Orofacial Sciences.* 2006;1:57-59.

20. Shoaib L, Deery C, Ricketts DN, Nugent ZJ. Validity and reproducibility of ICDAS II in primary teeth. *Caries Res.* 2009;43(6):442-448.

21. Lara-Carrillo E, Montiel-Bastida NM, Sanchez-Perez L, Alanis-Tavira J. Effect of orthodontic treatment on saliva, plaque and the levels of *Streptococcus mutans* and *Lactobacillus*. *Med Oral Patol Oral Cir Bucal*. 2010 Nov;15(6):e924-929.
22. Baeshen HA, Lingstrom P, Birkhed D. Effect of fluoridated chewing sticks (Miswaks) on white spot lesions in postorthodontic patients. *Am J Orthod Dentofacial Orthop*. 2011 Sep;140(3):291-297.
23. Aljehani A, Yousif MA, Angmar-Mansson B, Shi XQ. Longitudinal quantification of incipient carious lesions in postorthodontic patients using a fluorescence method. *Eur J Oral Sci*. 2006 Oct;114(5):430-434.
24. Andersson A, Skold-Larsson K, Hallgren A, Petersson LG, Twetman S. Effect of a dental cream containing amorphous cream phosphate complexes on white spot lesion regression assessed by laser fluorescence. *Oral Health Prev Dent*. 2007;5(3):229-233.
25. Al Mulla AH, Kharsa SA, Kjellberg H, Birkhed D. Caries risk profiles in orthodontic patients at follow-up using Cariogram. *Angle Orthod*. 2009 Mar;79(2):323-330.
26. Sanpei S, Endo T, Shimooka S. Caries risk factors in children under treatment with sectional brackets. *Angle Orthod*. 2010 May;80(3):509-514.

# 10. Concluding Remarks

This study was divided into two articles. The first article investigated the relationship between patient related characteristics and the development, number and severity of white spot lesions (WSLs) in orthodontic patients. It also evaluated the use of the DIAGNOdent pen (KaVo, Biberach, Germany) compared with the ICDAS II in assessing WSLs in orthodontic patients. The second article evaluated the use of a saliva test, the Saliva-Check Buffer Kit (GC Corp., Belgium), in being able to diagnose the development, number and severity of WSLs.

The results from the first article indicated that a patient's reported number of tooth brushings per week, the number of restored molars present and their compliance with attending appointments are indicative of their WSL experience in terms of development, number and severity. Also, the DIAGNOdent pen was found to correlate well to the ICDAS II index. The second article showed that the pH of stimulated saliva, quantity of stimulated saliva produced in five minutes and the pH unstimulated saliva may indicate the development and severity of WSLs. The Saliva-Check Buffer Kit failed to recognise those patients with a large number of WSLs.

This study was the first to consider risk factors and their associations to the presence, extent of demineralisation and number of WSLs graded via the ICDAS II in orthodontic patients. It is was also the first to find that the most common type of WSL found in orthodontic patients is an ICDAS II

grade 2 lesion. This study was also the first to evaluate the use of the Saliva-Check Buffer Kit to detect WSLs on orthodontic patients using the ICDAS II and the DIAGNOdent pen.

WSLs continue to be a common complication related with orthodontic treatment. The ideal clinical study of WSL during orthodontic treatment would be longitudinal investigation over an entire course of fixed orthodontic treatment rather than the cross-sectional design of the present study. Many difficulties are posed during longitudinal studies such as time constraints, increased cost and loss of patient compliance. Despite the design limitations in the present study, some significant results have been found. Due to the cross-sectional nature of the study, care must be taken before inferences between cause and effect of variables and WSL formation are concluded. Future research is required in the area of saliva tests and the use of laser fluorescence devices, such as the DIAGNOdent pen, during orthodontic treatment. Further questions on patient variables to determine susceptible individuals may also answered in terms of disease incidence between right and left sides, socio-economic and compliance variables.

Ideally, a chair-side test might be developed in the future to identify orthodontic patients who will develop WSLs prior to the commencement of treatment.

# Appendix

## **SALIVA-CHECK BUFFER KIT(1, 2)**

The Saliva-Check Buffer Kit is a test that provides 6 results describing saliva quality and quantity which are proposed to indicate a patient's expected caries risk and general oral health. It uses a light indication system where a red light is considered a high disease risk, a yellow light indicates a moderate risk and a green light indicates a normal to low risk.

Each package contains:

- 20 pH test strips.
- 20 Saliva dispensing cups.
- 20 wax gum pieces for saliva stimulation.
- 20 Saliva dispensing pipettes.
- 20 Buffer test strips.

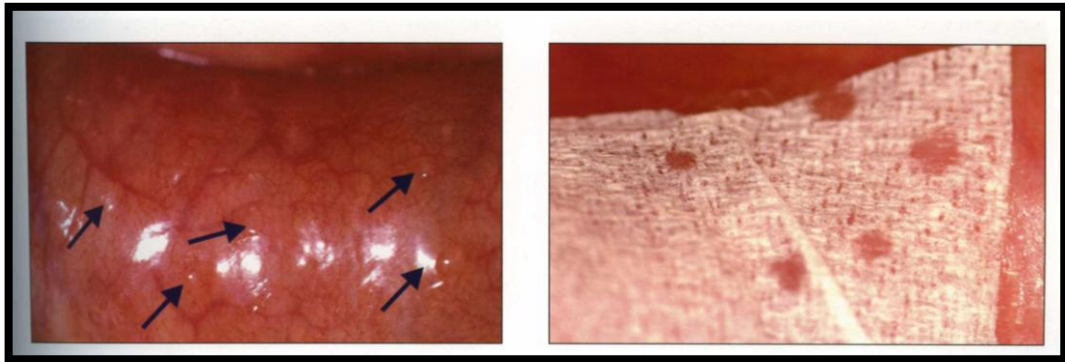
Prior to using any of these tests, the manufacturer recommends that the patient does not smoke, consume food or drink, brush their teeth or use a mouthwash for an hour prior to the appointment time.

### **Test 1: Visual inspection of hydration**

This involves assessing the lower lip labial gland secretion to determine unstimulated salivary flow. The lower lip is everted, gently blotted with gauze and observed under good light for the formation of saliva from the



minor salivary glands (Figure 1). The time taken for visible production of saliva is assessed. Greater than 60 seconds is considered a “red light” of low resting saliva flow and less than 60 seconds is considered a “green light” of normal resting saliva flow.



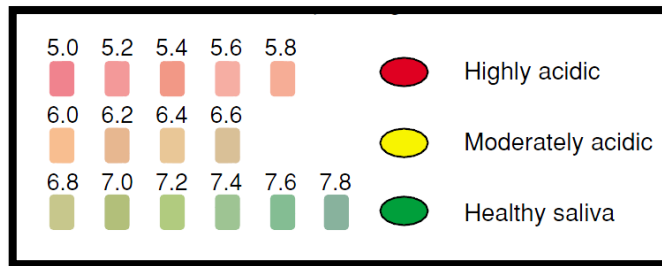
**Figure 1: Labial Saliva Gland Secretion (3)**

### **Test 2: Saliva consistency**

This involves assessing the resting saliva consistency. Sticky, frothy saliva residues are considered a “red light” for increased viscosity. Frothy, bubbly saliva is a “yellow light” for slightly increased viscosity, and watery clear saliva is a “green light” for normal viscosity.

### **Test 3: Resting pH measurement**

The patient expectorates any pooled saliva into the collection cup. A pH strip is placed into the resting saliva for 10 seconds. The colour of the strip is compared to the colour chart available in the package (Figure 2).



**Figure 2: pH Colour Chart (1)**

#### Test 4: Testing of Stimulated Saliva Flow

The patient chews on a piece of wax to stimulate the salivary flow. After 30 seconds they expectorate into the dispensing cups. Chewing is continued for 5 minutes and saliva collected into the cup at regular intervals. The quantity of saliva is measured by the marking on the side of the cup.

If the quantity of saliva at 5 minutes is:

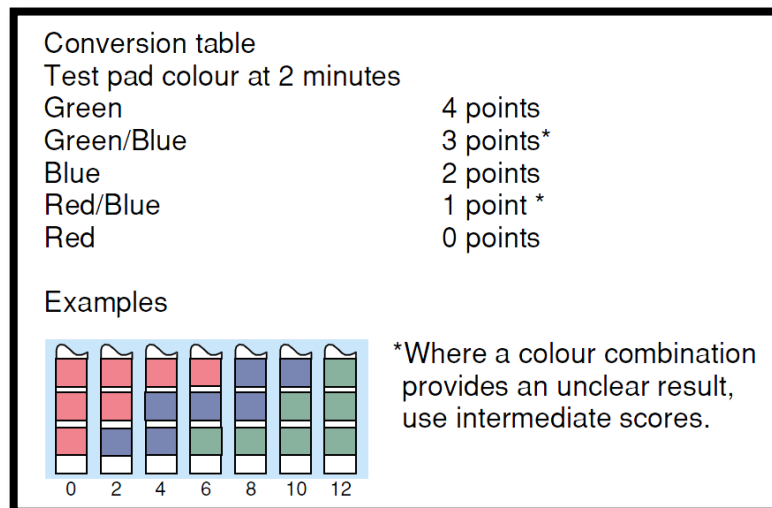
- Less than 3.5mL then this is a “red light” and considered very low.
- Between 3.5mL and 5mL then this is a “yellow light” and considered low.
- Over 5mL then this is a “green light” and considered normal.

### Test 5: The pH of Stimulated Saliva

A pH strip is placed into the cup of stimulated saliva for 10 seconds. The colour of the strip is compared to the colour chart available in the package shown in Figure 3.

### Test 6: Buffering Capacity

In this test, the pipette provided is used to draw up saliva from the previous test and dispense 1 drop onto each of the test pads on a buffer test strip. Following this, the strip is placed at 90 degrees to an absorbent tissue to soak up excess saliva. At two minutes each of the 3 colour pads is assigned points depending on the colour (Figure 3).



**Figure 3: Buffering Capacity Points (1)**

If the combined total of points range from 0-5, this is considered a “red light” indicating a very low buffering capacity of the saliva. A score ranging from 6-9 is a “yellow light” and is thought to be a less than ideal buffering capacity. A score of 10-12 is a “green light” and hence indicates a normal to high buffering capacity.

## **REFERENCES**

1. GC. Saliva Check Buffer. In: LTD. SKSBBTC, editor. Hong Kong: GC corporation; 2001.
2. Walsh LJ. Saliva testing: Good Practice, Good Sense. GC Asia; 2002.
3. Mount GJ, Hume WR. Preservation and Restoration of Tooth Structure. 2nd Edition ed: Brighton, Qld. : Knowledge Books and Software; 2005.

## Information sheet for participants regarding the project titled “An evaluation of tests that predict early decay in patients undergoing orthodontic treatment”

The purpose of this study is to evaluate marketed dental decay tests for the prediction of cavity development in teeth during treatment with fixed braces. This research is going towards a thesis for a Doctor of Clinical Dentistry.

If you would like to participate, we will collect some de-identified information and/or samples of saliva at your orthodontic appointment. The saliva will first be looked at in your mouth and then you will be asked to spit into a cup so that we can test your saliva. We will also ask you to chew on a neutral tasting piece of wax for 5 mins while spitting regularly into a cup and also test this saliva. Finally we will look at your teeth with a mirror, probe and a small hand held laser to see if there are any signs of early dental decay. We expect the total time to take approximately 20-30mins on top of your normal orthodontic visit. It is important that you do not smoke, consume food or drink, brush your teeth or use a mouthwash for an hour prior to the appointment time.

If any of these tests are successful, they may be used in the future as a quick way to determine if a patient is at a high risk of developing tooth decay during orthodontic treatment. As we are analysing the successful use of these tests, a decision on how useful they are will not be completed during the course of your treatment. This means we will not be able to analyse the results gained from testing you to assess your individual decay risk.

Your participation in this research project is strictly confidential and personal details will not be included. Any reporting of the research results is not open to the public.

You are able to withdraw from the study at any time without notice. This will not affect your orthodontic treatment in any way. If you have any complaints please see the attached independent complaints sheet

### Contact details

Research candidate

Balya Sriram, ph. 83033102

SCHOOL OF DENTISTRY

FACULTY OF HEALTH SCIENCES

THE UNIVERSITY OF ADELAIDE

SA

5005

Research Supervisors

Professor Wayne Sampson, ph. 83035153

Associate Professor Craig Dreyer, ph. 83035153

Dr Neville Gully, ph. 83033887

Associate Professor John Kaidonis, ph. 8303 3297

THE UNIVERSITY OF ADELAIDE HUMAN RESEARCH ETHICS COMMITTEE

**STANDARD CONSENT FORM  
FOR PEOPLE WHO ARE PARTICIPANTS IN A RESEARCH PROJECT**

1. I, .....(*please print name*)  
consent to take part in the research project entitled:  
*An evaluation of tests that predict early decay in patients undergoing orthodontic treatment*
2. I acknowledge that I have read the attached Information Sheet entitled:  
*Information sheet for participants regarding the project titled "An evaluation of tests that predict early decay in patients undergoing orthodontic treatment"*
3. I have had the project, so far as it affects me, fully explained to my satisfaction by Balya Sriram. My consent is given freely.
4. Although I understand that the purpose of this research project is to improve the quality of dental care, it has also been explained that my involvement may not be of any benefit to me.
5. I have been given the opportunity to have a member of my family or a friend present while the project was explained to me.
6. I have been informed that, while information gained during the study may be published, I will not be identified and my personal results will not be divulged.
7. I understand that I am free to withdraw from the project at any time and that this will not affect dental advice in the management of my health, now or in the future.
8. I am aware that I should retain a copy of this Consent Form, when completed, and the attached Information Sheet.

.....  
(signature) (date)

**WITNESS**

I have described to .....(*name of subject*)  
the nature of the research to be carried out. In my opinion she/he understood the explanation.

Status in Project: .....

Name: .....

.....  
(signature) (date)

THE UNIVERSITY OF ADELAIDE HUMAN RESEARCH ETHICS COMMITTEE

**STANDARD CONSENT FORM**  
**For Research to be Undertaken on a Child, the Mentally Ill, and those**  
**in Dependant Relationships or Comparable Situations**  
*To be Completed by Parent or Guardian*

1. I ..... (please print name)  
consent to allow ..... (please print name)  
to take part in the research project entitled:  
*An evaluation of tests that predict early decay in patients undergoing orthodontic treatment*
2. I acknowledge that I have read the attached Information Sheet entitled:  
*Information sheet for participants regarding the project titled "An evaluation of tests that predict early decay in patients undergoing orthodontic treatment"*
- and have had the project, as far as it affects ..... (name)  
fully explained to me by Balya Sriram. My consent is given freely.
- IN ADDITION, I ACKNOWLEDGE THE FOLLOWING ON BEHALF OF  
..... (name)
3. Although I understand that the purpose of this research project is to improve the quality of dental care, it has also been explained to me that involvement may not be of any benefit to him/her.
4. I have been given the opportunity to have a member of his/her family or friend present while the project was explained to me.
5. I have been informed that the information he/she provides will be kept confidential.
6. I understand that he/she is free to withdraw from the project at any time and that this will not affect dental advice in the management of his/her health, now or in the future.
7. I am aware that I should retain a copy of this Consent Form, when completed, and the attached Information Sheet.
- ..... Parent/Guardian .....  
... (signature and please indicate relationship) (date)

**WITNESS**

I have described to ..... (name of parent/guardian)  
the nature of the research to be carried out. In my opinion she/he understood the explanation.

Status in Project: .....

Name: .....

.....  
(signature) (date)

THE UNIVERSITY OF ADELAIDE  
HUMAN RESEARCH ETHICS COMMITTEE

*Document for people who are participants in a research project*

CONTACTS FOR INFORMATION ON PROJECT AND INDEPENDENT COMPLAINTS  
PROCEDURE

The Human Research Ethics Committee is obliged to monitor approved research projects. In conjunction with other forms of monitoring it is necessary to provide an independent and confidential reporting mechanism to assure quality assurance of the institutional ethics committee system. This is done by providing research participants with an additional avenue for raising concerns regarding the conduct of any research in which they are involved.

The following study has been reviewed and approved by the University of Adelaide Human Research Ethics Committee:

Project title: An evaluation of chairside caries tests in the prediction of whitespot lesions in patients undergoing orthodontic treatment.

1. If you have questions or problems associated with the practical aspects of your participation in the project, or wish to raise a concern or complaint about the project, then you should consult the project co-ordinator:

*Name: Dr Balya Sriram*

*Telephone: (08) 83033102*

*Name: Professor Sampson*

*Telephone: (08) 83035153*

2. If you wish to discuss with an independent person matters related to
  - making a complaint, or
  - raising concerns on the conduct of the project, or
  - the University policy on research involving human participants, or
  - your rights as a participant

contact the Human Research Ethics Committee's Secretary on phone (08) 8303 6028